Cytochrome-oxidase blobs and intrinsic horizontal connections of layer 2/3 pyramidal neurons in primate V1

NEUSA H. YABUTA AND EDWARD M. CALLAWAY
Systems Neurobiology Laboratories-C, The Salk Institute for Biological Studies, La Jolla
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Abstract

Pyramidal neurons in superficial layers of cerebral cortex have extensive horizontal axons that provide a substrate for lateral interactions across cortical columns. These connections are believed to link functionally similar regions, as suggested by the observation that cytochrome-oxidase blobs in the monkey primary visual cortex (V1) are preferentially connected to blobs and interblobs to interblobs. To better understand the precise relationship between horizontal connections and blobs, we intracellularly labeled 20 layer 2/3 pyramidal neurons in tangential living brain slices from V1 of macaque monkeys. The locations of each cell body and the cell’s synaptic boutons relative to blobs were quantitatively analyzed. We found evidence for two cell types located at characteristic distances from blob centers: (1) neurons lacking long-distance, clustered axons (somata 130–200 μm from blob centers) and (2) cells with clustered, long-distance axon collaterals (somata <130 μm or >200 μm from blob centers). For all cells, synaptic boutons close to the cell body were located at similar distances from blob centers as the cell body. The majority of boutons from cells lacking distal axon clusters were close to their cell bodies. Cells located more than 200 μm from blob centers were in interblobs and had long-distance clustered axon collaterals selectively targeting distant interblob regions. Cells located less than 130 μm from blob centers were found within both blobs and interblobs, but many were close to traditionally defined borders. The distant synaptic boutons from these cells were generally located relatively near to blob centers, but the neurons closest to blob centers had synaptic boutons closer to blob centers than those farther away. There was not a sharp transition that would suggest specificity for blobs and interblobs as discrete, binary entities. Instead they appear to be extremes along a continuum. These observations have important implications for the function of lateral interactions within V1.

Keywords: Visual cortex, Blobs, Local circuits, Macaque monkey, Clustered connections

Introduction

Long-distance horizontal connections provide a substrate for lateral interactions within the cerebral cortex. Such connections are formed by layer 2/3 pyramidal cells and have been studied extensively in the visual cortex. These connections extend for several millimeters parallel to the cortical surface, crossing the cortical columns, and often terminate in a highly selective and patchy manner (Gilbert & Wiesel, 1979, 1983; Rockland & Lund, 1983; Martin & Whitteridge, 1984; Rockland, 1985). In primate V1, layer 2/3 is also characterized by the presence of cytochrome-oxidase (CO) blobs—distinctive regions of high metabolic activity that are organized in rows which lie directly above the ocular dominance bands in layer 4C (Horton, 1984). Morphologically, the dendritic arbors of neurons in blob areas do not show any peculiarity distinguishing them from others outside blobs (Hubener & Bolz, 1992; Malach, 1992), but physiologically they are distinct (Livingstone & Hubel, 1984a; Ts’o & Gilbert, 1988; Tootell et al., 1988a–c; Edwards et al., 1995). Neurons in blobs tend to be monocular, tuned to low spatial frequencies, and have higher contrast sensitivity. On the other hand, neurons in interblob regions tend to be binocular, tuned to higher spatial frequencies, and have lower contrast sensitivity. These differences can apparently be explained by the organization of connections from magnocellular and parvocellular recipient divisions of layer 4C relative to the blobs (see Callaway, 1998, for review). It has also been suggested that blob and interblob neurons differ in their color and orientation selectivity, but these findings are more controversial.

Several studies have focused on the relationships between the patches formed by horizontal connections and modular features of cortical organization such as orientation columns, ocular-dominance columns, and CO blobs (Livingstone & Hubel, 1984b; Ts’o et al., 1986; Ts’o & Gilbert, 1988; Gilbert & Wiesel, 1989; Malach et al., 1993; Weliky et al., 1995; Yoshioka et al., 1996). These experiments suggest that, as a general rule, horizontal connections link neurons with similar receptive-field properties. Most notably tracer
injections in blobs or interblobs resulted in preferential labeling in other similar regions (Livingstone & Hubel, 1984b; Yoshioka et al., 1996). The generality of this rule is supported by developmental studies showing that horizontal connections are preferentially formed/maintained between neurons with temporally correlated activity (Lowel & Singer, 1992; Schmidt et al., 1997). However, recently Yoshioka et al. (1996) analyzed neural labeling in a large sample of extracellular biocytin injections in layer 2/3 of macaque V1. They noted that connections were blob-specific and ocular-dominance-specific overall, but there were many examples that provided clear exceptions to these rules. In some cases, biocytin labeling was more prominent in ocular-dominance columns corresponding to the opposite eye, and in other cases labeling was not blob-specific. These observations suggest that the horizontal connections could have a more complex organization or might not always be specific for functionally similar regions.

The work reported here was aimed at better understanding how horizontal connections might mediate lateral interactions in layer 2/3 of macaque V1, by elucidating the detailed anatomy of horizontal connections and their relationship to CO blobs. To obtain a high-resolution view of these relationships, individual layer 2/3 pyramidal neurons in living tangential brain slices were intracelularly labeled with biocytin during whole-cell recording. The axonal and dendritic arbors of 20 neurons were reconstructed and the positions of synaptic boutons and CO blobs marked with the aid of a computerized reconstruction system.

We found that individual neurons vary considerably in the arrangement of their horizontally projecting axon collaterals. While most neurons had long, horizontal axon collaterals with periodic clusters, 25% of the cells had shorter horizontal collaterals that lacked distal axon clusters. These same cells also tended to have smaller cell bodies and less extensive dendritic arbors. Interestingly, the cells lacking distal clusters were all located at distances of 130–200 μm from blob centers, and cells with distal clusters were typically not found at these distances from blob centers. Analyses of cells with distal axon clusters showed that their distant synaptic boutons contributed connections that are blob-specific as long as transition from blob centers to interblobs are considered gradual. But when blobs and interblobs are considered discrete entities, this arbitrary division makes the horizontal axons of many cells near blob/interblob edges appear to lack specificity. These observations are consistent with a general principle of functional specificity of long-distance horizontal connections and support the hypothesis that functional transitions from blob centers to interblobs are gradual, not discrete (Edwards et al., 1995). Furthermore, the nature of horizontal interactions within V1 is highly dependent on position relative to blobs.

Materials and methods

Individual neurons in the primary visual cortex of macaque monkeys (Macaca mulatta) were intracellularly labeled in tangential living brain slices. The axonal and dendritic arbors of labeled neurons were reconstructed and the positions of synaptic boutons and CO blobs were marked with the aid of a computerized reconstruction system. The positions of their cell bodies and distributions of their synaptic boutons relative to blobs were quantitatively analyzed.

