Comparison of Intrinsic Connectivity in Different Areas of Macaque Monkey Cerebral Cortex

We have used small injections of biocytin to label and compare patterns of intraareal, laterally spreading projections of pyramidal neuron cells in a number of areas of macaque monkey cerebral cortex. In visual areas (V1, V2, and V4), somatosensory areas (3b, 1, and 2), and motor area 4, a punctate discontinuous pattern of connections is made from 200-μm-diameter biocytin injections in the superficial layers. In prefrontal cortex (areas 9 and 46), stripe-like connectivity patterns are observed. In all areas of cortex examined, the width of the terminal-free gaps is closely scaled to the average diameter of terminal patches, or width of terminal stripes. In addition, both patch and gap dimensions match the average lateral spread of the dendritic field of single pyramidal neurons in the superficial layers of the same cortical region. These architectural features of the connecional mosaic are constant despite a twofold difference in scale across cortical areas and different species. They therefore appear to be fundamental features of cortical organization. A model is offered in which local circuit inhibitory “basket” interneurons, activated at the same time as excitatory pyramidal neurons, could veto pyramidal neuron connections within either circular or stripe-like domains; this could lead to the formation of the pattern of lateral connections observed in this study, and provides a framework for further theoretical studies of cerebral cortex function.

It is clear from our own studies and those of other investigators (Gilbert and Wiesel, 1983; Rockland and Lund, 1983; Livingstone and Hubel, 1984; Levitt et al., 1992; Yoshioka et al., 1992a,b) that the visual cerebral cortex of the primate is characterized by orderly, repetitive intraareal connections; these connections, forming lattice-like arrays concentrated in the superficial layers, arise from pyramidal neurons (Rockland and Lund, 1983), and terminate largely upon pyramidal neurons (Rockland, 1985; McGuire et al., 1991).

We have been curious to see what general features might be shared by these patterned connections in different cortical areas in the macaque, and whether a comparison of their anatomical structure across areas might provide clues to both their function and the rules governing their formation. Recently, we have found that a new tracer substance, biocytin (Horikawa and Armstrong, 1988; King et al., 1989; Lachica et al., 1991), provides excellent labeling of these connections in cerebral cortex, and we have employed it in this study to compare intrinsic patterns of connectivity in three visual areas, V1, V2, and V4, as well as in motor (area 4), somatosensory (areas 1–3), and prefrontal (areas 9 and 46) cortices.

In area V1, the pattern of local connectivity is known to be a patch-to-patch repeating system within two clearly different territories (Rockland and Lund, 1983; Livingstone and Hubel, 1984) that are defined by being either rich or poor in cytochrome oxidase (CO; the blob and interblob zones). Our own work on V4 and V2 (Levitt et al., 1992; Yoshioka et al., 1992a) suggests that a patch-to-patch lattice may also characterize the visual association cortices; the studies of Juliano et al. (1990) and Huntley and Jones (1991) show a patchy connectivity in somatosensory-motor areas as well, though different labeling techniques were employed. We felt that analyses and comparison between areas should be made using a common tracer substance and a similar range of injection sizes in order to provide comparable data.

The current view of such intrinsic, presumed excitatory, projections is that they are created during postnatal development by both new growth and pruning from widely spreading axon collateral branching (Callaway and Katz, 1990; Löwel and Singer, 1992), and that in area V1 they link cell clusters that share common functional properties (Ts'o et al., 1986; Ts'o and Gilbert, 1988; Gilbert and Wiesel, 1989). We ask in this study what might determine the scale and pat-
tern of intrinsic connections, what relationship the pattern of these laterally spreading connections may have to patterns of afferent fiber termination and to the distribution of efferent neuron populations within each area, and whether the neurons giving rise to them lie in common laminar locations in each region. We have observed in prior studies in tree shrews and macaque V1 that the periodicity of the long-distance intrinsic connectivity systems matches the average dendritic spread of pyramidal neuron dendritic arbors in the same laminar environment (Rockland et al., 1982; Lund and Yoshioka, 1991). We, and others, have suggested such a relationship might be an important feature of the visual cortex. We therefore made measures of pyramidal neuron dendritic field dimensions in each area, in those layers giving rise to and receiving the lattice connections, to compare the average dimensions of the axon terminal zones with the spread of single pyramidal neuron dendritic fields (Rockland and Lund, 1983; Lund and Yoshioka, 1991; Malach, 1992). We have also found it of interest to compare our findings in the macaque cortex with the organization of connections in visual cortices of cats, tree shrews, and rats to determine if this aspect of cortical organization is unique to primates or rather is a more universal architectural feature.

