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DEVELOPMENT OF LOCAL CIRCUITS IN MAMMALIAN VISUAL CORTEX

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INTRODUCTION

Most of the synapses in the mammalian cerebral cortex are components of local circuits. Not only do these local, or intrinsic, connections dominate in numerical terms, but such synapses are directly responsible for generating neuronal codes that convey sensory data and elicit behavior. In the mammalian primary visual cortex, the precise arrangement of local circuits in both vertical and horizontal dimensions is crucial for such fundamental properties of cortical neurons as detecting the orientation and direction of movement of visual stimuli. The elaboration of local connections, therefore, is probably a key factor in the emergence of computationally competent neural circuits.

Despite the central role of local circuits in neuronal processing, until recently several factors conspired to limit insight into how precise local excitatory and inhibitory interconnections emerge during development. First, the patterns of intrinsic circuitry in many brain areas were either unknown or known only in crude outline, which greatly hindered interpretation of developmental studies. Second, techniques used to elucidate local circuits were limited, as they relied almost exclusively on Golgi staining. In recent years, advances in techniques have improved our understanding of adult local circuits and have provided powerful tools for detailed investigations of local circuit development. These studies have been particularly

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fruitful in the mammalian primary visual cortex, in which the arrangements of local circuits in the adult, and the response properties generated by these circuits, have been examined in considerable detail. Furthermore, the availability of at least some information concerning the emergence of specific response properties and behaviors during postnatal development offers an opportunity to correlate the elaboration of local connections to the emergence of distinct physiological properties.

In this review, we concentrate on the visual cortex to examine the emergence of intrinsic processing machinery in the mammalian neocortex. We define the general mechanisms and constraints on the elaboration of local axonal connections and focus primarily on the differentiation of excitatory links within and between the cortical layers. These excitatory synapses arise principally from spine-bearing neurons: the pyramidal cells outside of layer 4 and the spiny stellate cells within layer 4. The developmental history of subplate and layer I neurons has been reviewed by others (Marin-Padilla 1988; Shatz et al 1988). Although we emphasize development of the visual cortex, we also present additional information garnered from other brain regions that illuminate some of the basic mechanisms.

TECHNICAL ADVANCES IN THE STUDY OF LOCAL CIRCUITS

Most information about adult and developing cortical circuitry has been inferred from Golgi studies of immature animals. Although adult myelinated axons usually stain poorly, the unmyelinated processes of young animals stain well. Even in young animals, however, the Golgi technique suffers several well-known limitations. It stains capriciously, which makes it difficult to study a restricted neuronal population in isolation. Also, the completeness of axonal staining is always in question because of the pronounced age-dependence of impregnation. This is particularly troublesome in developmental studies, in which the growth, elaboration, and retraction of processes are critical events.

Intracellular Staining in Brain Slices

Intracellular staining, with either horseradish peroxidase (HRP), biocytin, or fluorescent dyes, overcomes many limitations of the Golgi method, thus allowing detailed visualization of the complete axonal arbors of selected subsets of cells in visual cortex (e.g. Gilbert & Wiesel 1979, 1983; Martin & Whitteridge 1984). Coupling intracellular staining with in vitro brain slice preparations has overcome many limitations of both Golgi staining and

intracellular staining in vivo (Katz 1987). Specific neurons in specific locales (such as a cortical layer) can be targeted for injection, and the yield of filled cells is dramatically higher than with in vivo staining. Intracellular staining in brain slices has been successfully employed to visualize developing neurons in a variety of systems, including the visual cortex (Callaway & Katz 1990; Friauf et al 1990; Katz 1991), the optic tectum (Katz & Constantine-Paton 1988), the retina (Dann et al 1988; Ramoa et al 1987), and the hippocampus (Rihn & Claiborne, 1990). The high yield of filled neurons allows systematic assessment of the morphological consequences of specific manipulations to a developing system; by using directed intracellular injections, one can visualize dozens of similar neurons in each animal. For example, Katz et al (1989) examined the influence of ocular dominance column boundaries on cortical stellate cells in primate visual cortex; they required only a few animals to obtain enough cells to complete the study. The capricious nature of Golgi staining, or the restricted yield of intracellular staining in vivo, would make such investigations otherwise impossible.

Intracellular staining in slices has its own limitations, however, including the severing of long collaterals and difficulties in staining long processes in their entirety. Therefore, complementary anterograde and retrograde tracing techniques for visualizing local patterns of connections are frequently required.

New Fluorescent Tracers for Visualizing Local Circuits

The structure of the developing brain limits the usefulness of conventional tracing reagents; they usually diffuse widely in the large extracellular space in young animals and produce injection sites much larger than the entire extent of a developing local projection. Several new fluorescent tracers that overcome these problems have proven useful in the analysis of local cortical circuits. Retrogradely transported fluorescent latex microspheres, or "beads" (Katz et al 1984; Katz & Iarovici 1990) are composed of relatively large, hydrophobic particles that diffuse very little after injection into very young brains, thus providing the resolution necessary for developmental studies. Retrogradely transported beads are also retained in living cells for many months following uptake, thus permitting visualization of the eventual fate of neurons connected to particular locales in the developing animal. However, this approach does not reveal the detailed axonal or dendritic arborization of labeled neurons.

The fluorescent carbocyanine dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, popularly known as DiI, and DiA and DiO, which are of the same family, are also very powerful tools for analyzing developing local circuits. These dyes travel both anterogradely

and retrogradely and they fill axons, including growth cones and fine branches, exceptionally well. In addition, these dyes diffuse along fibers even in fixed tissue (Godement et al 1987), which overcomes many of the problems of making tracer injections in prenatal animals or in difficult to access targets. Finally, these dyes allow detailed analysis of circuits in the human brain (Burkhalter & Bernardo 1989), which previously relied almost exclusively on the Golgi technique.