We labeled and analyzed 20 layer 2/3 pyramidal neurons from one of the hemispheres of each of three macaque monkeys, 13 to 15 months old, of both sexes. Coronal slices from the opposite hemisphere were used for unrelated studies.

Cortical slice preparation

Animals were anesthetized with sodium pentobarbital, V1 removed, and the animals were euthanized by sodium pentobarbital overdose using methods identical to those described previously (Callaway & Wiser, 1996; Wiser & Callaway, 1996). After chilling the cortical tissue in oxygenated artificial CSF (ACSF; composition in mM: NaCl 124, KCl 5, KH2PO4 1.25, MgSO4 2, CaCl2 3, NaHCO3 26, d-glucose 10, and kynurenic acid 1, pH 7.4) for ~1 min, it was placed on filter paper saturated with ACSF, the pia was removed, and the tissue was cut into four blocks, each ~8 × 8 mm. Two, 400-μm-thick tangential slices were prepared from the most superficial layers of each block using a vibratome. The slices were then transferred to an interface holding chamber warmed to 34°C for later intracellular filling. We used tissue from the entire exposed surface of area V1, from the V1/V2 border anteriorly to the occipital pole posteriorly, and from the midline to ~2.5 cm laterally.

Intracellular labeling and tissue processing

Methods for intracellular labeling and tissue processing were also identical to those described previously (Callaway & Wiser, 1996; Wiser & Callaway, 1996). Briefly, cells were labeled with biocytin during whole-cell recording (Blanton et al., 1989) using electrodes filled the following solution: 130 mM K-gluconate, 1 mM EGTA, 2 mM MgCl2, 0.5 mM CaCl2, 2.54 mM ATP, 10 mM HEPES, and 2% biocytin (Sigma, St. Louis, MO), pH 7.3. After labeling, slices were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer for 12–14 h, sunk in 30% sucrose in 0.1 M phosphate buffer, and then sectioned on a freezing microtome to a thickness of 50–100 μm. Sections were then stained for CO by incubation in a solution of 30 mg cytochrome C, 20-mg catalase, and 50-mg diaminobenzidine (DAB) per 100 ml of PBS, for 2–4 h at 40–50°C (Horton, 1984) and then for biocytin by incubating them in a horseradish peroxidase (HRP)-conjugated avidin-biotin complex (ABC; Peroxidase Standard Kit, Vector Laboratories, Burlingame, CA), prepared at the standard concentration in PBS and 0.75% Triton X-100, for 2 h. The intracellularly injected biocytin was revealed by reacting the solutions in a solution containing 50 mg of DAB, 2.8 ml of 1% CoCl2, 2.0 ml of 1% nickel ammonium sulfate, and 10 μl of 30% H2O2 per 100 ml of PBS for 5–15 min. This procedure resulted in a dense, black reaction product in labeled neurons, against the reddish brown reaction product from the CO stain.

Analyses of labeled neurons

Stained sections were scanned to locate labeled neurons, and suitably labeled cells were identified. Such cells were located in layer 2/3, as judged by laminar differences in the pattern of CO staining, and labeled well enough that the most distal axonal processes could easily be seen with the 10× objective and synaptic boutons clearly seen with a 60× objective. Layer 2/3 was easily identified by the presence of CO blobs, and the CO “honeycomb” pattern in layer 4A clearly defined the bottom of layer 2/3 (Lund, 1988). Analysis and drawing was done using a Neurolucida (MicroBright-Field, Inc., Colchester, VT; version 2.0) computerized system under light microscopy. Each neuron’s axonal arbor and its relation to the cortical layers and CO blob and interblob regions of layer 2/3 were carefully reconstructed in two dimensions using a 60× oil immersion objective. Adjacent tangential sections were aligned by
matching blood vessels and neuronal processes through the light microscope. Slight rotation and shrinkage differences between sections were always corrected using the Neurolucida computer program. Locations of both en passant and terminaux synaptic boutons (see Fig. 1) were reconstructed and analyzed quantitatively. The location of each synaptic bouton was stored in \( x,y \) coordinates. When necessary, a 100× objective was used to verify locations of synaptic boutons. For each cell, we calculated the distances from the cell body and from each synaptic bouton to the nearest blob center (see below). All dendritic branches and cell bodies were also reconstructed using a 60× objective; however, thickness of dendrites and other details such as dendritic spines were not recorded.

**Results**

Twenty, biocytin-labeled, layer 2/3 pyramidal neurons were suitable for reconstruction and quantitative analysis. Their cell bodies, dendrites, axons, and synaptic boutons were clearly visible and CO blobs readily identifiable in the same tissue sections (Fig. 1). The computerized reconstructions of nine of these neurons and the neighboring CO blobs are shown in Figs. 2–10.

**Two cell types**

We noted two morphologically distinct types of pyramidal neurons. Both types had basal dendrites extending radially around the cell body and several horizontally projecting axon collaterals extending from the main descending axon. For most neurons (15 of 20), the horizontal axon collaterals branched at periodic intervals to form one or more clusters of collateral branches several hundred microns from the cell body (Figs. 2–6, and 8–10). Clusters were either separated from one another by regions that contained no axons or were linked by long, unbranched collaterals. However, the five remaining neurons (cells F and H–K, see below) lacked distal clusters (e.g. Fig. 7). The horizontal axon collaterals of these cells tended to be shorter, usually extending less than 600 \( \mu \)m from the cell body, but occasionally up to 800 \( \mu \)m.

For both groups of cells, long, unbranched axon collaterals occasionally left the plane of the brain slice before giving rise to distal branches. Axon collaterals also left the plane of the slice closer to the cell body. Thus, the extent of distal axonal arborization observed in the brain slice preparations is an underestimate. In view of this, the five neurons without distal clusters might simply represent cells whose axons were cut off during the preparation of brain slices. However, several lines of evidence argue strongly against this possibility.