Materials and Methods

Seventeen macaque monkeys (Macaca fascicularis, M. mulatta) were used in these experiments. For biocytin transport studies, all animals were M. fascicularis. For the Golgi impregnation studies, M. fascicularis material was used where available (V1, V2, and V4). Only M. mulatta material was available for other areas. However, comparison of the two species in Golgi preparations from the visual areas (see Table 1) revealed no major differences in dendritic field size, and therefore we feel reasonably confident in including Golgi data from the M. mulatta animals. In somatosensory and motor cortices, both infant (2 months) and adult Golgi material was examined; dendritic spread differed by no more than 10% between the two age groups. Adult means are shown in Table 1. The methods employed in making small biocytin injections to visualize intracellular patterns of cortical connectivity were outlined in detail in a previous study (Yoshioka et al., 1992a). We varied the size of injections, making small iontophoretic injections (with core of the deposit measuring 200 μm or less) as well as larger (300-1500 μm) iontophoretic or pressure injections of 4% biocytin (0.2-1.0 μl) in each area, and prepared serial sections cut in both pia-to-white matter and tangential-to-pia planes. Individual injections within each area were spaced at least 3-6 mm apart to prevent overlap of label from adjacent injection sites. Each area, apart from V2, was an exposed surface region of cortex, and each presented a reasonably flat surface for tangential sections. Area V2 was dissected free and physically flattened. We mapped the resulting labeled fibers, terminal arbors, and retrogradely labeled neurons through the section series, and adjusting for different angles of section, the label was reflected in columnar fashion to the cortical surface for purposes of map comparison between areas. In areas V1 and V2, a cytochrome oxidase (CO)-reacted series was interleaved with the sections reacted for biocytin to identify blobs in V1 and CO-rich and CO-poor stripes in V2. A section series from area V2 was reacted for Ca2-301 (DeYoe et al., 1990) to allow verification of the position of CO-rich thick stripes. In some cases in V1 both biocytin and CO reactions were carried out on single sections following the procedure of Lachica et al. (1991). A CO series was also interleaved in the somatosensory and motor cortex section sets to help identify laminar boundaries.

Biocytin label was mapped with light microscopy at both low and high power, and maps from serial sections were matched by blood vessels in the case of tangential series, or by reference to the mid-line or sulcus edges in the case of pia to white matter section series. Figure 1 summarizes injection sites in each area used for this study. The injection sites were defined as a core zone where dense biocytin deposit made recognition of cell or fiber structure impossible. Surrounding this core in each case was a narrow zone of intense cell and fiber label. The limit to the actual region of deposit of biocytin was taken to be where the cell and fiber labeling became first apparent. We measured patch dimensions across both the widest axis and orthogonal to that; the mean of these two measures was taken to be patch diameter. Stripe-like label was measured across the axis orthogonal to stripe trajectory. Spacing between labeled regions was measured as the shortest distance from center to center of neighboring labeled patches or stripes (see Table 1). In V2, spacing was measured between patches clustered in single stripe domains. In all areas, we measured a minimum of 30 patches and their center-to-center distances resulting from at least two separate injections.

We used Golgi preparations to determine the av-
layer 3 of each of the other areas from our extensive collection of Golgi impregnations of adult monkey cerebral cortex; the neurons chosen had basal dendrites spreading in layer 3—not down into layer 4 or upturned into layer 2—and we measured the average width of their layer 3 basal dendritic fields. The correction factor (+12%) was then applied to the measurements obtained from Golgi material to compare their dimensions to those of the biocytin-labeled terminal patches.

Results

We have found that the biocytin technique as described here is highly reliable and reproducible, giving successful orthograd e transport in over 90% of injections. The size of the injections can be precisely controlled, and deposit of label restricted to particular laminae. However, we found the biocytin technique to vary in the success of intraareal retrograde cell labeling among areas. This seemed to depend on fiber diameter; for instance, in motor cortex, many more cells labeled retrogradely than in prefrontal cortex, where labeled fibers were considerably finer than in motor cortex. Fiber diameter was, however, 1 μm or less in all areas studied, and we feel electron microscopy is needed to give accurate figures on labeled fiber diameters. This inconsistency of retrograde labeling has also been observed by others using biocytin (King et al., 1989).

It was clearly evident from the biocytin material that distributed, discontinuous intraareal orthogradely labeled projections were present in all areas of the monkey cortex that we examined. Labeling of incoming afferents was very rarely apparent, and clearly distinguishable from the intrinsic pyramidal neuron relays on the basis of axonal morphology and diameter, as well as laminar distribution. The patchy intraareal terminations were made chiefly within layers 1–3 in each area, with most retrogradely labeled neurons lying in layers 2–3 (see Figs. 2, 3 for representative injections). Layer 5 participated in these connections to a much lesser degree. Patchy terminal clusters were clearly evident, even in single sections, in the three visual areas we examined (Fig. 2), while in somatosensory, motor, and prefrontal areas (Fig. 3) the clustering of terminals in layers 1–3 was less obvious, and often required serial reconstruction to be completed before the patchy or stripe-like pattern was recognized. It was also apparent that similarly sized injections yielded quite different labeling patterns in each area; we observed differences in number of filled fibers, total distance over which the intraareal connections spread, overall spatial distribution of terminal label, and number of retrogradely filled cells. We also noted differences among areas regarding the degree to which the axon projections were closely channeled to reach specific, spatially distinct terminal patches without collateralization en route. Some injections seemed to yield a more diffuse fiber distribution with considerable numbers of bifurcations and further collateralization of fibers at apparently randomly distributed points along their trajectories from the injection.

Figure 2. Photomicrographs of representative biocytin injection sites in visual cortical areas V1 (A), V2 (B), and V4 (C). The cortex is seen in tangential view, and the arrows in each panel indicate terminal patches. Scale bars: 100 μm for A, 200 μm for B, and 400 μm for C.
site to final termination points across the cortex. Different-sized injections in any one area produced changes in dimensions of patches: large injections produced distinct patches of labeled terminals, while smaller injections tended to produce patches of smaller size but similar repeat distance. We include measures of patch dimensions resulting from a similar range of large and small injections in each area.

