Local Circuit Neurons of Macaque Monkey Striate Cortex: II. Neurons of Laminae 5B and 6

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This study investigates the intrinsic organization of axons and dendrites of aspinous, local circuit neurons of the macaque monkey visual striate cortex. These investigations use Golgi Rapid preparations of cortical tissue from monkeys aged 3 weeks postnatal to adult. We have earlier (Lund, ’87) described local circuit neurons found within laminae 5A and 4C; this present account is of neurons found in the infragranular laminae 5B and 6. Since the majority of such neurons are GABAergic and therefore believed to be inhibitory, their role in laminae 5B and 6, the principal sources of efferent projections to subcortical regions, is of considerable importance. We find laminae 5B and 6 to have in common at least one general class of local circuit neuron—the “basket” neuron. However, a major difference is seen in the axonal projections to the superficial layers made by these and other local circuit neurons in the two laminae; lamina 5B has local circuit neurons with principal rising axon projections to lamina 2/3A, areas whereas lamina 6 has local circuit neurons with principal rising axon projections to divisions of 4C, 4A, and 3B. These local circuit neuron axon projections mimic the different patterns of apical dendritic and recurrent axon projections of pyramidal neurons lying within laminae 5B and 6, which are linked together by both dendritic and axonal arbors of local circuit neurons in their neuropils extending between the two laminae. The border zone between 5B and 6 is a specialized region with its own variety of horizontally oriented local circuit neurons, and it also serves as a special focus for pericellular axon arrays from a particular variety of local circuit neuron lying within lamina 6. These pericellular axon “baskets” surround the somata and initial dendritic segments of the largest pyramidal neurons of layer 6, which are known to project both to cortical area MT (V5) and to the superior colliculus (Fries et al., ’85). Many of the local circuit neurons of layer 5B send axon trunks into the white matter, and we therefore, suspect them of providing efferent projections. The axons of lamina 6 local circuit neurons have not been found to make such clear-cut contributions to the white matter.

Key words: vision, inhibition, interneuron, visual cortex, primate

This study continues a morphological description of the organization of local circuit neurons with smooth or sparsely spined dendrites in the monkey primary visual cortex (Lund, ’87). The local circuit neurons are believed to be responsible for inhibitory or modulatory control of the excitatory neurons in the cerebral cortex—the pyramidal neurons and stellate neurons—which retain marked dendritic spine populations into maturity (Lund, ’73). A recent study on the population of neurons within striate cortex that contain the inhibitory transmitter substance γ-aminobutyric acid (GABA) or its synthetic enzyme glutamic acid.
decarboxylase (GAD) shows layers 5 and 6 neuron populations each to contain about 12% of GABA or GAD positive neurons (Fitzpatrick et al., '87). This is quite comparable to their density in laminae covered in our earlier study of interneuron organization (laminae 4Cg about 14%, and 4Cc about 15%). Since lamina 4Cg has almost twice the cell density of 5B, the density of GABAergic neurons may be more a reflection of the total number of neurons in a particular lamina than being determined on some other basis. Crucial data on the precise number of particular kinds of local circuit neuron are, however, largely lacking, except for the relatively sparse number (less than 10%) that can be identified by the colocalization of various peptides (Hendry et al., '83, '84; Jones and Hendry, '85, '86). There is some concern even here, however, that numbers of neurons obtained by using antibody labels may be minimum figures since activity level determines whether or not the label appears significant to the human eye—and activity level may vary from cell to cell (see, for instance, Hendry and Jones, '86).

It is clear that single pyramidal neurons may receive input from the axons of several different varieties of interneuron. For instance, there are chandelier neurons (Somogyi, '77; Fairén and Valverde, '80) that target pyramidal neurons in the infratemporal segments; "basket" cells that target the somata and initial dendritic segments (Freund et al., '83), and other local circuit neurons seem to show a preference for the more distal dendritic segments (Somogyi and Cowey, '81, '84); in addition, the apical dendrite can pass to other laminae where different local circuit neurons may contact its surface compared to the basal dendritic field. We have, therefore, examined laminae 5B and 6 for interneuron classes that might target different regions of the pyramidal neurons within these laminae.

The comparison of local circuit neuron populations in laminae 5B and 6 also involved us in the question of to what degree layers 5B and 6 interact with each other and how the local circuit neurons might participate in that interaction. Another question also arose: whether particular functional sublaminae exist in layers 5B and 6, which might be the targets of specific populations of interneurons. We also looked for interlaminar projections by the local circuit neurons in laminae 5B and 6 to more superficial layers. We were interested to see whether the neurons of 5B and 6 participate differently in these projections (since interlaminar axon projections by local circuit neurons had rarely, if ever, be used as a defining characteristic of neuron variety or class. It is the quality and pattern of distribution of the axon that most clearly provides an identity to the neurons we describe below.