If the lack of distal axon clusters for a subpopulation of neurons resulted from cutting of axons during slice preparation, there should be no systematic relationship between the locations of their cell bodies and the locations of blob centers. Nevertheless, these five cells were all found at similar distances of 130–200 \( \mu \)m from blob centers. Of the remaining 15 cells, 14 were found outside of this range (five closer to blob centers, nine more distant). Only one neuron with distal clusters was located in this range and it was just

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**Fig. 1.** (a) Low-power photograph illustrating the typical quality of blob staining in sections from tangential brain slices. The darker gray areas are CO dense blobs and the small round, white regions are blood vessels. Biocytin-labeled dendritic arbors from two individual neurons are clearly seen in this photograph. Most axonal processes in this section are too small to be seen at this magnification. Scale bar = 400 \( \mu \)m. (b) A high-power photograph of an axon from a neuron labeled intracellularly with biocytin. A terminal bouton (bouton terminaux) is indicated by the large arrowhead and an en passant bouton is indicated by the arrow. Scale bar = 10 \( \mu \)m.
136 \mu m from a blob center (Fig. 6). This cell had pronounced distal clusters and therefore clearly did not belong to the group lacking such clusters. A statistical analysis reveals that the spatial distributions of the two cell types relative to blob centers is not random. The probability that five of six cells falling within a particular range will be of one type while all 14 cells outside the range are of the other type is extremely low (\( P < 0.0004, \) Fisher exact test). The cells located at distances of 130–200 \mu m from blob centers therefore appear to be a unique type that lacks long-distance clustered horizontal connections.

Further supporting the interpretation that these cells are of a different type than those with long, clustered axonal arbors is the observation that they tend to have smaller cell bodies and less extensive dendritic arbors. Although there was overlap between the populations for all measures of cell size and dendritic extent, the values for cells with short axons were consistently much smaller.
The mean cell body area for the five neurons with short axons was 156 $\mu$m$^2$ (S.E.M. = 15.2 $\mu$m$^2$), compared to a mean of 250 $\mu$m$^2$ (S.E.M. = 31.8 $\mu$m$^2$) for the 15 cells with long, clustered axons (comparison between distributions: $P = 0.067$, Mann-Whitney U-test). There was also a slight difference in the number of primary dendrites: mean of 6.6 (S.E.M. = 0.81, $n = 5$) for cells with short axons versus 8.8 (S.E.M. = 0.82, $n = 15$) for cells with long axons ($P = 0.2$, U-test). Differences in dendritic branching and length were, however, more dramatic. The total numbers of dendritic branches per cell were 16.2 ± 3.5 (mean ± S.E.M., $n = 5$) for cells with short axons compared to 26.0 ± 2.5 ($n = 15$) for cells with long axons ($P = 0.067$, U-test). The total dendritic length per cell was 1725 ± 368 $\mu$m (mean ± S.E.M., $n = 5$) for cells with short axons compared to 3021 ± 292 $\mu$m ($n = 15$) for cells with long axons ($P = 0.036$, U-test).

The different patterns of axonal arborization are also not attributable to differences between brain slices, such as the plane of cutting, the depth of the labeled neuron within the brain slice, or the depth of the brain slice relative to the pial surface. Cells without distal clusters were found in the same brain slices as other cells that had long, clustered axons, and both cell types were located at similar depths, within the upper 200 $\mu$m of the brain slices. Fur-

![Fig. 3. Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell C. The cell body is located in a blob, 60 $\mu$m from the nearest blob center. Long-distance horizontal axon collaterals form branches predominantly in blobs and/or near blob centers. The histograms at the top, right of the figure show that most of the synaptic boutons are located close to blob centers, although the distant boutons are located somewhat farther from blob centers. Conventions are as in Fig. 2. Scale bar = 200 $\mu$m.](image)
thermore, all five cells with short axons were labeled in the upper slice from the parent tissue block (see Materials and methods), as were 11 of 15 cells with long axons. Thus, cells with short axons were not sampled preferentially from deeper in layer 2/3 (where axons tend to extend more obliquely and upward, possibly increasing the probability of axon cutting). The only systematic differences between the two cell types were their patterns of axonal arborization, positions relative to blob centers, cell body sizes, and extent of dendritic arbors.

In our analyses of the relationships between synaptic boutons and blob centers, described below, results from the five cells lacking distal axon clusters are illustrated separately. In addition, two of the cells with distant axon clusters (identification letters L and M) formed only a single cluster 500–600 μm from the cell body (not shown). These two cells probably belong to the same group as those with multiple distal clusters, but for the purpose of analyzing the relationships between blob centers and synaptic boutons distant from cell bodies these cells are far less useful than those with multiple distal clusters. Thus, for such analyses they are also illustrated separately.

**Cytochrome-oxidase blobs**

Before describing in detail the relationships of axonal arbors to CO blobs, it is first useful to consider the organization of CO blobs in V1. The appearances of individual blobs and the geometric relationships between blobs have important implications for implementing and interpreting quantitative analyses of their relationships to horizontal axonal arbors.

The patterns of CO staining in sections from our tangential brain slices are as expected from previous studies of tangential sections through V1 from perfused macaque monkeys (cf. Wong-Riley, 1994). Blobs are present in superficial layers (2–3B) and the
transition to layer 4A is easily discernible as the blobs give way to
the characteristic layer 4A honeycomb pattern. Blobs are seen
isolated, connected by bridges, or nearly confluent with one an-
other (Horton, 1984; Ts’o & Gilbert, 1988; Edwards et al., 1995;
see Fig. 1). Their shapes are in general, elliptical, but sometimes
can be nearly circular. We traced CO blobs, drawing borders where
staining appeared to drop to background levels (Figs. 2–10). How-
ever, as the CO staining density decreases gradually from peaks

Fig. 5. Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell E. This cell is located in a blob region, but close
to its “edge,” 112 μm from the nearest blob center. Distant clusters of axon collaterals are found in both blob and interblob regions.
The histograms at the top, left of the figure show that most of the synaptic boutons are located at intermediate distances from blob
centers. Conventions are as in Fig. 2. Scale bar = 200 μm.
that vary from blob to blob (Edwards et al., 1995), these contours are somewhat arbitrary and are shown primarily for illustrative purposes and to allow comparisons to previously published results.

The identification of individual blobs is, however, less subjective. We analyzed cell body positions and distributions of synaptic boutons relative to both geometric centers of blob outlines and centers defined by the darkest CO staining in each blob, as marked by stars in Figs. 2–10. Since the blob outlines, and therefore their geometric centers, are subjective it is not surprising that our quantitative analyses of synaptic bouton distributions (see below) were best correlated to the centers defined by staining density. We therefore present only our analyses relative to the centers defined by staining density. Edwards et al. (1995) found a similar relationship between blobs and functional properties of layer 2/3 neurons. The locations of the "centers" observed in our study were not always at the geometric centers of blob outlines, but were in the middle of blobs as well as closer to the borders.