LINKING LAYERS: THE DEVELOPMENT OF VERTICAL CONNECTIONS

One of the earliest noted features of adult cortical organization was the strong tendency for connections to run vertically between cortical layers (Cajal 1911; Lorente de No 1944). For many years, vertical connections were considered the predominant, if not exclusive, dimension of connectivity in the cortex (Lorente de No 1933). The discovery of orientation columns, in which radially aligned groups of neurons shared preference for the orientation of a visual stimulus, was also attributed to the strong vertical links between cortical layers. Hubel & Wiesel (1962) observed that cells with increasingly complex response properties were located in different layers and hypothesized that interlaminar connections were used to construct more elaborate physiological properties from simpler ones. For example, they proposed that several "simple" cells in layer 4 converged on overlying cells in layer 3 to generate neurons with "complex" receptive fields. In this hierarchical scheme of cortical processing, specific intrinsic vertical connections between excitatory neurons (spiny stellate and pyramidal cells) in different layers form the neural circuitry required to analyze information from a local portion of the visual field. The use of Golgi, extracellular, and intracellular staining techniques provided an anatomic framework for the hierarchical theory and revealed the basic patterns of excitatory interlaminar connections in the adult visual cortex of cats and primates (reviewed in Gilbert 1983).

Except for the recent work of Bolz & Gilbert (1986, 1989), who tested the physiological roles of vertical connections by inhibiting the activity of one layer while recording responses in another, the exact contribution of interlaminar connections to receptive field properties remains largely unknown. In this light, developmental studies may provide some insight into the role of interlaminar connections: If the time course of the emergence of a specific vertical connection is known, the differentiation of the circuit can be correlated with the emergence of physiological response properties.

Development of Vertical Connections: Specificity vs. Exuberance

When a developing pyramidal cell completes its migration, it has only a single process, its efferent axon, which either has reached or is en route to distant targets (Cajal 1911; Miller 1988). The transition from one simple process to the exquisite order and remarkable laminar specificity of adult vertical excitatory connections could involve several distinct cellular mechanisms. For example, consider the pattern of vertical connections of pyramidal cells in layer 2/3. In adult cats and primates, the main efferent axons of these cells descend through all the cortical layers (except layer 1) on their way to making long-distance connections to extrastriate cortical areas. Collateral branches are primarily present in layers 2/3 and 5; layer 4 contains few, if any, branches (Gilbert & Kelly 1975; Gilbert & Wiesel 1979, 1983; Lund & Boothe 1975; Tigges & Tigges 1982). The specificity of this connection, like that of any vertical connection in cortex, could be achieved in at least three ways: selective "pruning" of initially elaborate and poorly specified collaterals present in all layers; sprouting of collaterals in all layers, followed by differential growth of collaterals situated in appropriate layers; or initially specific outgrowth of collaterals exclusively in the appropriate layers. Ample precedents exist for the role of all three mechanisms in the development of the nervous system in general and the visual system in particular. Extensive pruning of geniculocortical afferents has been implicated as the mechanism that underlies the segregation of inputs to form the ocular dominance stripes in striate cortex (LeVay & Stryker 1979; LeVay et al 1980). Elimination of immature sprouts may be involved in the refinement of geniculocortical topography in hamster visual cortex (Naegele et al 1988), and differential outgrowth of appropriately situated terminal arbors (coupled with elimination of inappropriate sprouts) appears to be one mechanism by which lamina-specific arborizations are formed by retinal ganglion cell axons in the lateral geniculate nucleus (Sretevan & Shatz 1984, 1986). The formation of the specific pattern of intrinsic vertical connections in cerebral cortex, however, does not seem to involve formation of sprouts or collaterals in inappropriate layers. Instead, studies of cats, monkeys, and humans indicate that interlaminar connections are generally highly specific from the initial elaboration of local collaterals.

Lund et al (1977) first noted that specific patterns of laminar connections could be achieved by specific outgrowth, rather than by regressive events. They studied the emergence of local circuits in macaque cortex by using Golgi staining. In layer 6 of late postnatal and young adult brains, at least four cell types can be differentiated, based on highly specific and

stereotyped patterns of laminar arborizations of their dendrites and axons. These patterns may be related to the patterns of inputs from magnocellular and parvocellular layers of the lateral geniculate nucleus, which subserve very different functions in visual processing. Lund et al examined these cells at embryonic day 127 (E127) and noted that the specific dendritic and axonal arborizations were already present by that age. In many cases the sprouts in the "appropriate" layers were < 50 μm long, but no sprouts or growth cones were observed in incorrect layers. Although the emergence of the laminar patterns was not followed from the very earliest stages (i.e. before the emergence of any collateral arbors), Lund et al concluded that "the specific pattern is attained in the initial growth of the neuron, not apparently by later loss of processes or 'pruning' of a more random initial growth." Thus, at E127, well before birth at E165 and certainly before the onset of any visual experience, the specificity of this interlaminar circuit has already been achieved.

One could argue that a period of exuberance for these intrinsic connections in the monkey occurs before E127, or that the Golgi technique might fail to impregnate very immature, fine sprouts in the inappropriate layers. These possibilities seem less likely in view of observations of layer 2/3 neurons in cat and human visual cortex, which started at younger developmental stages and used different staining techniques. In the adult cortex, layer 2/3 pyramidal cells form extensive collateral arbors within both layer 2/3 and layer 5, but they almost completely avoid the intervening layer 4. Intracellular staining with Lucifer yellow in brain slices prepared from neonatal cats demonstrated that this specific pattern of collaterals was present from the very earliest times that collateral sprouts could be detected (Katz 1991). Even extremely immature cells, in which only growth cones emerged from the main efferent axon, had sprouts exclusively in the appropriate layers. Furthermore, the number of primary collaterals remained constant throughout postnatal development, which suggests that little, if any, elimination of primary collaterals occurred. By using extracellular staining in fixed fetal human brains, Burkhalter et al (1990) also concluded that the formation of interlaminar connections of layer 2/3 cells involved specific outgrowth in the appropriate layers. At 24–26 weeks' gestation, crystals of DiI, which were placed in the upper layers, labeled only a radial band of fibers, with no evidence of collateral spouts in layers 2/3 or 5. Only at 29 weeks were collaterals first visible in layer 5; no sprouts were evident in layer 4.

In addition to the extrinsically projecting pyramidal cells, other cells have demonstrated early specificity. In the adult monkey, for example, spiny stellate cells in layer 4 (the geniculate afferent recipient layer) have stereotyped projections to layers 3 and 5. Lund et al (1977) found that the

specificity of these characteristic patterns of connections developed early; by E127, the rudiments of the adult connections were already in place. No cells with widespread or unusual collateral systems were present. Investigations of the development of vertical connections by layer 4 spiny stellate cells in the cat, which used either Golgi (Meyer & Ferres-Torres 1984) or intracellular staining (Katz & Callaway 1990), similarly found that the laminar specificity characteristic of these cells was present from the earliest times that collaterals began to form.