Prefrontal Cortex—Areas 9 and 46
The region injected in each case (see Fig. 1) lay on the exposed cortical surface between mid-line and principal sulcus, anterior to the arcuate sulcus. We could detect no boundary in terms of the spread of connections across the cortex that might distinguish a border between area 9, lying more medial, and area 46, lying more lateral. Figure 4A is a map of the label resulting from a narrowly focused (core zone, 200–300 μm width) columnar pressure injection (0.7 μl biocytin) filling neurons at all cortical depths but not entering the white matter; the pattern of label was reconstructed from serial coronal (pia to white matter) sections. Axon processes traveled away from the injection column in all directions, mainly in the superficial cortical layers and in layer 5; the fibers traveling farthest and of largest diameter arose in layer 3. The spreading horizontal fibers gave rise to collaterals that formed columns or slabs of fine terminal label in layers 1–3 with terminal-free gaps between. Farther from the injection site, the terminal label became restricted to more superficial layers 1 and 2, but these patches were still served by fibers traveling in both deeper and superficial layers. The pattern of terminal label reconstructed and mapped in tangential view formed a pattern of broken, stripe-like territories that ran anteroposteriorly and slightly obliquely, orthogonal to the axis of the principal sulcus. The spacing between terminal territories averaged 537 μm center to center, and the spacing between patches or bands.
increased somewhat from medial to lateral. The terminal territories, measured across their narrower width, averaged 267 µm, with narrower strips appearing medial and wider stripes lateral to the injection site. A long, unbroken, 300-µm-wide stripe of dense terminal label extended anteriorly and posteriorly from the injection site; lighter terminal label and many fibers filled 470-µm-wide flanking bands each side of this stripe before the patchy bands of longer-range axon terminal fields began. The total area containing terminal label formed a long oval in the mediolateral direction from mid-line to lip of the principal sulcus, measuring 9.4 × 3 mm. Retrogradely labeled cells away from the immediate vicinity of the injection site were extremely rare and were consistently pyramidal neurons in deeper layer 3 when they were found. These cells were scattered evenly across the whole area of the field containing terminals and were usually, but not always, coincident with a terminal patch.

Smaller iontophoretic injections into layers 2–3 (measuring 150–200 µm across) produced prominent bands of terminal label immediately surrounding the injection, as well as more distant lightly labeled bands or patches. While in most cases, following either larger pressure or small iontophoretic injections, label did not extend into area 9 on the medial bank or laterally into area 46 in the depths of the principal sulcus, in some instances we did observe spread of label into these regions.

Label from a larger pressure injection (approximately 400 µm in diameter but not reaching the white matter) was reconstructed from sections cut tangential to the pia, and this reconstructed map is shown in Figure 4B. A wealth of fibers spread away from the injection sites with profuse collaterization terminating in arrays of band-like territories and elongate patches around the injections. The bands sometimes appear interconnected by cross-links of axon terminal fields, and both broad (350–400 µm) and narrow (50–150 µm) terminal band widths were evident. The narrow band width is most prominent around small injections, or interleaved between the broader bands in the case of larger injections. A preliminary account of the organization of intrinsic contacts of this region is given by Lund et al. (1992).

**Motor Cortex—Area 4**

Label from two injections (one pressure, with central focus less than 420 µm, and one iontophoretic, with central focus less than 250 µm) confined to layers 2–3, but also filling pyramids in layer 5 via their apical dendrites, is mapped in Figure 5. Injection locations are marked in Figure 1 by asterisks 1 (larger injection, placed in region of trunk and leg representation) and 2 (smaller injection, placed in wrist and forearm representation) on the precentral gyrus (Bindman and Lippold, 1981). The sections were cut tangential to the pia in each case. Both injections produced broad swathes of terminals and fibers close to the injection site, and patches of terminal label spread over a considerable distance away from the injections. Patches of terminal label extended over an area of 7.4 × 5.2 mm (with the longer dimension running anteroposteriorly) for the larger injection, and over an area of 5.9 × 5.2 mm for the smaller injection (again, extending farther anteroposteriorly than mediolaterally). Elongated patches of terminals concentrated in layers 1–3 averaged 470–670 µm in diameter (950 µm center to center) anterior to the large injection, and 370 × 630 µm in diameter (860 µm center to center) anterior to the small injection. Posterior to the smaller injection, patches measured 320 × 490 µm in diameter (900 µm center to center). Retrogradely labeled pyramidal neurons (mainly in layers 2–3) were most numerous close to the injection sites, but were found across the total extent of anterograde label, usually but not exclusively spatially coincident with anterograde terminal label. Fibers traveled away from the injections chiefly in layers 1, 3, and 5.