It has been noted previously that the distance between blobs varies from animal to animal and that within a single hemisphere the density of blobs increases considerably from regions of V1 representing foveal visual fields to peripheral representations (cf. Wong-Riley, 1994). We did not explicitly keep track of which of our tangential brain slices were from V1 regions with peripheral versus central-field representations, but we did sample neurons from slices representing the entire, exposed dorsal surface of V1 for every hemisphere (see Materials and methods). Thus, it is not surprising that the spacing of blobs in our sample varied considerably from slice to slice. The average distance between blob centers for a given slice ranged from 358 μm to 496 μm. In addition to variation between slices there was also substantial variation within each slice. For the blobs within range of the horizontal axonal arbor of a given cell, the minimum and maximum distances from a blob to its nearest neighbor ranged from 200–432 μm (range of minimums) to 498–781 μm (range of maximums), respectively. The differences between minimum and maximum values were greatest when the average distances were greater.

As implied by the irregular spacing of blobs, they do not form regular hexagonal arrays. Instead they tend to form rows directly above the ocular-dominance columns in layer 4C (Horton, 1984). We did not mark ocular-dominance columns in our material, but others have reported that blob centers are spaced farther apart within columns (about 450–550 μm) than between adjacent col-

Fig. 6. Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell G. This cell is located in a blob, but close to its "edge," 136 μm from the nearest blob center. Distant axonal patches are found predominantly in interblob regions, but generally close to the boundaries. The histograms at the top, right of the figure show that most of the synaptic boutons are located at intermediate distances from their nearest blob centers. Conventions are as in Fig. 2. Scale bar = 200 μm.
umns (about 350 \( \mu m \)). The spacing within columns varies with eccentricity, but the spacing between columns does not (Wong-Riley, 1994). This is consistent with our observation that blob spacing is more variable in slices with a greater average distance between blobs. These sources of variability constrain the types of quantitative analyses that can be used to assess the relationships between synaptic boutons and CO blobs (see below for details).

**Axonal arbors and synaptic boutons**

Synaptic boutons were recognized reliably under light-microscopic observation (see Fig. 1). Both *en passant* and *terminaux* boutons were observed at regular intervals along all axon collaterals. Boutons *terminaux* were far less common than *en passant* boutons (1 to 7.6 ratio), but both types were observed on all collaterals without any apparent spatial segregation by bouton type. For all further analyses, the two types of boutons were therefore pooled. The densities of synaptic boutons on long, unbranched axon collaterals were compared to those on collaterals in clusters distant from the cell body. For any given cell, no difference was observed in the density of boutons per unit of axon length at the two locations. However, the average distance between boutons (and conversely bouton density) varied two-fold from neuron to neuron, ranging from 2.8 \( \mu m \) to 5.6 \( \mu m \), with an average distance of 3.7 \( \mu m \).

Thus, for any given cell, boutons are distributed evenly over the axonal arbor with respect to axon length, but the density varies

**Fig. 7.** Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell J. This cell is located in an interblob region, near a blob edge, 190 \( \mu m \) from the nearest blob center. This cell lacks clusters of axon collaterals distant from the cell body. The histograms at the top, right of the figure show that nearly all synaptic boutons are located more than 150 \( \mu m \) from their nearest blob center, with the majority being located more than 200 \( \mu m \) away. The boutons distant from the cell body (lower histogram) are biased toward even greater distances from blob centers than for the overall distribution. Conventions are as in Fig. 2. Scale bar = 200 \( \mu m \).
from cell to cell. Due to the even distribution on each cell, a reliable visual impression of bouton density can be conveyed by illustrating the locations of axon collaterals without explicitly plotting the location of each synaptic bouton (e.g. Figs. 2–10). We prefer this type of display because the high densities of boutons result in plots of bouton locations which either contain symbols too small to identify or which overlap too extensively to allow resolution of individual markers. We therefore do not explicitly plot bouton locations in Figs. 2–10. It should be kept in mind, however, that the long, unbranched collaterals contribute substantial numbers of synapses—as many per unit of axon length as collaterals in patches—and should not be ignored.

**Horizontal connections and CO blobs**

For each of the 20 cells in our sample, the distance of every synaptic bouton to its nearest blob center was measured. Histograms plotting these distances for each cell are shown in Fig. 11, as well as in insets of Figs. 2–10. These distributions provide information about the overall synaptic output of a particular cell relative to blobs. But since a large proportion of the synapses from each cell are located near the cell body, it is apparent that the distances of these synapses from blob centers will tend to mirror the distance of the cell body from the nearest blob center. Thus, in order to address the specificity of long-distance clustered connections relative to blobs, it was necessary to consider synapses close to the cell body separately from those far away.

To accomplish this, separate plots of the distributions of bouton positions relative to blob centers were made, this time only considering synapses located more than 300 μm from the parent cell body. We chose a cutoff of 300 μm for boutons to be considered “distant,” because analyses of the distances of boutons from cell bodies revealed substantial numbers 200 μm away and secondary distant peaks were observed as close as 400 μm away. The distributions of distant synaptic boutons are shown in Fig. 12. For most of the cells, these distant boutons accounted for a sizeable portion of the overall number and they were found in distinct clusters. But seven of the cells in the sample lacked multiple distal clusters of

![Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell N. This cell is located in an interblob region, 267 μm from the nearest blob center. Distant axonal patches are located in interblob regions, far from blob centers. The histograms at the top, right of the figure show that most of the synaptic boutons are located far from blob centers. Conventions are as in Fig. 2. Scale bar = 200 μm.](image)
axon collaterals and therefore provide less information about the relationship between distant boutons and blob centers. The bouton distributions for these seven cells are indicated by histograms with unfilled or hatched bars (Figs. 7, 11, and 12). These include the five cells that lacked distant axonal clusters (cells F and H–K; unfilled bars) and two cells that had just a single distal cluster (cells L and M; hatched bars; see above).

In the histograms showing distant synaptic bouton distributions (Fig. 12), the Y axis is plotted with two different scales. The scale at the left indicates the percentage of distant boutons as a function of all boutons from the same cell (both near and distant), while the scale to the right indicates the percentage of distant boutons as a function of just the number of distant boutons from the same cell. Thus, the percentages shown at the right are always greater than at

Fig. 9. Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell P. The cell body is located 292 μm from the nearest blob center, in an interblob region. Clusters of long-distance horizontal axon collaterals are primarily in interblob regions and far from blob centers. The histograms at the top, left show that synaptic boutons tend to be located far from their nearest blob centers. Conventions are as in Figure 2. Scale bar = 200 μm.
Fig. 10. Light-microscopic reconstruction of biocytin-labeled layer 2/3 pyramidal cell S. The cell body is located 331 μm from the nearest blob center, in an interblob region and distant axonal branches are also located predominantly in interblobs. The histograms at the top, left of the figure show the distributions of synaptic boutons relative to the nearest blob center. Most of the synaptic boutons are located far from blobs. Conventions are as in Fig. 2. Scale bar = 200 μm.
the left and these scales vary from cell to cell depending on the proportion of the cell's synapses that were located nearby versus distant from the cell body.