The vertical connections formed by other nonprojecting neurons also seem to develop highly specifically. In the adult cortex, about 20% of neurons are GABA-containing inhibitory interneurons. These cells fall into an array of cell types, which are principally distinguishable by their local axonal connections (reviewed in Fairen et al 1984; Lund 1988). The laminar organization of these inhibitory connections is as precise as that of excitatory neurons. Golgi studies strongly suggest that the laminar specificity of inhibitory connections, like that of excitatory vertical connections, also arises via specific outgrowth, rather than through a process of elimination. When the development of a particular class of inhibitory neurons in monkey layer 4 was analyzed, the characteristic laminar distribution of collaterals was present by E127 (Lund et al 1977). A similar pattern of development was observed in the cat (Meyer & Ferres-Torres 1984).

Timing of Specific Collateral Outgrowth

There are large differences in the age of neurons in different cortical layers, and one might expect that the emergence of intrinsic vertical circuits would reflect these maturational differences. The cell layers in the mammalian cortex (except for layer 1 and the subplate zone) are formed in an "inside-out" pattern, in which later-generated cells migrate past postmigratory cells to occupy positions near the top of the cortical plate (Angevine & Sidman 1961). In animals with increasingly long gestation times, these differences can be dramatic. In the cat, more than one month elapses between the time that layer 6 cells reach their position and the layer 2 cells reach theirs (Luskin & Shatz 1985); in monkeys, at least 50 days separate the arrival times of layer 6 and layer 2 cells (Rakic 1974). Recent observations indicate that when timing differences do exist, arborizations sometimes form first in the more mature layers. Very young layer 4 spiny stellate cells form arbors in "older" layers 4 and 5 before extending collaterals into the "younger" layer 2/3 (Katz & Callaway 1990; Lund et al 1977; Meyer & Ferres-Torres 1984). In humans, layer 2/3 cells may form collaterals in the older layer 5, several months before forming collaterals within layer 2/3 (Burkhalter et al 1990). Some investigators have reported that inhibitory interneurons in deeper layers appear to differentiate before

those located more superficially (Miller 1986). The patterns of connections between cortical areas can also reflect a similar age-dependence, as older cells form arbors in older layers first (Coogan & Burkhalter 1988).

Alternatively, local circuits in the entire visual cortex might develop more or less in concert, perhaps initiated by some cortex-wide cue (Lund et al 1977; Parnavelas et al 1978). Developing layer 6 neurons form equally extensive arbors in layer 4 and layer 3, with no obvious delay in either layer (Lund et al 1977). Similarly, layer 2/3 cells in cat form sprouts and collaterals simultaneously in layers 2/3 and 5 (Katz 1991). Rakic et al (1986) have observed that cortical synaptogenesis, which should at least partly reflect the formation of local connections, proceeds simultaneously in all cortical layers, and not in an inside-out fashion.

Although the "gradient rule" and the "simultaneity rule" are each consistent with the development of some vertical circuits, clearly no single rule applies to all. On a sufficiently coarse time scale, circuits may develop in near synchrony. However, as the time course of circuit differentiation of specific cell types is examined in more detail, discontinuities in the apparent simultaneity are revealed. When the differentiation of some nine different types of spine-free cells in the cat was examined at closely spaced intervals, different types of these inhibitory interneurons had apparently matured at dramatically different rates, even within the same cortical layer (Meyer & Ferres-Torres 1984). Distinctions in the timing of local circuit formation may be more readily apparent in animals with protracted cortical development, like cats and humans. In the next section, we consider some of the possible cues that could produce layer-specific branching patterns.

Cues for Generating Vertical Specificity

To relate the mechanisms involved in local circuit formation to the formation of other connections in the brain more closely, it is worthwhile to distinguish between activity-independent and activity-dependent events during the development of neuronal connections. In general, the pathfinding events and cues that are involved in the navigation of axons towards their correct targets elsewhere in the brain involve a complex interplay between mechanical constraints, diffusible growth factors, and specific molecules on neuronal and nonneuronal cell surfaces. Neuronal activity during pathfinding does not appear to play a significant role. In the vertebrate visual system, for example, axons of retinal ganglion cells make appropriate pathway choices at the optic chiasm, and locate their appropriate targets, even in the presence of TTX, which blocks neuronal activity (reviewed in Udin & Fawcett 1988).

In contrast, the mechanisms that determine how developing axons form specific patterns of terminal arborizations within target areas generally require either evoked or spontaneous neuronal activity (for recent reviews, see Harris & Holt 1990; Shatz 1990). Well-known examples of synaptic rearrangements within the visual system include the selection of appropriate layers in the lateral geniculate nucleus by ingrowing retinal ganglion cell axons and the formation of the segregated pattern of ocular dominance columns by geniculocortical axons in the visual cortex. In contrast to pathway finding cues, blocking neuronal activity by TTX application prevents the normal segregation of retinal ganglion cell terminals into appropriate geniculate layers (Shatz & Stryker 1988; Sretevan et al 1988), prevents the formation of ocular dominance columns in cortex (Stryker & Harris 1986), and reduces the specificity of retinotectal topography (Meyer 1983; Schmidt & Edwards 1983).

The limited evidence currently available suggests that the mechanisms for the formation of specific vertical connections have more in common with the pathway choices observed in other parts of the brain, than with activity-dependent reorganization of synapses. Unlike the formation of cortical ocular dominance columns, vertical connections develop specifically from the outset of axonal differentiation. Furthermore, the development of this specificity may not require either patterned visual experience or spontaneous retinal activity. Continuous binocular deprivation for the first few postnatal months had no discernable effect on either the pattern of interlaminar connections of layer 4 stellate cells or the specificity of the vertical connections of layer 2/3 pyramidal cells; cells still formed normal numbers of branches in the appropriate layers and avoided forming sprouts in inappropriate layers (Katz & Callaway 1990 and unpublished observations). Physiological experiments also suggest that patterned visual activity is not required for the development of specific vertical connections. End-stop inhibition, which relies on vertical connections from layer 6 to layer 4, is present in visually inexperienced animals (Braastad & Heggelund 1985). At least until the end of the first postnatal month, neither binocular deprivation nor dark rearing disrupts the appearance of oriented cells throughout the cortical layers. And, these manipulations do not degrade the columnar organization of orientation selective cells (Sherk & Stryker 1976; Braastad & Heggelund 1985).