**Somatosensory Cortex—Areas 3b, 1, and 2**

Figure 6 illustrates two somatosensory cortex injections, both centered in layer 3. Their locations are indicated in Figure 1 by asterisks 3 (in region of trunk and foot representation) and 4 (in region of wrist and
digit representation) on the postcentral gyrus (Kaas et al., 1981). The larger, a pressure injection in area 1 on the dorsal surface, had a core size measuring less than 390 μm, and the smaller, more laterally placed iontophoretic injection (also in area 1) had a core size less than 200 μm. The smaller injection produced clusters of terminal label over an area measuring 7.1 × 6.3 mm, extending into area 3b anteriorly and down the floor of the intraparietal sulcus posteriorly; the terminal patches averaged 270 × 390 μm in diameter (795 μm center to center) in area 3b anterior to the injection and 290 × 450 μm (715 μm center to center) in area 2 posterior to the injection. The larger injection, placed closer to the central sulcus, gave terminal label over a region measuring 5.8 × 5.5 mm; the majority of terminal patches for this injection were located posterior to the injection site extending into area 2, and the patches measured 300 × 450 μm in diameter (800 μm center to center). The terminal patches were unevenly distributed in islands, with patch-free zones between, for both injections. Most terminal label occurred in layers 1–3, particularly in layers 2–3. Retrogradely labeled neurons, found in layers 2–3, were most numerous close to the injections, but scattered cells were observed across the whole extent of the terminal label zone on the exposed postcentral gyrus, both coincident with and outside of labeled terminal patches. Anterior to the large injection, retrogradely labeled neurons predominated in area 3a in the posterior bank of the central sulcus, and terminal label was sparse, more typical of retrograde labeling of an interareal projection. Orthograde terminal label from the smaller injection in area 2 in the anterior bank of the intraparietal sulcus was not accompanied by retrogradely labeled neurons, more typical of an anterograde interareal projection. Neither of these interareal projections is included in the summary maps of intraareal projections seen in Figure 6.

**Visual Areas—VI, V2, and V4**

Table 1 lists the terminal patch sizes and interpatch distances in visual areas V1, V2, and V4 (plus other areas studied) following iontophoretic injections into layers 2–3 (core size < 300 μm). In general, the total area containing patch label was smaller, and there were fewer terminal patches per injection in any of these three visual areas than was seen for similarly sized injections in somatosensory, motor, or prefrontal cortices. Fewer neurons were retrogradely labeled than in somatosensory and motor cortices, though the labeled neurons were again localized in layers 2–3 and were found predominantly but not always within the same regions as terminal label. Larger pressure injections were required to label the patch system for distances equivalent to somatosensory and motor cortex. V2 label in the lunate sulcus (Fig. 7) was preferentially extended mediolaterally (over 4 mm away from the injection site), orthogonal to the CO-rich and -poor stripe system, rather than down the length of the stripes. Most V4 label (Fig. 8) also spread farther mediolaterally than anteroposteriorly (extending up to 6 mm from the injection); in V1 (Fig. 9), the label was often preferentially extended orthogonal to the rows of CO blobs aligned with the axis of ocular dominance stripes, rather than down their length (i.e., spread parallel to the V1–V2 border, for distances over 3 mm; see also Yoshioka et al., 1992b). In all three of these visual areas, the anisotropy of spread in biocytin label around the injections was in the direction of visual field map anisotropies, suggesting a general relationship of these connections to the topography of the retinal representation.

**Dimensions of Pyramidal Neuron Dendritic Fields in the Monkey Cortex**

We also wished to compare the scale of these patchy intrinsic connections with that of the dendritic field spread of pyramidal neurons in each of these areas. The greatest lateral spread of the pyramidal neuron dendritic field is in nearly all cases made by the basal dendrites. We therefore examined samples of Golgi-impregnated neurons in layers 2–3 (as these are the layers in which patchy terminations are most prominent) of motor cortex, prefrontal cortex areas 9 and 46, somatosensory cortex, and visual areas V1, V2, and V4, and determined the average width of their basal...
Table 1
Pyramidal neuron dendritic field diameter, biocytin-labeled terminal patch size and spacing in different cortical areas

<table>
<thead>
<tr>
<th>Species and cortical area</th>
<th>Mean terminal patch size or stripe width</th>
<th>Mean patch (or stripe) center-to-center distance</th>
<th>Mean layer 2–3 pyramidal neuron basal dendritic spread</th>
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</thead>
<tbody>
<tr>
<td>Macaque monkey (all M. fascicularis except where indicated as M. mulatta by Mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>227.5 ± 54.4</td>
<td>430.0 ± 139.2</td>
<td>237.6 ± 23.4</td>
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<tr>
<td></td>
<td>R = 130–370</td>
<td>R = 210–765</td>
<td>R = 202–283</td>
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<tr>
<td></td>
<td></td>
<td>(242) Mm</td>
<td>(R = 196–283)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R = 175–500)</td>
<td></td>
</tr>
<tr>
<td>V2</td>
<td>339.8 ± 80.8</td>
<td>634.9 ± 200.0</td>
<td>257.1 ± 31.7</td>
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<tr>
<td></td>
<td>R = 175–500</td>
<td>R = 325–1050</td>
<td>R = 180–327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(314) Mm</td>
<td>(R = 235–369)</td>
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<tr>
<td></td>
<td></td>
<td>(R = 220–460)</td>
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<td>V4</td>
<td>345.0 ± 58.2</td>
<td>678.3 ± 254.2</td>
<td>374.8 ± 50.2</td>
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<td>R = 220–460</td>
<td>R = 375–1300</td>
<td>R = 300–460</td>
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<td></td>
<td></td>
<td>(355) Mm</td>
<td>(R = 246–437)</td>
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<td></td>
<td></td>
<td>(R = 215–740)</td>
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<td>Somatosensory (areas 3b, 1, 2)</td>
<td>399.8 ± 104.4</td>
<td>732.8 ± 220.2</td>
<td>351.5 ± 49.1 Mm</td>
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<td></td>
<td>R = 195–740</td>
<td>R = 290–1160</td>
<td>R = 250–406</td>
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<tr>
<td>Prefrontal (areas 9–46)</td>
<td>267.3 ± 114.1</td>
<td>536.7 ± 235.8</td>
<td>351.1 ± 60.2 Mm</td>
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<td></td>
<td>R = 100–700</td>
<td>R = 230–1200</td>
<td>R = 246–440</td>
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<tr>
<td>Motor (area 4)</td>
<td>481.0 ± 144.1</td>
<td>949.5 ± 337.5</td>
<td>484.0 ± 40.2 Mm</td>
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<td></td>
<td>R = 180–850</td>
<td>R = 450–1550</td>
<td>R = 408–554</td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>V1*</td>
<td>300</td>
<td>600</td>
<td>311.1 ± 50.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R = 224–392</td>
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<tr>
<td>Tree shrew</td>
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<tr>
<td>V1*</td>
<td>230</td>
<td>475</td>
<td>243.9 ± 33.3</td>
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<td></td>
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<td>R = 190–314</td>
</tr>
</tbody>
</table>