Cells near blob centers

Three of the 20 neurons in our sample (cells A–C) had cell bodies within 100 μm of a blob center. The reconstructions of two of these, and their relationships to blobs, are illustrated in Figs. 2 and 3 (cells B and C, respectively). Each of these cells has long-distance axon collaterals with clusters largely confined to traditionally defined blob regions, as indicated by the gray oval outlines in the figures.

The preference of connections from these cells for locations near blob centers (marked by stars) is apparent in the quantitative analyses of synaptic bouton distributions. For cells B and C, the percentages of boutons located at various distances from their nearest blob center are illustrated in the histogram insets in Figs. 2 and 3, respectively. The histograms at the top illustrate the distributions for all synaptic boutons, while those at the bottom include only the distant boutons. The same histograms, as well as the histograms for cell A, are also illustrated in Figs. 11 and 12.

Note that in comparison to other cells with somata farther from blob centers (e.g., cells N–T), the locations of synaptic boutons tend to be close to blob centers (Figs. 11 and 12) and the median distances are also smaller (Fig. 14). (Distances from somata to nearest blob centers are indicated by arrowheads in each histogram of Figs. 2–12.) Most notably, cells A and B have more than 60% of their distant synaptic boutons within 150 μm of a blob center (Fig. 12). This contrasts with cells located far (>200 μm) from blob centers which typically have fewer than 25% of their distant synaptic boutons within 150 μm of a blob center (see further below). But the locations of distant boutons from cell C are not as well correlated with the position of the cell body. Instead the boutons are shifted toward greater distances from blob centers. Nevertheless, they are still closer to blob centers than the boutons from most cells that are far from blob centers.

For all three cells located within 100 μm of a blob center (cells A–C), it is clear that the synaptic boutons (both nearby and distant) tend to be located farther from blob centers than the cell body (Figs. 11 and 12). For example, there are roughly twice as many boutons at distances of 100–150 μm from blob centers than at less than 50 μm (Figs. 11 and 12), and the median values for all three cells are greater than 100 μm (Fig. 14). Thus, these histograms give the impression that the cells preferentially make synapses at distances farther from blob centers than the distances between their cell bodies and the nearest blob center.

But these analyses can be misleading because they are not adjusted for differences in area corresponding to each bin in the histograms. The area corresponding to a circle of 50-μm diameter immediately surrounding a blob center is three-fold smaller than the area in the ring at 50–100 μm from the blob center, and five-fold smaller than the ring at 100–150 μm. For cells located close to blob centers (within half the typical distance between blob centers—about 150 μm), it is straightforward to adjust for the effects of differences in area: the numbers of distant boutons in each range of distances from blob centers were divided by the area corresponding to that range, to obtain a synaptic bouton density within that range. The values for each cell were then normalized to the peak density (the largest value for any bin) and plotted in the histograms shown in Fig. 13. It can now be seen that for all three cells within 100 μm of a blob center (A–C), the peak density of distant boutons is within 50 μm of blob centers (Fig. 13). (Note that the precise peak density might be greater than 50 μm if the value were calculated at a higher spatial resolution.)

Cells far from blob centers

Nine of 20 neurons had somata at distances greater than 200 μm from the nearest blob center (cells L–T). Because two of these (cells L and M) did not have multiple distal axon clusters, they provide little information about long-distance clustered connections. But the remaining seven neurons (cells N–T) had long-distance horizontal axon collaterals with multiple distal clusters. Reconstructions of three representative neurons (cells N, P, and S) are illustrated in Figs. 8–10. In keeping with their greater distances from blob centers, the somata of all of these neurons are located in traditionally defined interblob regions. Distant axon clusters are also found predominantly within interblob regions. Such clusters are apparent for cells N, P, and S (Figs. 8–10). Just one of the clusters from these cells impinges significantly on a blob region (two collaterals to the left of cell P in Fig. 9) and these axon collaterals are located at the edge of the blob, not at the blob center.

Quantitative analyses of synaptic bouton distributions also reflect the tendency for these cells, located far from blob centers, to have synaptic boutons that are far from blob centers. This relationship holds for both the distributions of all synaptic boutons (Fig. 11) and for just the distant synaptic boutons (Fig. 12). For all of these cells (except cell T) fewer than 5% of all of their synaptic boutons are within 100 μm of a blob center and the bouton distributions are shifted toward locations far from blob centers (Fig. 11). The distributions for synaptic boutons distant from the cell body are similar to the overall distributions (Fig. 12), although the boutons tend to be closer to blob centers. Again, fewer than about 5% of the distant boutons are located within 100 μm of a blob center, but for many of the cells the percentage located at 100–150 μm from a blob center is somewhat higher for distant boutons than for the overall population. This difference between the distributions of boutons near (<300 μm) and distant (>300 μm) from the cell body is most apparent in comparing the median values from each cell (Fig. 14). The median values for distant boutons from these seven cells are all at about 175–250 μm from blob centers while those for nearby boutons are typically more than 250 μm.

Furthermore, the median distant bouton distances tend to be smaller than the distances of the cell bodies from the nearest blob center (Fig. 14). This is analogous to the relationship between median bouton distance and cell body location for neurons close to blob centers, where the medians are at greater distances from blob centers than the cell bodies. For those cells, the difference could be attributed to the smaller areas of regions corresponding to locations closer to blob centers (see above). Similar geometric considerations could account for the mismatch for more distant cells. As rings progressively farther from blob centers are considered, they begin to overlap at roughly half the distance between blob centers, effectively reducing the area corresponding to larger distances from blob centers. But because there is widespread variability in the distances between blob centers (see above), it is not straightforward to normalize for the reduced area distant from blobs. These problems had a minimal effect on calculations of density distributions for cells close to blob centers (cells A–G) because they have relatively few boutons far from blob centers (see Figs. 11 and 12). But these problems precluded such an analysis for more distant cells.

Nevertheless, the overall effect of such a bias can easily be appreciated. It is expected to reduce the number of boutons at distances from blob centers greater than half the distance between
Distributions of All Synaptic Boutons

FIGURE 11.
blobs. This skews the distant bouton distributions for cells that are far from blob centers toward smaller values. But less skewing occurs for boutons close to the cell body because the fact that the cell body is very far from a blob center assures that any boutons near it will also be very far from a blob center (cf. Fig 14).