Numerous cell surface molecules, which either stimulate or inhibit neurite outgrowth, have been described throughout the nervous system. The absence of sprouts of layer 2/3 pyramidal cells in layer 4 could result from the presence of inhibitory factors on either neuronal or nonneuronal cells, as described in optic tectum (Walter et al 1987a,b). However, even while layer 2/3 cells fail to sprout in layer 4, collaterals from both layer 4 and

layer 6 cells are growing well within the layer. Therefore, if inhibitory cues are involved, they must be specific for one group of cells.

Not surprisingly, some aspects of the development of vertical circuits within the cortex display a striking similarity to the selection of specific targets by other cortical cells. Pyramidal cells in layer 5 project to the brainstem, where they form specific connections to the basal pontine nuclei. These connections are formed by "interstitial sprouting": The axon first grows past the target nucleus; at a later point in development, sprouts emerge and grow specifically into the appropriate subregion of the target. The specificity of the terminal arborization, therefore, emerges via specific outgrowth from a main axon, and not via selection of appropriate collaterals (O'Leary & Terashima 1988). The descriptions of interstitial sprouting in the corticopontine system are reminiscent of interstitial sprouting in the formation of interlaminar connections. Recent in vitro evidence suggests that this interstitial sprouting is induced by a diffusible factor secreted by the pons (Heffner et al 1990). Although the sprouting factors that might be responsible for specific interlaminar connections in cortex have not yet been identified, such factors may be responsible for targeting geniculocortical afferents to layer 4 (Bolz et al 1990).

Differentiation of Vertical Connections and the Emergence of Response Properties

Because the inputs from individual neurons in the lateral geniculate nucleus carry unoriented, monocular information, almost all of the critical cortical response properties must be synthesized within the cortex itself. From a developmental standpoint, physiological studies can answer two important questions: Are response properties innately specified, or are they modified by the animal's visual experience? What is the relationship between the emergence of specific connections and the appearance of distinct response properties?

There is now widespread agreement that the basic response property of orientation selectivity, and the arrangement of cells with similar orientations into columns, is present very early in postnatal development. Many subsequent studies in the cat have confirmed the initial observations of Hubel & Wiesel (1963), who found that orientation selective cells can be detected as soon as the eyes are open, about postnatal day 8, and that these cells are organized into columns (Albus & Wolf 1984; Blakemore & Van Sluyters 1975; Braastad & Heggelund 1985; Frégnac & Imbert 1978; Wiesel & Huble 1974). These studies suggest that at least the rudiments of the local circuitry necessary for generating orientation selectivity are present at eye opening.

Close parallels apparently exist between the emergence of specific

response properties and the differentiation of vertical connections. A detailed laminar analysis of the emergence of response properties in the cat revealed that during the first two postnatal weeks, only cells that resemble simple cells were visually responsive; these were only found in layers 4 and 6, which receive direct geniculate input (Albus & Wolf 1984). Cells with complex receptive fields were only detected in layers 2/3 and 5 after the beginning of the third postnatal week (> P14). The emergence of complex cells may be related to the formation of connections from layer 4. Until about P12, the proportion of layer 4 cells that form vertical connections to the overlying layer 2/3 is very small. The number of cells that form such connections only increases to near adult values toward the beginning of the third week, even though the strength of the 4–2/3 connection is still much less than in the adult (Katz & Callaway 1990). Because complex receptive field properties emerge at approximately the same time that layer 4 cells grow into layer 2/3, this connection may be vital for conveying the information necessary to generate complex receptive fields. Furthermore, the temporal difference in the emergence of simple and complex receptive fields, coupled with the anatomic results, supports the idea that complex receptive fields may be generated, at least initially, by inputs from simple cells in layer 4 (for an alternative hypothesis for generating complex receptive fields in the adult, see Malpeli 1983). The simultaneous emergence of complex cells in layers 2/3 and 5 also seems to echo the simultaneity in the development of connections from layer 2/3 to 5, which supports the idea that the receptive fields of layer 5 cells may be at least partially generated from inputs from layer 2/3.

CONNECTING COLUMNS: DEVELOPMENT AND MODIFICATION OF HORIZONTAL CONNECTIONS

Although Golgi techniques strongly emphasized vertical connections that link different layers, newer tracing techniques have revealed extensive horizontal connections over distances of several millimeters within individual cortical layers. These connections, variously termed tangential, intralaminar, or horizontal, are especially prominent in layers 2/3 and 5 of the visual cortex in primate and nonprimate species. Individual pyramidal neurons injected with HRP form numerous periodic aggregates of synaptic terminals spaced at about 1 mm intervals in the tangential plane, over a distance of up to 6 mm from end to end (3 mm from the cell body) (Gilbert & Wiesel 1979, 1983; Martin & Whitteridge 1984). These periodic connections are reciprocal: Small cortical injections of mixed retrograde and anterograde tracers label distinct clusters of neurons and synaptic

terminals, also spaced at about 1 mm intervals over similar distances, in cats, tree shrews, and primates (Burkhalter & Bernardo 1989; Gilbert & Wiesel 1989; Livingstone & Hubel 1984; Rocklund 1985; Rocklund & Lund 1982, 1983).

Several roles have been suggested for these connections (Mitchison & Crick 1982), although their precise functions are unknown. Long horizontal connections may generate the elongated receptive fields encountered in layer 6 (Bolz & Gilbert 1989). Their relationship to functional cortical organization suggests that long horizontal connections are involved in integrating visual information across columnar boundaries to form a cohesive map of the visual field. In the cat, and probably in tree shrews, horizontal connections in layer 2/3 specifically interconnect columns that share the same preferred orientation (Gilbert & Wiesel 1989). In primates, horizontal connections specifically link the cytochrome oxidase rich "blobs" to one another, and "interblob" regions to each other (Burkhalter & Bernardo 1989; Livingstone & Hubel 1984). Gray & Singer (1989) have reported that synchronized oscillatory responses to same-orientation stimuli extend over many millimeters. They also propose that the synchrony of the responses is mediated by tangential connections. Because the horizontal connections span a cortical area greater than that corresponding to the classical receptive field of an individual neuron, such connections may be important in modifying a neuron's responsivity according to the context of surrounding stimuli (e.g. Allman et al 1985; Gilbert & Wiesel 1990; Gray et al 1989;
Malsburg & Schneider 1986). Thus, the emergence of perceptual capabilities that require integration of information from distant points in the visual field may also require the development of appropriate horizontal links within a cortical layer.