**Variety 5B-1**

The neurons of variety 5B-1 (Figs. 1b, 2b) have cell body and dendrites confined to lamina 5B with a roughly radial dendritic spread of 250-300 μm lateral extent. The axon arises on the pial side of the soma and stout horizontal trunks emerge from this rising axon within lamina 5B and spread laterally about 450 μm from the soma within 5B, giving a lateral spread of at least 800-900 μm to the local axon arbor in 5B. The terminal beaded collaterals of the axon arbor are somewhat more dense in the vicinity of the dendritic field and largely omit lamina 5A in their distribution. The stout rising axon trunk reaches lamina 3A without forming collaterals, then rapidly divides into a number of branches, forming a dense, laterally confined (200-300 μm) terminal arbor within 3A and 2. A fine descending axon process enters lamina 6 and may enter the white matter without contributing significantly to lamina 6. The general form of the axon arbor within 5B suggests this neurons from obstacles to such studies. For these reasons the Golgi impregnations of infant monkey striate cortex (where myelination has not yet blocked axonal impregnation) here provides material for at least the beginnings of a detailed study of the organization of these important neurons.

Short reports of some aspects of this work have appeared earlier (Lund et al., '87a,b).

**MATERIALS AND METHODS**

These are reported in full in Lund ('87) and are not repeated at length here. Golgi Rapid impregnations, cut at 90 μm from blocks of infant *Macaca nemestrina* and *M. fascicularis* monkeys, were used. The processes of individual neurons were often traced through a number of consecutive sections and Figure 1A-D in Lund, '87 illustrate the laminar numbering system for monkey cortex used in the following description. The neurons are grouped in terms of varieties which are classified first in terms of the lamina in which the soma customarily lies, and then a number that implies nothing more than the investigators’ order of description of neurons in that lamina. The term class is used to identify neurons that are suggested to have a common physiological action by virtue of their anatomical form and other information gathered about such cells. For instance, the neurons of "chandelier" morphology (Somogyi, '77) would form a class (see Lund, '87 for a more detailed discussion of these terms).

**RESULTS**

**Local circuit neurons of lamina 5B**

It is clear from our studies of local circuit neuron morphology in striate cortex that dendritic morphology can rarely, if ever, be used as a defining characteristic of neuron variety or class. It is the quality and pattern of distribution of the axon that most clearly provides an identity to the neurons we describe below.

**Variety 5B-2**

The variety 5B-2 neurons (Figs. 1a, 2a) have a similar dendritic arbor to variety 5B-1. Their dense local axon arbor
Fig. 1. (a) Local circuit neuron of the 5B-2 variety. The axon characteristics are a dense local arbor, not much wider in lateral extent than the dendritic field, within lamina 5B and a stout axon trunk that rises to the superficial laminae giving a sparse arbor in 4A/3B and a profuse arbor in the 2/3A region. The 5B-2 variety usually has descending axon trunks that enter the white matter with only a few weak collaterals in lamina 6. (Another example of this variety is seen in Fig. 2a.) (b) Local circuit neuron of the variety 5B-1. The axon characteristics are a wide-spreading axon, perhaps of the "basket" axon type, within lamina 5B and a rising axon trunk ascending unbranched until a rather laterally restricted terminal arbor in lamina 2/3A. The dendrites of these neurons appear very similar in form and extent to the 5B-2 variety (see Figs. 1a, 2a). (Another example of the axon arbor of the 5B-1 variety is shown in Fig. 2b.) Both (a) and (b) are from Golgi Rapid impregnations of 3-week-old Macaca nemestrina. Scale bar = 100 μm.
Figure 2
is formed from branches that emerge from the stout axon trunk shortly after it leaves from the pial side of the soma. The local axon within 5B differs from that of the 5B-1 variety in that it barely exceeds the width of the dendritic arbor (about 300 μm in lateral spread) and the beaded collaterals are somewhat finer and denser in their arbor. The rising axon trunk reaches lamina 4A before sparse to moderate numbers of collaterals emerge, but the main superficial arbor occurs in laminae 3A and 2. The width of this arbor may match or exceed the axon in 5B. Occasional cells occur where the soma lies high in lamina 5, but the dendrites still bridge the width of the lamina 5 division; the local axon arbor favors the upper half of 5 and the rising axon trunk begins its terminal arbor somewhat lower, in upper lamina 3B. The 5B-2 neurons have one or two descending axon trunks that pass down through lamina 6, where they give off a few fine collaterals, then pass into the white matter.

**Variety 5B-3**

Variety 5B-3 (Fig. 3) is of the chandelier class and typically the soma lies near the base of 5B on the border with lamina 6. The dendrites characteristically bear forked distal tips and may either reach up through the whole depth of lamina 5B or may not reach the upper border; the dendrites do, however, generally bridge the 5-6 border. The axon emerges from the white matter aspect of the soma and spreads locally at the 5-6 border and upward to the base of lamina 5A, having a lateral spread of 200–250 μm. Whereas the processes of this neuron variety intrude into upper lamina 6, both axon and dendrites are biased in their distribution to lamina 5B and this class of cells has not yet been found with soma well into lamina 6, so these cells are here designated a variety of lamina 5B.