Data are means ± SD, plus ranges (R). Note that sample sizes were a minimum of 30 patches, and 15 Golgi-impregnated cells per area (see Materials and Methods).


* From Rockland et al. (1982).

dendritic fields (as described in Materials and Methods). The average dimensions of the basal dendritic field of these pyramidal neuron populations are given in Table 1, together with average biocytin-labeled terminal patch width (or stripe width for prefrontal cortex), and average patch center to patch center (or stripe spacing) distances for each area. In each area, the distribution of all these parameters appeared unimodal and symmetric about the mean.

The primates data in Table 1 show a very close similarity in size between the average dendritic spread of single pyramids likely to contribute to the lattice system, and the average terminal patch size (or stripe width in prefrontal cortex). This is true despite an almost twofold size difference in average layer 2–3 pyramidal neuron dendritic spread between area V1 and motor cortex. Figure 10.4 plots the data from Table 1 of terminal patch size against dendritic field spread. These parameters are well correlated (r =

Figure 7. Reconstructed maps of terminal label following two (A, B) koniocellular biocytin injections into area V2 in the lunate sulcus. Injection sites are indicated by the stippled circles; terminal label, by the black regions. V2 has been flattened and sectioned tangentially; the solid lines indicate the borders of the O1 stripe compartments (K: thick; N: thin; P: pale). Both of these injections were made in pale stripes, and most (though not all) terminal label was in pale stripes. Scale bar, 1 mm.
Figure 8. A large pressure injection of biocytin in area V4, not encroaching on the white matter. This map was reconstructed from serial sections cut tangential to the pial surface. The core of the injection is represented by the central black bar, and the immediately adjacent dense radiation of fibers by the hatched area. M, medial; L, lateral; A, anterior; P, posterior. Scale bar, 1 mm. Reproduced with permission from Yoshikawa et al. (1992a).

Visual Cortex (area V1)

Figure 9. Small isoconcentric injections of biocytin (fringed stippled circles) made into macaque area V1. Biocytin-labeled terminal patches (black) are reconstructed in relation to CO-rich "blobs" (with borders marked by broken lines). In A, a "blob" injection gives labeling mainly in blobs. B-D are injections on the edges of CO-rich blobs, and their projections are mainly but not exclusively to "edge" positions. Note that orthograde terminal patches can be much smaller than the area of single blobs. Scale bar, 1 mm.

Figure 10. A. Data from Table 1 are used here to plot the relationship between the average diameter of terminal patches (or width of stripe-like zones) and the average lateral spread of the basal dendritic field of single layer 2–3 pyramidal neurons. Error bars indicate SDs of data points. The dashed line is a least-squares regression line through the monkey data. Prefrontal data point (elongated terminal zones rather than patches) is marked by asterisk (see Results). There is a significant correlation between these measures (r = 0.779, p < 0.05), and the data fall very near to a line of slope 1, which indicates that the size of these terminal zones is scaled almost precisely to the size of the local pyramidal neuron basal dendritic field. We have also included data from area V1 of cat and tree shrew (TS) (indicated by arrows) to illustrate that similar constraints seem to hold in these nonprimate species as well. B. Data again taken from Table 1 to plot patch center-to-center spacing against patch size for the same macaque cortical areas. The least-squares regression line through the monkey data is indicated by the dashed line. These measures were highly correlated (r = 0.983, p < 0.001), indicating that the spacing of these terminal zones across the cortex is matched to the size of the patches themselves, thus maintaining nearly equivalent coverage by the lattice in each cortical area. Data from cat and tree shrew (indicated by arrow) are again plotted for comparison.

0.779, p < 0.05). The dashed line drawn through the data points is the least-squares regression line for the monkey data (small rectangular symbols); the slope of this line is very close to one, indicating that the size of these terminal patches is closely scaled to the size of the local pyramidal neuron basal dendritic field. For comparison we have included similar measures from tree shrew and cat area V1 patches (labeled with HRP; from Rockland et al., 1982; Luhmann et al., 1986), and pyramidal neuron dendritic field sizes from our own Golgi material (with +12% correction). In these species as well, the same correlation between
patch or stripe width and single layer 2-3 pyramidal neuron arbor size is apparent. The basal dendritic field measure for prefrontal cortex stands out in Figure 10A (data point marked by asterisk) as being less well correlated with patch size than the other measures. We suspect that this may be due to the dendritic field being constrained in only one direction (i.e., across the stripe width); if the field is more extended along the length of the stripe, our measures from Golgi material (where we do not know the orientation of the local stripe arrays) may be greater than this constrained direction. This may explain the greater deviation of the prefrontal measure from the expected value indicated by the regression line.