Normal Development of Horizontal Connections in Visual Cortex

In very young cats and humans, clustered horizontal connections are absent (area 17: Burkhalter et al 1990; Callaway & Katz 1990; Luhmann et al 1990a; area 18:
in cats, retrograde tracers injected into the superficial layers of cortex result in a continuous pattern of labeling over a limited tangential domain; similarly, DiI injections in neonatal human brains revealed no clustering, even at four months postnatal. By the end of the first postnatal week in the cat, tracer injections reveal crude clusters of retrogradely labeled cells over a somewhat greater tangential extent than seen only a few days earlier. These crude clusters emerge simultaneously in layers 2/3 and 5. There is some controversy over the subsequent process of cluster development.

Luhmann et al (1986, 1990a) claim that the number, spacing, and tangential extent of clusters, which result from a single retrograde tracer injection, all increase steadily during the first postnatal month, until the most distant clusters are up to 10 mm from an injection site. After the fourth postnatal week, both the number and tangential extent of labeled clusters decline to adult levels. Thus, according to these investigators, the development of early postnatal clusters involves reductions in both the length of tangential fibers and the number of clusters linked to a single site. In contrast, Callaway & Katz (1990), who used sequential tracer injections at precisely the same cortical locus, observed that the number, tangential extent, and location of clusters labeled from a single site remained unchanged between P15 and P30, at which time labeling was indistinguishable from that in adult animals (Figure 2). The tangential extent of clusters reached its adult values (3–4.5 mm from the injection site) by eight days postnatal, and there was no subseqent increase or reduction. However, Callaway & Katz found that the early clusters were "crude;" as many retrogradely labeled cells were present in the spaces between clusters. As animals matured, the proportion of labeled cells between clusters gradually diminished, until the adult organization—distinct clusters of labeled cells with few cells between clusters—appeared by the end of the first postnatal month (see Figure 1).

Some of the differences between the observations of the two groups are probably methodological. The failure to differentiate between crude and refined clusters may be attributable to the use of a neuronal tracer (WGA-HRP) that produces injection sites in excess of 1 mm in diameter. These injections are far larger than the distance between adjacent clusters; therefore, labeling between clusters would be expected regardless of the precision of connections between them. Luhmann et al (1986, 1990a) also analyzed their data with an image processor that employed a band-pass filter designed to remove contributions from labeling between clusters.

Differences related to the tangential extent of retrograde label are more difficult to reconcile. In more than 20 experiments involving microsphere injections in animals aged P12–P21, Katz & Callaway never observed intrinsic label extending more than 4.5 mm from an injection site. Luhmann et al (1990a) reported label extending more than 10 mm from a microsphere injection in a few cases, but considerably less in others. Using current source density analysis, these investigators report that they saw no direct electrophysiological evidence for such extensive horizontal collaterals (Luhmann et al 1990b). Indirect evidence for changes in the length of tangential arbors was obtained by using extracellular single-unit recordings, which occasionally revealed cells with ectopic receptive fields. The distance of such fields from the receptive field center was comparable to

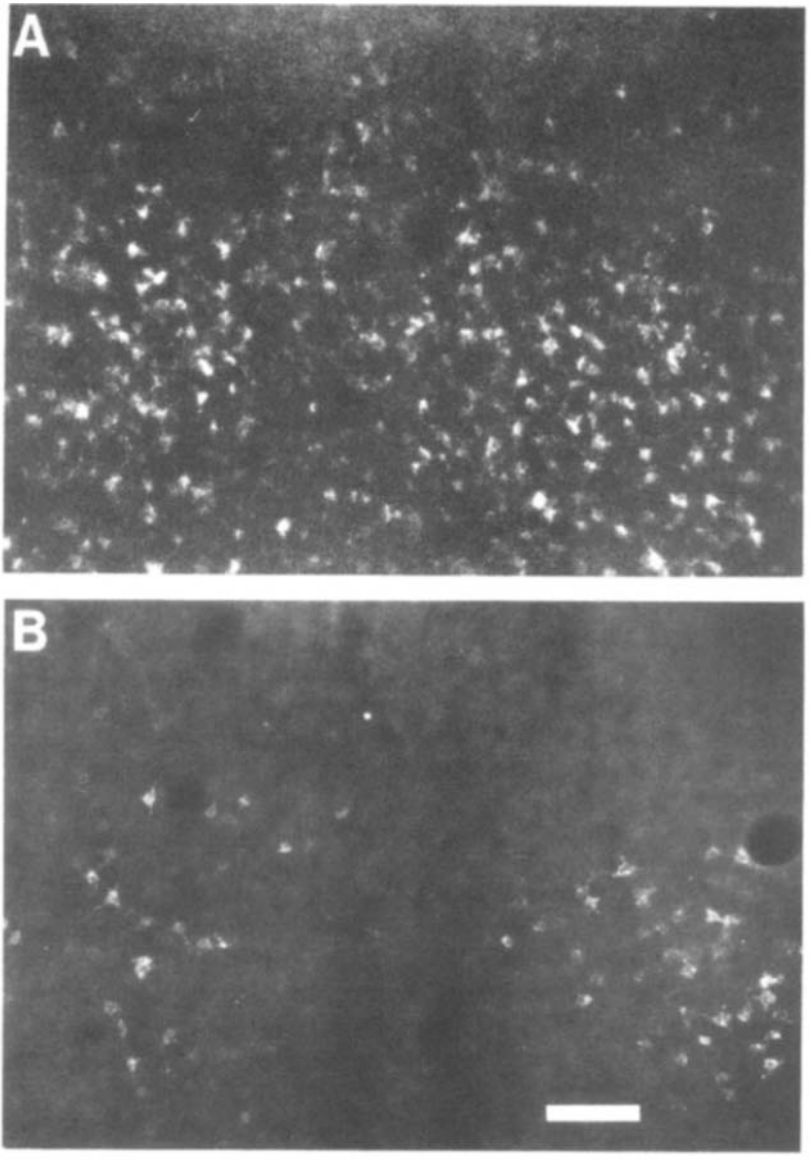


Figure 1 Crude and refined clusters in tangential sections of developing cat visual cortex. (*A*) Part of the pattern of retrograde labeling following a single microsphere injection in a postnatal day 12 cat. Two crude clusters are visible as zones of dense, bright retrograde labeling; between these zones are numerous labeled cells that have made inappropriate connections to the injection site. (*B*) At postnatal day 38, a similar injection results in highly refined clusters of labeled cells; clusters are smaller and few cells between clusters are labeled. Scale bar: 100 μm. (From Callaway & Katz 1990, with permission.)