**Cells at intermediate distances from blob centers**

Eight of 20 neurons (cells D–K) had somata 100–200 μm from the nearest blob center. Five of these neurons (cells F and H–K) lacked distant clusters of axon collaterals (see above). Reconstructions of the remaining three (D, E, and G) are illustrated in Figs. 4–6. Each of these cells had many long-distance horizontal axons giving rise to clear clusters distant from the cell body. These distal axon patches clearly do not conform to the traditionally defined blob borders. Nevertheless, these cells have distant synaptic boutons that are located far from blob centers (cells A–C), and are clearly at shorter distances than for cells located more than 200 μm from a blob center (e.g. cells N–T) (Figs. 11, 12, and 14).

Quantitative analyses of the distributions of synaptic boutons relative to blob centers, however, reveal a preference of each of these three cells (cells D, E, and G) to connect to locations at a distance from blob centers that corresponds to the distance from the soma to the nearest blob center. This is apparent for the distributions of distant synaptic boutons alone (Figs. 12D, 12E, and 12G) as well as for distributions of all boutons (Figs. 11D, 11E, and 11G). The distributions of bouton distances from blob centers for these three cells tend to be shifted toward greater values compared to cells located within 100 μm of blob centers (cells A–C) and are clearly at shorter distances than for cells located more than 200 μm from a blob center (e.g. cells N–T) (Figs. 11, 12, and 14).

**Gradual shifts in bouton distributions**

We have so far shown that cells far from blob centers tend to have “distant” synaptic boutons that are located far from blob centers, and conversely cells near blob centers tend to have distant boutons closer to blob centers. These observations suggest that the clustered long-distance horizontal axons giving rise to clear clusters distant from the cell body are “blob-specific.” But we have also noted that the three cells at intermediate distances from blob centers (cells D, E, and G) have distant axon clusters that clearly do not respect the traditionally defined blob borders. Nevertheless, these cells have distant synaptic boutons whose distributions relative to blob centers are intermediate to those of cells located either more distant or closer to blob centers.

Closer scrutiny of our results suggests that for cells within 200 μm of blob centers (cells A–E, and G), relationships between distant bouton positions and blob centers shift gradually as the cell body positions move away from blob centers. These shifts are illustrated in Figs. 12–14. The relationships between distant bouton locations and cell body positions are best seen as illustrated in Fig. 14 (bottom panel). The cells of interest (A–E and G) are all located less than 200 μm from a blob center and are represented by the six filled black squares to the left of the 200-μm tick-mark. Within this group of cells, it can be seen that the cells closest to blob centers have boutons closer to blob centers and as the cell body distance gets larger, the median bouton distance from blob centers gets larger.

Despite this clear trend, it is apparent that the coupling between cell body location and median bouton distance is not particularly tight; there is scatter along the trend line. The greatest departure is for cell C. This cell is nearly as close to a blob center as cells A and B, but its bouton distribution is shifted toward greater distances so that it more closely resembles the distribution for cell D or G (Fig. 12).

Interestingly, this departure from the trend is not present when the normalized densities of distant synaptic boutons are considered (Fig. 13). The highest density of synaptic boutons is within 50 μm of blob centers for cell C as well as cells A and B. And the density distribution for cell C is clearly shifted toward shorter distances from blob centers than for cells D–G. The density distribution for cell C is also toward greater distances from blob centers than for cell A or B.

Thus, the overall gradual trend for cells closer to blob centers to have distant boutons closer to blob centers is apparent in the distributions of normalized bouton densities. The close relationship between bouton distribution and cell body position is also more striking for the normalized bouton densities (Fig. 13) than for the raw values (Fig. 12). Cells A–C are closest to blob centers (distances of 38–60 μm) and their highest bouton densities are within 50 μm of blob centers. The relative bouton densities for all three cells fall to about 80% of the peak densities at distances of 50–100 μm. For cells A and B, these densities fall to about 40–50% and 20–25% of the peak densities at distances 100–150 μm and 150–200 μm, respectively. But for cell C, the densities at these distances remain at about 80% of the peak value. The next two cells, D and E, are farther from blob centers (107 and 112 μm) and have corresponding shifts in the locations of their peak bouton densities. Cell D’s peak is at 100–150 μm from blob centers, with the density falling off evenly at either side of the peak. Cell E has a peak nearer to blob centers (50–100 μm), but the density at 100–150 μm is nearly as high as the peak. Finally, the soma of cell G is the farthest from a blob center for this group of cells and its...
Distributions of Distant Synaptic Boutons

FIGURE 12.
peak bouton density is also at the greatest distance, 150–200 μm from blob centers.

**Cells without distal axon clusters**

We have so far not considered the quantitative analyses of the five cells (F and H–K) that are located 130–200 μm from blob centers and lack clustered long-distance axon collaterals. These cells also have a good correspondence between the locations of their cell bodies and the positions of all of their synaptic boutons relative to blob centers (Fig. 11). But for these cells this relationship results from the fact that the majority of boutons are located near the cell body. This becomes apparent when the relationships between cell body position and median distance of boutons to blob centers for these cells is compared for nearby (<300 μm from the cell body) versus distant boutons (Fig. 14). For nearby boutons there is a very tight correspondence between cell body position and median bouton distance (Fig. 14, top panel, open circles). This can be attributed to the obvious fact that boutons close to a cell body at a particular distance from a blob center will tend to be located at a similar distance from that blob.

But this is not true for boutons far from the cell bodies. Since the cell bodies are located 130–200 μm from blob centers, the distant boutons will either be about 100–200 μm on the other side of, or even more distant from, the same blob center. The blob center closest to these distant boutons is usually not the same blob center that is closest to the cell body. It is therefore noteworthy that the distant synaptic boutons from these cells tend to be located quite far from blob centers, particularly for cells I, J, and K (Figs. 7, 12, and 14—bottom panel, open squares). For these cells (I–K) distant boutons account for substantial proportions of the overall population (25–45% of all boutons), greater than the proportion of distant boutons for some cells with distal axon clusters. These cells therefore provide a substantial link to locations distant from their cell body that tend also to be far from blob centers. The other two cells without distal clusters (F and H) have fewer distant boutons (<20% of the total, cf. Fig. 12). Thus, their distant boutons are too few to have a dramatic effect on the overall distributions. These observations suggest that cells 130–200 μm from blob centers make very different contributions to lateral interactions in V1 than cells at other locations relative to blobs.