Figure 10B plots the center-to-center spacing of terminal patches or stripes (measured in regions of densest periodic label) in the primate, cat, and tree shrew (TS) against patch (or stripe) size. These parameters are highly correlated ($r = 0.989, p < 0.001$). The dashed line, once again the least-squares regression for the monkey data, is also a good descriptor of cat and tree shrew cortices. This suggests that, in all areas studied, the spacing between regions of local connections is evenly matched to the dimensions of these zones themselves (and therefore, again, to the size of the pyramidal neuron dendritic arbor) so as to maintain a roughly 33% coverage of the cortical surface by lattice patch connections, and 50% coverage in the case of lattice stripe connections (as in prefrontal cortex and tree shrew V1).

Discussion

Interpretation of Biocytin Label

A crucial issue in considering the patterns of biocytin-labeled pyramidal neuron axons in the macaque monkey is whether the projections arise only from the injected point, or also include collateral projections from retrogradely labeled neurons. Since the label includes retrogradely labeled pyramidal neurons to a greater or lesser extent in each region, it is possible that some of the terminals could arise from the axon collaterals of these neurons, sometimes positioned far distant from the injection site (see Kisvárday and Eysel, 1992, in studying cat cortex intrinsic connections). The interpretation of the patterns of label would then change; instead of assuming them to arise only from neurons within the small injected region, they might also arise from a widely scattered population of pyramids either that project to the injection region or whose axon trunks may have passed through the injection site. In studying the retrogradely labeled neurons in the macaque cortex at high magnification, their axons appeared rather faintly labeled even when the dendritic label was quite intense. Collaterals from their axons were hard to find, and if present were even more faintly labeled than the parent axon. They generally became too faint to follow not far from their origin. We believe, therefore, that the terminal label observed following these injections arose largely (but perhaps not entirely) from neurons in or very close to the injection focus. (This depended on the region; e.g., there were very few retrogradely labeled neurons in prefrontal cortex but many more in somatosensory and motor cortex—the success of retrograde filling may depend on the diameter of the axon and its collaterals.)

Laminar Pattering of Lateral Projections

The laminar location of most retrogradely labeled cells was layer 3, with a lesser contribution from layer 2 and very occasional cells in layer 5; this pattern was common to all areas examined, suggesting a common laminar origin to the periodic intraareal connections of cerebral cortex (for detailed examination of this issue, see Yoshioka et al., 1992a, for primate area V4, and the studies of Ojima et al., 1991, 1992, for cat auditory cortex). The precise distribution or weight of terminals in depth could vary but it always lay between layer 1 and the base of layer 3, suggesting that factors leading to the formation of these intraareal connections are most strongly expressed in these layers. However, this does not rule out a monosynaptic link by thalamic (or corticocortical) afferents to the layer 2-3 pyramids providing the lattice connections. In prefrontal cortex or V2, for instance, pyramids in lower 3 can have basal dendrites extending down through layer 4; thalamic afferents may invade the superficial layers (e.g., the blob zones of V1), or thalamic and cortical afferents may terminate in columnar form throughout the cortical depth including both the granular layer 4 and layers 1-3 (V2, prefrontal cortex). There is also the possibility of two modes of processing afferent information—one highly localized and emanating in columnar fashion from layer 4, and the other spread widely via the collaterals of the layer 2-3 pyramids through these intrinsic lattices.

Species Comparisons

We applied the same techniques we used in the monkey to make small biocytin injections in different layers of rat visual and somatosensory cortex (in both albino and pigmented strains). Although we observed excellent local and long-distance transport of label, we were unable to demonstrate patterns of intraareal connections resembling those observed in monkeys, cat, and tree shrew. Burkhalter (1989) and Burkhalter and Charles (1990) described a periodic intrinsic pattern of increases in fiber density and terminals in rat V1 using HRP and *Phaseolus* lectin. However, their illustrations show, and their descriptions stress, that the rat intrinsic connections are not organized as isolated terminal patches as in other species, and are found in layers 2-6. In the rat, layers 2-3 are considerably thinner than in the other species we have considered, and it is possible that these layers lack the same substrate for the intrinsic periodic connectivity seen in other species. It has been reported (Dragger, 1975; Mangini and Pearlman, 1980) that the layout of orientation specificity in mouse V1 cortex does not show the regular layout and repeating character apparent in cat, monkey, or tree shrew V1. It is possible that the lack of clearly defined lattice arrays in the superficial layers is another reflection of an оргa-
izational difference between rodent cortex and the cortices of these other mammals.

**Patterning of Lateral Intrareal Connectivity**

It is clear that in each of the areas investigated in the macaque, a discontinuous distribution of terminal labeling was produced in layers 1–3, and sometimes to a much lesser extent in layer 5 as well, around both large and small injections. The overall patterns of terminal label observed were predominately patch-like in all areas except the prefrontal cortex, where more elongate stripe-like terminal zones were observed. As the injection size was increased (300–400 μm), there was some tendency for the patches to merge in V2, somatosensory, and motor cortex to form elongate patches or irregular stripes closer to the injection, and fusion appears to occur between stripes in the prefrontal cortex. Despite this tendency for patches to fuse together, there was an impression in all areas of a persistence of discontinuous stripe or patch-like organization with a fixed upper limit in width even after large injections. This is perhaps most clearly seen in V1 (Fig. 11), where even after a massive injection (1 μl) of biocytin whose core covered many blob and interblob zones, a clear patch structure of retrograde and orthograde label remained. While we would argue for a continuum of these lattice connections across each area, since every injection produced discontinuous regions of terminal label around it, it is difficult then to understand why a patch system should persist and gaps not fill in when large injections are made. Mitchison and Crick (1982) have put forward a model to explain this phenomenon of discontinuous label even after large injections in the tree shrew cortex; their model is based on each point having very strict spatial constraints on its projections across the cortex, and it remains to be seen if their arguments could also explain all the patterns seen in the primate cortex.