the tangential spread of collaterals. Ectopic fields were extremely rare in the adult (Luhmann et al 1990c). The loss of ectopic receptive fields could represent loss of tangential arbors, but could also result from other maturational changes in striate cortex during the first months of life.

Based on this work, it is not clear whether a postnatal reduction in the length of horizontal connections actually occurs. This issue was directly addressed by making closely spaced or overlapping injections of red microspheres at an early age (P14–15), followed by green microspheres at a later age (P29 or P38). The retrograde labeling that resulted from the 2 injections was invariably coextensive, and always less than 4.5 mm from the injection sites. When the injection sites were superimposed directly, the refined clusters caused by the later injection were located in the middle of crude clusters from the earlier injection (Callaway & Katz 1990) (Figure 2). These data argue against a postnatal reduction in horizontal axon length, or change in the number or position of clusters between P14 and P38. Single time point experiments indicate that the adult extent and pattern of clusters are attained even before P38, which suggests that there is no overall change in these parameters in the developmental history of clusters.

Mechanisms of Cluster Refinement

Over the past decade it has become clear that regressive phenomena, such as cell death, process elimination, and synapse elimination, play a major role in shaping the patterns of connectivity in the developing nervous system (for review, see Purves & Lichtman 1985). In the development of specificity in several corticocortical projections, both selective cell death and process elimination have been implicated (Innocenti & Caminiti 1980; O'Leary et al 1981; Price & Blakemore 1985a,b). In the normal development of clustered intrinsic horizontal connections, at least two transitions potentially mediated by regressive phenomena have been identified: the change from an unclustered distribution of retrograde labeling to a crudely clustered distribution and the change from crude to refined clusters. The use of persistent cellular labels to follow developing horizontal connections indicates that neither transition involves the selective death of incorrectly situated cells (Callaway & Katz 1990).

The use of the above-mentioned double-injection paradigm, in which different tracers were injected at different times to label crude and refined clusters, demonstrated that selective process elimination is responsible for cluster refinement. Neurons located between clusters, which are labeled by early injections, are still clearly visible after clusters have refined, but no longer make connections to the original injection site. This indicates that

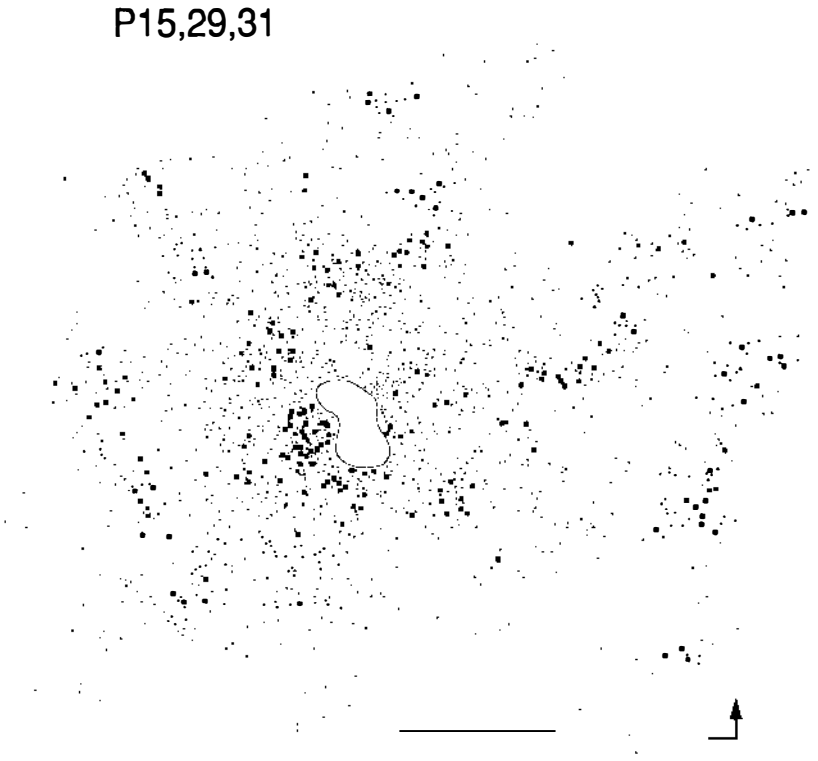


Figure 2 The patterns of intrinsic retrograde labeling in a tangential section of layer 2/3 of cat striate cortex, following injections of fluorescent microspheres at a single site at two different times. The injection site is within the central blank area. Red microspheres were injected on postnatal day 15, followed by green microspheres on day 29. The animal was perfused on day 31. Dots mark cells labeled only by the P15 injection, and squares indicate cells labeled by both the P15 injection and the P29 injection. Triangles indicate cells labeled only at P29. Cells labeled by the P15 injection were arranged in the crude clusters typical of this age, whereas the double labeled cells—those that made an appropriate connection at P15 and maintained it until P29—are arranged in refined clusters. This indicates that the refinement of clusters results from elimination of collaterals, and not cell death. The double-injection also reveals that the number, position, and tangential extent of clusters does not change between P15 and P29. Scale bar: 1 mm. (From Callaway & Katz 1990, with permission.)

those cells that made "inappropriate" connections have selectively lost certain horizontal collaterals (Figure 2).