**Discussion**

**Overview**

Long-distance intrinsic horizontal connections are found throughout the visual cortex and generally link functionally similar regions. In macaque primary visual cortex, they link regions of similar orientation selectivity and ocular dominance (Malach et al., 1993). Previous studies also demonstrate specificity relative to CO blobs (Livingstone & Hubel, 1984b; Yoshioka et al., 1996). Using intracellular labeling to identify the contributions of single cells to horizontal interactions in primate V1, we identify layer 2/3 pyramidal neurons with two distinct patterns of lateral axonal arborization. While most cells contributed long-distance clustered horizontal axons, one-fourth of the cells in our sample had lateral axons of more limited length that lacked distinct clusters. Surprisingly, these cells were all located within a similar range of distances from blob centers. Cells with distal clusters were not detected between 140 μm and 200 μm from blob centers, suggesting that lateral interactions originating from these locations are more limited. Outside of this range, individual neurons contributed distal axon clusters that are likely the source of clustered anterograde label observed at long distances from extracellular tracer injections (e.g. Livingstone & Hubel, 1984b; Yoshioka et al., 1996).

Consistent with these extracellular labeling studies, we found that neurons far from blob centers made long-distance connections that preferentially targeted regions far from blob centers and neurons located closer to blob centers had distal synaptic boutons located closer to blob centers. Also consistent with the findings of Yoshioka et al. (1996), we found that many cells located at intermediate distances from blob centers, near traditionally defined blob/interblob boundaries, had distal axon clusters that clearly did not respect these borders. But quantitative analyses of the synaptic bouton distributions from these neurons revealed that they preferentially connect to regions at intermediate distances from blob centers. The distribution of distant synaptic boutons appears to shift gradually away from blob centers as the distance from the cell body to its nearest blob center increases.

Insofar as the cytochrome-oxidase blobs are anatomical markers of functional organization within V1, these findings suggest that long, clustered horizontal connections selectively link neurons with similarities in functions that are organized around blob centers. Properties organized in a graded fashion around blob centers include spatial-frequency selectivity and contrast sensitivity (Edwards et al., 1995). But the functional specificity reflected in the blob specificity of horizontal connections may not be restricted to these response properties. These connections might also specifically link neurons with similar specificities for other response properties that have been reported to be related to blobs, such as orientation bias and color sensitivity (e.g. Livingstone & Hubel, 1984a; Ts’o & Gilbert, 1988). Furthermore, our findings suggest that any such properties might also have graded organizations relative to blob centers.

**Comparison to previous studies**

Livingstone and Hubel (1984b) first demonstrated that tracer injections centered on blobs resulted in anterograde and retrograde label predominantly “in blobs,” and injections located far from blobs resulted in label far from blobs. But in both cases there was also substantial label in noncorresponding regions. This nonspecific label could have been interpreted as an artifact of large injection sites or a low incidence of nonspecific connections.

More recently, Yoshioka et al. (1996) used smaller injections of biocytin and based on a large sample (25 injections) they found...
that anterograde label relative to blobs was blob-specific overall, but often highly nonspecific. For injections confined to blob or interblob regions, the percentage of distal axon patches located in “same CO compartment” ranged from 50% (nonspecific) to 100% (highly specific, mean of about 70%). Injections at blob edges resulted in more than half (53%) of the patches in interblob regions and only 30% at edges; the remaining patches (22%) were in blobs. Thus, edge cells did not appear to make edge-specific connections.

Our findings provide a more detailed view of how individual neurons are likely to contribute to these extracellular labeling patterns. The tendency for blobs to be linked to blobs and interblobs to interblobs is clear in all three studies. But our findings suggest that the lack of specificity observed in a minority of cases might be due to differences in methods of analysis (see further below). Most interesting, however, is the consideration of the contributions of cells at “blob edges.” Cells located near blob edges in our sample include those somewhat closer to blob centers and extending long-distance clustered axons, as well as cells slightly more distant from blob centers that lacked distal clusters. Yoshioka et al.’s (1996) “edge” injections likely labeled both cell types. This would explain the slight preponderance of label in interblobs following edge injections. We found that many of the cells without distal clusters extended axons preferentially into interblobs. The mixture of cell types labeled might also explain why Yoshioka et al. were unable to detect a preference for long-distance connections originating from blob edges to target edges. Our quantitative analyses of distant boutons from individual “edge” cells with long, clustered axons indicate a preferential distribution of boutons within edge regions, about 100–200 μm from blob centers.

More generally, apparent discrepancies between previous studies and our results can be attributed to differences in labeling resolution (single cell vs. extracellular tracer injections); resolution of axonal analyses (synaptic bouton location vs. axonal patch boundaries); or methods of quantitative analysis relative to blobs (distance from blob centers vs. sharp blob/interblob boundaries). Specifically, in the analyses of Yoshioka et al. (1996), blobs and interblobs were considered to be binary compartments. Also, only label within distal axon patches was considered; regions with substantial label not organized into patches were discounted. Furthermore, the contributions for all patches were assumed to be similar despite variability between patches in the density of labeling and the subjective assignment of patch boundaries.

Fig. 13. Histograms showing the normalized densities of “distant” synaptic boutons at various distances from their nearest blob centers, for cells located within 150 μm of blob centers. Each histogram shows the distribution of synaptic boutons for one cell, with the cell’s identifying letter indicated at the upper right of the histogram. The horizontal axes indicate distances of boutons from their nearest blob centers (μm) and the vertical axes indicate the normalized densities of synaptic boutons in each bin. Bouton densities are the numbers of boutons divided by the relative areas corresponding to the circle or ring at each range of distances. The values indicated are normalized to the peak density for each cell. The arrowheads indicate the distances of the cell bodies from their nearest blob centers. The horizontal axes indicate the distances of cell bodies from their nearest blob centers. The distances of the cells from blob centers increase (from A–G), the distributions shift gradually toward higher bouton densities at greater distances from blob centers.
In another previous study, McGuire et al. (1991) described the horizontal axonal arbors of two layer 2/3 pyramids in macaque V1. These cells were intracellularly labeled in vivo, precluding the possibility of axon loss due to cutting, and the receptive-field properties of the cells were also characterized. By our criteria, one of these cells belongs to the class that we identify as lacking long, clustered axons (McGuire et al., 1991; see their Fig. 4). The most distant axon collateral is only about 500 μm from the soma, the cell body is located in an interblob, about 185 μm from the nearest blob center (within the 130–200 μm range characteristic of our sample), and its axons preferentially target interblob regions. Interestingly, the receptive field of this cell was strongly end-inhibited, while the cell with long, patchy axons was not end-inhibited. These observations suggest the possibility that end-inhibited cells might be preferentially located at 130–200 μm from blob centers and contribute to horizontal interactions over only relatively short distances.