**Factors That May Determine Connectivity:**

*Match to Pyramidal Neuron Dendritic Arbor Size*

The question of what factors determine the patch or stripe size remains unresolved. We suspect from our work, particularly in the visual cortex, that there is a maximum patch size or stripe width for the intrareal connections in each region, but that terminal contributions from individual axons can be considerably smaller than the full patch extent or stripe width. Figure 9 shows most V1 terminal patches are about one quarter to one third the size of GO blobs (which match the size of fully filled patches that surround a large injection; see Fig. 11). This same phenomenon may be seen in the varied stripe widths in prefrontal cortex between large and small injection sizes. The full patch (or stripe) width matches well with the average pyramidal neuron dendritic width in each area. However, labeled terminals from single small injection regions can occupy much less space than this dimension. This suggests that the developmental constraints that mold these synaptic connections (Callaway and Katz, 1990) must operate very locally in the dendritic field rather than being spread across an entire cell's dendritic Arbor, much as has been proposed by those studying synaptic modification at dendritic spines (e.g., Shepherd et al., 1985; Brown et al., 1991; Muller and O'Connor, 1991). We do not, however, propose that small patches of terminal label are concentrated on any single pyramidal neuron; the pyramidal dendrites probably provide a continuum of axonal available surface through the neuropil, and the axons forming small patches of terminals may still contact a few sites on each of several postsynaptic neurons.

The close match between pyramidal cell dendritic arbor size and maximum patch or stripe width for each region does, however, suggest that some other constraint must keep the patch-to-patch system scaled to a dimension equivalent to the dendritic arbor spreads of single pyramids in each region. While the apparent close match in scale could be fortuitous, it might also reflect an essential organizational feature of cerebral cortex that emerges even from a system comprised of continuous overlapped dendritic fields.

**Inhibition as a Factor Shaping Patchy Connections**

The importance of inhibition in shaping the responses of cortical neurons has been emphasized in many studies (Sillito, 1984), and abolition of inhibition produces marked changes in receptive field size (generally enlarging it considerably) as well as in other aspects of the cells' responses (generally a loss of specificity of response properties). This inhibition seems to arise quite local to the neuron (within 1 mm). We know from our own studies (e.g., Lund and Yoshioka, 1991) and those of others (Jones and Hendry, 1984) that pyramidal neurons in both superficial layers and in layer 5 are accompanied by local circuit neurons with "basket" axons that reach out far enough to create an inhibitory zone around each pyramid (see Fig. 12). The scale of the spread of the axons of these inhibitory neurons in at least V1 (Lund and Yoshioka, 1991), motor, and somatosensory cortices (Jones and Hendry, 1984), and the prefrontal region (Lund and Lewis, 1992) seems sufficient (each spreading over an area of three times the diameter of the local pyramidal neuron dendritic fields) to account for the absence of terminals with their Arbor.
Figure 12. A. Golgi impregnation of a basket neuron in layer 4B of macaque area V1. The cell's dendritic arbor is drawn to the left of the open arrow, and the axonal arbor to the right. Reproduced with permission from Lund and Yoshida (1981). B. Golgi impregnation of a basket neuron of macaque prefrontal cortex. The cell's dendritic arbor is drawn to the left, and the axonal arbor to the right. Note the extensive lateral spread of each cell's axonal arbor. Scale bar, 1 mm. Reproduced with permission from Lund and Lewis (1982).

Figure 13. A. Diagram of intraregional cortical connectivity suggested to explain offset patch-like distribution of pyramidal neuron axon terminals. The cortex is viewed from the surface, and one pyramidal neuron is indicated spatially colocalized with an inhibitory "basket" neuron. The basket neuron axon spreads over a region limited by the innermost...
Coactivation of both pyramidal and basket neurons would drive inhibition within the inner hatched circle, thereby making it less likely that the pyramidal neuron would find any other simultaneously active pyramidal neurons within that region, and thus regarding the establishment of synaptic connectivity between different pyramids in this zone during development. The pyramidal neuron makes connections to zones (small circles marked by plus signs) outside the range of the coactivating inhibitory axon field. However, here again, each other pyramidal neuron it contacts will have a similar inhibitory surround from basket neurons colocatalized with them (outer dashed circles), and the excitatory connectivity is restricted to a series of six points, in hexagonal form across the cortex. These constraints are the same for any point, so the same hexagonal connectional matrix would be found around any single pyramidal cell, thus forming a continuum across the cortex. A diagram of cortical connectivity suggested to explain stripe-like discontinuous distribution of intraareal pyramidal neuron connectivities in macaque prefrontal cortex. Here the basket neuron colocatalized with the pyramidal neuron provides an elongate inhibitory area field (marked by minus signs); this permits the colocatalized and coactivating pyramidal neuron to make local excitatory connections to simultaneously active pyramids in a band orthogonal to the long axis of the basket neuron inhibitory axon field. The presence of the inhibitory field versus synaptic connections being made within the flanking stripes and the pyramidal neuron axon "stripes" over a stripe-like region before establishing more distant terminal fields—again under the same constraint so that stripes of terminal label continue across the cortex.
from pyramids located at the same or immediately neighboring cortical points. This suggestion is based on the idea that correlated activity of pre- and post-synaptic elements during development is required for consolidation of synaptic connections (Löwel and Singer, 1992). Both inhibitory local circuit neurons and excitatory pyramidal neurons are likely to be driven simultaneously by common afferents if they are in close spatial proximity, and it is known that pyramidal neurons' axons terminate on both pyramidal and non-pyramidal neurons (McGuire et al., 1991).