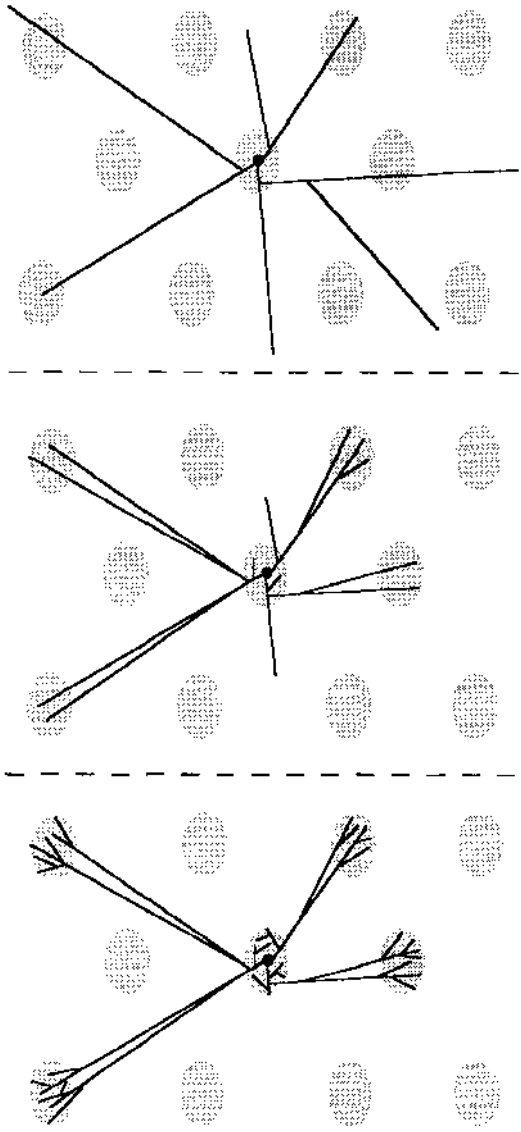
Retrograde labeling experiments can indicate the presence or absence of some sort of connection between two points, but leave a great deal unanswered about the types of modifications that horizontal axonal arbors

undergo during the emergence and refinement of clusters. Reconstructions of individual layer 2/3 pyramidal cells from cat striate cortex show that early in development, cells extend long, relatively unbranched horizontal axon collaterals several millimeters from the cell body (Figure 3, *top*). These branches lack the clusters of fine distal branches characteristic of adult cells (Callaway & Katz 1990, 1991). During the time at which retrograde tracing experiments show no clusters, the directions of collateral growth appear random. Even three weeks postnatally, when crude clusters are clearly present, cells still lack obviously clustered horizontal collaterals. This apparent contradiction between the appearance of single cells and the results of retrograde tracing studies suggests that the differentiation of crude clusters may result from a rearrangement of synapses along axon collaterals without any major redistribution in the positions of the collaterals themselves. Reorganization of axon collaterals is observed between three and six weeks postnatally, as the pattern of retrograde labeling refines: The long horizontal collaterals of a single cell become grouped, and clusters of distal branches are elaborated at the 1 mm intervals typical of adult cells (Figure 3).

Role of Neuronal Activity in Cluster Emergence and Refinement

The initial emergence of crude clusters does not require patterned visual experience. Neither binocular deprivation, nor dark rearing, nor binocular intraocular injections of TTX prevent crude clusters from forming (Luhmann et al 1986, 1990a; Callaway & Katz 1991 and unpublished observations). Apparently, patterned visual activity does not carry the critical cues for establishing crude clusters. This result does not imply that neuronal activity is not involved in the emergence of clusters, as it has become increasingly apparent that even intrinsic activity can provide the cues necessary for the refinement of connections in the visual and other systems. Spontaneous activity of retinal ganglion cells during prenatal life appears to be sufficient and necessary to allow retinal afferents to segregate into eye-specific layers in the lateral geniculate nucleus (Shatz & Stryker 1988; Sretavan & Shatz 1984, 1986; Sretavan et al 1988). Geniculate afferents in the developing monkey segregate into ocular dominance patches before birth and before the onset of patterned visual input (Rakic 1976). It is certainly possible that spontaneous activity in either the geniculate itself, or perhaps within the cortex, is sufficient to organize the pattern of crude clusters. At this point, however, it is equally possible that activity-independent cues in the cortex provide the basis for the organization of a crude system of orientation columns and clusters.

The refinement of clusters, on the other hand, shows a clear dependence



on patterned visual activity. Prolonged binocular deprivation or dark rearing severely degrades or eliminates the clustered organization of horizontal connections. Deprivation during the first two postnatal months does not cause degeneration, but may alter the number and extent of clusters (Luhmann et al 1990a). Deprivation also prevents the normal progression from "crude" to "refined" clusters (Callaway & Katz 1991). Thus, the pattern of clusters that results from a small tracer injection in a P38 binocularly deprived cat resembles, at least superficially, a similar injection in a normal P14 animal. Nevertheless, binocular deprivation does not simply arrest the development of these local connections at a less mature state. Intracellular due injections reveal that individual cells reorganize their axonal arborizations, much like normal cells: The long horizontal collaterals group together, and aggregates of distal branches are elaborated. However, the precision with which these changes occur is much reduced from normal. Thus, each cell makes connections over a region that represents a larger than normal range of orientations.

As with the establishment of ocular dominance columns, there may be a "critical period" in early postnatal life during which the precision of horizontal connections is modifiable by visual experience (Hubel & Wiesel 1970). In the cat, the critical period for response to monocular lid suture in layer 4 extends from about three weeks to three months postnatally. Within layer 2/3, which is rich in long distance horizontal connections, the critical period extends considerably longer (Daw & Fox 1991). This may reflect continued plasticity of intrinsic circuits in these layers for some time after plastic changes in thalamic afferents are no longer possible.