Layer 2/3 neurons which appear to lack long, clustered axons have also been reported in the cat striate cortex (Gilbert & Wiesel, 1983). Five of nine layer 2/3 neurons in their sample were “not clustered,” but the authors point out that clustering could have been missed since the axons were viewed in two-dimensional reconstructions from sections perpendicular to the cortical layers rather than in tangential sections.

**Horizontal connections and functional architecture**

We have found that the spatial distribution, relative to blobs, of synaptic boutons from long-distance horizontal axons is highly dependent on the location of the parent cell body. Cells near blobs preferentially connect to locations near blobs, those far away make more distant connections, and cells at intermediate distances have intermediate synaptic bouton distributions. As noted above, since several response properties of cortical neurons are closely linked to their positions relative to blobs, these observations imply that horizontal connections preferentially link neurons with similar specificities for these properties. But in examining the precise distributions of synaptic boutons from individual neurons, it is clear that, although the majority of boutons are located at similar distances from blob centers as the cell body, there are many boutons that are located at noncorresponding distances.

There are several possibilities that might account for the existence of this minority population of “nonspecific” connections. One possibility is that location relative to blobs is only a crude predictor of the functional attributes of neurons—the horizontal connections are more specific with regard to functional attributes than with regard to distance from blob centers. However, comparison of our findings with quantitative studies of the relationships between each neuron’s cell body location and synaptic boutons relative to nearest blob center. For each neuron, the median distance of its synaptic boutons from blob centers is plotted as a function of the distance from the cell body to the nearest blob center. The upper panel plots the median values for boutons located close to cell bodies (within 300 μm; circular symbols) while the lower panel plots the values for distant boutons (more than 300 μm from the cell body; square symbols). Open symbols indicate neurons that lack axonal patches far from the cell body; hatched, gray symbols indicate cells with just one long terminal arbor; filled, black symbols indicate cells with two or more axonal clusters far from the cell body. The lower and upper bars extending from each symbol indicate the values for the first and third quartiles of each distribution. For boutons close to cell bodies (upper panel), the median distance mirrors the cell body position, increasing gradually as the distance from the cell body to the nearest blob center increases. For distant boutons (lower panel) there is a similar trend for cells with two or more terminal arbors (filled, black squares), but the trend flattens for the cells farthest from blob centers (see text). The distant boutons from several of the cells lacking long terminal arbors (open squares) tend to be located much farther from blob centers than the distances from the cell bodies to the nearest blob center.

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**Fig. 14.** X–Y plots showing the relationships between each neuron’s cell body location and synaptic boutons relative to nearest blob center. For each neuron, the median distance of its synaptic boutons from blob centers is plotted as a function of the distance from the cell body to the nearest blob center. The upper panel plots the median values for boutons located close to cell bodies (within 300 μm; circular symbols) while the lower panel plots the values for distant boutons (more than 300 μm from the cell body; square symbols). Open symbols indicate neurons that lack axonal patches far from the cell body; hatched, gray symbols indicate cells with just one long terminal arbor; filled, black symbols indicate cells with two or more axonal clusters far from the cell body. The lower and upper bars extending from each symbol indicate the values for the first and third quartiles of each distribution. For boutons close to cell bodies (upper panel), the median distance mirrors the cell body position, increasing gradually as the distance from the cell body to the nearest blob center increases. For distant boutons (lower panel) there is a similar trend for cells with two or more terminal arbors (filled, black squares), but the trend flattens for the cells farthest from blob centers (see text). The distant boutons from several of the cells lacking long terminal arbors (open squares) tend to be located much farther from blob centers than the distances from the cell bodies to the nearest blob center.
between horizontal connections and orientation columns suggests that specificity of connections for orientation is no sharper than the specificity that we observe for blobs. Malach et al. (1993) combined optical imaging to reveal orientation columns with extracellular biocytin injections to label horizontal connections. They observed that about half of the territory occupied by distal biocytin patches contained neurons tuned to orientations that were within 45 deg (or 25% of a full 180-deg cycle) of the injection sites. Since the average distance we observe between blobs is about 400 μm, 25% of a full cycle would be about 100 μm. Inspection of the distributions of distant synaptic boutons for individual neurons shown in Fig. 12 (right-hand scales) reveals that, typically, about half of the distant synaptic boutons are located within a 100-μm range of distances from blob centers. This argues that specificity for functional parameters related to blobs is likely to be roughly as sharp as specificity for orientation domains. In view of the likelihood that blob location is not as good a predictor of functional attributes as optical imaging is for orientation selectivity, the specificity for parameters related to blobs might even be sharper.

Regardless of the relative specificity of horizontal connections for orientation domains versus blobs, it is striking that many of the connections are not highly specific. How can these connections develop and persist in the face of developmental mechanisms that might be expected to lead to their elimination? One possibility is that they are specific for some other stimulus attribute.

Studies of the development of horizontal connections in the cat visual cortex have shown that their specificity is dependent on visual experience and that neurons with correlated activity tend to become selectively interconnected (Callaway & Katz, 1991; Lowel & Singer, 1992; Schmidt et al., 1997). If these studies are interpreted to imply that connections can be formed and persist only if presynaptic and postsynaptic activity is correlated and that any noncorrelated activity results in elimination of connections (cf. Reiter & Stryker, 1988), nonspecific connections should not be present. The fact that nonspecific connections do exist implies that perhaps the developmental studies should be more loosely interpreted.

One possibility is that some activity must be correlated in order for horizontal connections to be formed and maintained, but that unlike geniculocortical connections (Reiter & Stryker, 1988), horizontal connections are not necessarily eliminated if there is postsynaptic activity in the absence of presynaptic activity. This interpretation is consistent with results from developmental studies. Horizontal connections fail to form/persist between neurons with opposite ocular dominance in strabismic cats (Lowel & Singer, 1992; Schmidt et al., 1997), presumably because such neurons lack correlated activity. But under normal conditions, each neuron has selectivities for multiple functional attributes. Thus, different sets of neurons will have correlated activity under different viewing conditions. Neurons can be interconnected if they are concurrently active some of the time but need not have exclusively correlated activity. This scenario would allow horizontal connections to be selective for each of the multiple functional attributes that are mapped onto the cortex. Furthermore, it suggests that connections that are not highly specific with regard to one stimulus attribute (e.g. orientation selectivity) are likely to be specific instead for some other stimulus attribute (e.g. spatial-frequency tuning).

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References