In area V1, we find the axons of layer 3 basket neurons to spread 750–800 μm (Lund and Yoshioka, 1991), and in prefrontal cortex, layer 3 basket neurons spread their axons over a range of about 900–1000 μm (Lund and Lewis, 1992); these might in each case provide a prohibitive surround to any one colocalized pyramidal sufficient to create the “gap” between connected patches (in V1 the average layer 2–3 pyramid's basal dendritic field measures approximately 238 μm across, and in prefrontal areas 9–46 it measures approximately 355 μm). This argument presumes that the inhibitory surround prevents coactivation of pre- and postsynaptic neurons, but the question of what influences distribution of terminals within the framework of the patches or terminals at a very local level on the dendrites remains. A circular inhibitory field might be expected to produce an offset patch-like distribution of terminals (Gilbert and Wiesel, 1983; Kisvárday and Eysel, 1992) as illustrated in diagrammatic form in Figure 13A. Stripe-like arrays of terminals would be more difficult to accomplish unless the inhibition were unequally distributed, restricted to an axis orthogonal to the terminal stripes. Basket axons have been described (Marín-Padilla, 1974) in some cortical regions as having a biased axon distribution of this kind, and it remains to be seen if this bias could underlie the patterns of terminals that resemble stripes such as in prefrontal cortex. Figure 13B diagrams a possible framework for inhibition determining a stripe-like discontinuous distribution of terminals. Here the basket neurons provide an elongate inhibitory field. This permits pyramidal neurons activated at the same point to form local excitatory connections in a band orthogonal to the inhibitory field axis, but also requires that the pyramidal neuron axon “step over” a stripe-like zone or inhibition before establishing more distant terminal fields. The circular, fixed-distance inhibitory arbor pattern has also been used recently to model orientation specificity (Wörgötter et al., 1991); their model showed that isotropically arrayed circular fields of inhibition around a center point could induce a net functional cross-orientation inhibition at the center point. In prefrontal cortex, the linear terminal arrays with the suggestion of anisotropic inhibition present another form of circuitry that may have equally interesting functional consequences. Clearly, this model is overly simplified, but it may serve as a useful initial framework for further theoretical studies.

**Relationship of Intrinsic and Extrinsic Connection Systems**

In those regions where the interareal termination of afferents from other cortical regions, or inputs from the thalamus have been described, the intrinsic projections do not exactly mimic the distribution pattern of particular afferent axon populations. However, there are indications that the layout of afferents may be built simultaneously with, or might determine, some aspects of the geometry of the internal connectivity. For example, the pattern of thalamic inputs to the CO-rich blobs in V1 is to the same scale as the continuously repeating connectivity within V1; patches of intrinsic terminals are roughly the same size as CO-rich terminal patches (blobs), as is the spacing between patches or blobs in each of these two systems.

In area V2 also, the connectivity between CO stripe compartments (each with distinct corticocortical afferents) shows a patchy pattern with some targeting specificity between particular CO stripes of “like” and “unlike” afferent terminations (Levitt et al., 1992). Furthermore, the overall topography of zones of labeled patches seems to follow visual field anisotropies in the three visual cortical areas studied. However, the relationship of intrinsic connectivity to afferent topographies in the other regions is harder to discern. Afferents from other cortical regions and from the thalamus are laid out in interdigitating or overlapped stripe arrays in prefrontal cortex with repeat distances that are a little larger than the intrinsic connectivity patterns (300–1000 μm; median width, 685 μm; Goldman-Rakic and Schwartz, 1982; Bugbee and Goldman-Rakic, 1983). This suggests a general topographic relationship may exist between the intrinsic and afferent systems. However, while afferent terminals and efferent neuron sets are of fixed position, the intrinsic lattice appears to be a continuum across that fixed framework, sampling it with a slightly smaller spacing scale. This mismatch in scale is interesting because it should enable the intrinsic connectivity system to sample from several afferent territories simultaneously. If afferent territories were matched in scale to the intrinsic repeat distance, the intrinsic system might regularly sample only from single afferents. Interestingly, in the prefrontal cortex, we find a match between the average stripe width of the intrinsic connections and the spacing of lacunae in the distribution of efferent neurons projecting across the corpus callosum (Lund et al., 1992). Whether the intrinsic lattice is therefore more exclusive in its efferent projections from single points than in its sampling of afferents remains to be tested. In the somatosensory and motor cortices, patchy intrinsic connectivity has been described previously (Juliano et al., 1990; Huntley and Jones, 1991), but the extent and scale of projections labeled by our biocytin injections show intrareal connectivity to be more extensive than previously realized, and unlikely to match exactly the pattern and scale of afferent interareal connectivity (described as stripe-like; Jones et al., 1979; DeFelipe et al., 1986).
Notes
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