Although a critical period for alterations in horizontal connections apparently exists, the boundaries of this period are still poorly defined. If regular vision is restored to a binocularly deprived animal with crude clusters at six weeks of age, the clusters will refine to a normal adult state by three months of age (E. M. Callaway and L. C. Katz, unpublished observations). Because prolonged binocular deprivation causes degener-

Figure 3 Schematic diagram depicting the development of the horizontal axonal arbor of a layer 2/3 pyramidal neuron, as seen in the tangential plane at three postnatal ages. Stippled areas represent iso-orientation columns, lines represent axon collaterals, and the dot in the center stippled area represents the neuron's cell body. (*Top*) 0–2 weeks postnatal. The initial outgrowth of horizontal axon collaterals is random, and these collaterals project for several millimeters to both correct and incorrect orientation columns. At this time, axonal arbors lack distal collateral branches. (*Middle*) 2–4 weeks postnatal. Axon collaterals projecting to incorrect orientation columns have been selectively eliminated. For at least some neurons, long collaterals projecting to correct columns are added. (*Bottom*) > 5 weeks postnatal. Distal collateral branches have been added selectively within correct orientation columns. At 6 weeks, axonal arbors are indistinguishable from adult arbors.

ative changes in horizontal connections, manipulations that change the pattern of visual input but not the overall level, such as induced strabismus, (Hubel & Wiesel 1965), may be more appropriate for determining the period during which horizontal collaterals can be modified.

Cluster Refinement and the Development of Orientation Columns

Because of the direct relationship between orientation columns and clustered connections (Gilbert & Wiesel 1989), developmental studies of cluster development have provided several insights into orientation column development. Although there is abundant electrophysiological evidence that oriented cells are arranged in columns even early in development, it has proven difficult to visualize the overall organization of columns in developing animals. Studies with 2-deoxyglucose (Thompson et al 1983) revealed patches of labeling in layer 4 in 21-day-old cats, but the adult columnar organization was not apparent until about 35 days of age. The sequential retrograde tracer experiments described above have important implications for the development of the overall pattern of orientation columns. Because the positions of clusters reflect the positions of iso-orientation domains, the fact that the positions of clusters do not seem to change during postnatal development (Figure 2) implies that at least a crude overall map of orientation columns emerges by P8.

The postnatal refinement of clusters may be related to the increased precision of orientation tuning. If, as outlined above, we accept the premise that adult clusters in the cat label iso-orientation columns, then the cells located between the developing clusters represent inputs from other orientation domains. Thus, during the time that crude clusters are present, cells in layer 2/3 are much more likely to receive input from "incorrect" orientations than cells in the adult cortex. This could account for the consistent observations that the orientation tuning in young cats is considerably broader than in older animals. Adult values for tuning emerge at about one month postnatal (Albus & Wolf 1984; Braastad & Heggelund 1985; Imbert & Buisseret 1975), the time at which clusters achieve their adult level of refinement. Alternatively, cluster refinement could result from increased precision of orientation tuning following changes in other circuitry.

For many years after the discovery of plasticity in the ocular dominance column system, a sometimes acrimonious debate raged as to whether orientation specificity was innately determined or, like ocular dominance specificity, subject to modification by visual experience (reviewed in Frégnac & Imbert 1984). There is now general agreement that the basic outlines of orientation processing—including orientation specific cells, the pre-

cision of orientation tuning, and the organization of orientation columns—do not depend on patterned visual experience (Sherk & Stryker 1976; Stryker et al 1978). However, results showing that binocular deprivation reduces the specificity of horizontal connections indicate that some aspects of orientation-dependent visual processing may depend on patterned visual experience for their development. In this context, it would be of considerable interest to test deprived animals either psychophysically or with more complex visual stimuli to determine whether their ability to process more global stimuli has been affected.

Cross Columnar Correlations and the Development of Horizontal Connections

Mechanisms based on correlations between pre- and postsynaptic activity (Hebb 1949) have been strongly implicated in the development of ocular dominance columns in visual cortex (Hubel & Wiesel 1965; Stryker & Strickland 1984) and in the development of retinotectal circuitry in frogs and goldfish (reviewed in Constantine-Paton et al 1990). Hebb-like mechanisms have also been proposed to guide the development of clustered intrinsic connections (Callaway & Katz 1990; Luhmann et al 1990c). Some evidence suggests that such a mechanism may in fact be employed, particularly during the refinement of clustered horizontal connections (Callaway & Katz 1991).

The functional relationships between source and target populations suggest that correlated activity could regulate the development of horizontal connections. Because horizontal connections link columns with similar stimulus preference—iso-orientation columns in cats; colour-specific blobs versus interblobs in primates—the shared response properties could allow for the requisite correlation of activity between axons and their targets. Such correlations have been demonstrated in adult animals for both same-orientation columns (Gray et al 1989; Gray & Singer 1989; T'so et al 1986) and in the blob system in primates (T'so & Gilbert 1988). Furthermore, the correlations in primates appear to be not only for blob versus interblob regions, but also for color opponentcy and ocular dominance within the blob system, and again for orientation preference and ocular dominance in nonblob regions. If these correlations were present between the crude columns in young animals, they could mediate the subsequent refinement of clusters. This hypothesis is consistent with the available evidence; the absence of cluster refinement in lid-sutured cats could be explained by the fact that these animals do not experience oriented visual stimuli that would be necessary to drive the appropriately correlated responses.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

We have attempted to define some of the basic developmental rules that guide the formation of the exquisitely precise, three-dimensional lattice of the mature cortex. Patterns of vertical connections, which give rise to the basic processing capabilities of cortex, appear to be highly specified, possibly by molecular cues within the cortex. On the other hand, the links that unite cortical modules in the tangential plane are only crudely specified and subject to activity-dependent modifications. Despite recent progress, we are still woefully ignorant of the cues or mechanisms used to establish or modify local cortical circuits. We suspect that in vitro approaches—especially long-term cultures of developing cortical slices—will provide the control and accessibility over molecular cues and activity to enable a detailed understanding of the emergence and refinement of circuits. In addition to in vitro analyses, far more insight into the workings of developing cortical machinery is required. Conventional, microelectrode-based extracellular recording techniques are limited in developing systems, in which the primary mode of synaptic communication may be via subthreshold electrical events. New recording techniques, such as optical recording with voltage- or ion-sensitive dyes, may provide a new perspective on the emergence of cortical processing machinery in the intact animal.

Considering the cortical lattice from an evolutionary perspective, vertical connections may represent the primordial axis of cortical intrinsic circuits, whereas horizontal connections may represent relatively recent additions necessitated by increasingly complex forms of processing. Although stereotyped vertical connections are observed in all vertebrate cortices, highly specific horizontal connections are especially prominent in the primate cortex. The crude specification and subsequent activity-dependent modification of horizontal connections may offer opportunities for forging novel links between processing modules, thus providing an avenue for generating new perceptual or cognitive capabilities.

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