**Variety 5B-4**

Variety 5B-4 are local circuit neurons (Figs. 4b, 5) of lamina 5B that lack a rising axon trunk with arbor in the superficial layers. Their dendrites bridge the depth of 5B spreading laterally about 250 μm. Their axon can form a local arbor, within and a little wider than the dendritic arbor (approximately 300 μm; Fig. 4b) or, if the soma lies near the base of 5B, the axon may favor the 5-6 border region with somewhat wider lateral extent (600–600 μm) and show not much overlap with the dendritic field (Fig. 5). These neurons have descending axon trunks that enter the white matter without substantial contribution to layer 6.

**Variety 5B-5**

The 5B-5 variety of neuron (Fig. 6) is large compared to the other local circuit neurons of 5B, the soma measuring close to 20 μm in diameter and the dendrites spreading 350–400 μm in total lateral extent. The dendrites reach the upper margin of lamina 5 and may either extend down to the white matter or intrude only into the upper portion of layer 6. The axon that arises on the pial surface of the soma forms a cone-shaped descending arbor that includes both layers 5 and 6, widening its lateral extent (500–600 μm) as it spreads downward. The main axon trunks are stout and their beaded terminal collaterals have characteristics vertical and obliquely angled trajectories within laminae 5B and 6. A rising axon trunk reaches at least to upper 4C without branching, but so far its final target has not been identified—the impregnation ceasing within the section, probably as a result of early myelination. This variety of neuron does not clearly contribute axon trunks to the white matter.

**Comments on local circuit neurons of 5B**

As we endeavor to provide a description of neurons in the 5B region, it can sometimes be questioned whether neurons belong to the 5A region or are properly considered part of the 5B population (see Lund, ‘87 for a description of the 5A local circuit neurons). Figure 4a shows a neuron that probably belongs to the 5A region, although we did not describe a cell of quite this kind in our earlier study. Although the soma lies a little lower than the base of 5A, its dendritic field and axon trunks (predominantly in 5A and lamina 6) resemble those of variety 5A-2. The rising axon arbor resembles those of the 6-6, type i variety to be described that have fine diameter rising axons with collaterals in 5A, mid to upper 4C, and in 4A. We suggest that this neuron be classed as a variety 5A-2 neuron, and thus we broaden the 5-2 variety to include neurons that have rising axon projections to upper 4Cs, 4C, and 4A (5A-2i) as well as those with axon trunks targeting only 4C (5A-2ii) as described in Lund (‘87).

**Cells of the 5-6 border region: Variety 5B-6-1**

There is indication from the distribution of axons and dendrites of several varieties of local circuit neurons of laminae 5B and 6 that the zone where laminae 5 and 6 meet is a specialized region. There is, moreover, one group of local circuit neurons that largely restricts its dendritic and axonal process to this region. Variety 5B-6-1 (Fig. 7) has long, horizontally oriented dendrites that reach up to 250 μm from the soma—giving a lateral spread of 400–500 μm to the dendritic field. The axon, which generally arises from the white matter side or at a lateral pole of the soma, forms stout laterally spreading trunks with short, rather sparse beaded terminal collaterals that tend to have vertical trajectories. The axon trunks can reach at least 400 μm from the soma, giving a potential total spread of 800–900 μm, with a narrow depth to the axon field of approximately 150 μm. Whereas a three-dimensional analysis has not been carried out on these neurons, their processes do not seem to evenly cover a circular territory around the soma, but may have a particular trajectory relative to the overall pattern of the neuropil. The general form of the axons of these neurons suggests they belong to the "basket" neuron class.
Fig. 3. Two examples of neuron variety 5B-3. The axons for each cell have been drawn separately and displaced laterally for clarity. This variety is of the chandelier class and characteristically the soma lies on the 5-6 border with dendrites largely ascending into lamina 5B. Note the forked tips of the dendrites and compare with the similar dendritic form of neurons of variety 6-2 seen in Figures 10a and 11b. The axon arises from the side or white matter pole of the soma and has its principal arbor within 5B and along the 6-5B border. Both cells are from Golgi Rapid impregnations of tissue from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Fig. 4. (a) This neuron is believed to belong to the group called variety 5A-2 (see Fig. 10 of Lund, ’87, and text of that account). These neurons emphasize a dendritic arbor in 5A and descending dendritic branches that reach to the base of lamina 6. The axon distributes in 5A and in a descending cascade that has most collaterals within lamina 6. The axons of the cell illustrated here resembles those of our earlier described 5A-2 neurons in arborizing in divisions of lamina 4; but here the axon seems to contribute to upper 4Cα, 4Co, 4A, and lowermost 3, rather than just in 4Co, as our previous examples had shown. In its rather wide axon distribution, the 5A-2 example shown here resembles some of the 6-6, type (i), neurons (see Figs. 17, 19a), whereas the earlier 5A-2 examples had rising axons arborizing in lamina 4Co resembling other 6-6 neurons—here called type (ii)—(see Figs. 18, 19a, 20). These similarities in axon distribution between the 5A-2 and 6-6 varieties are believed to illustrate parts of circuits that closely link laminae 6, 5A, divisions of 4C, and 4A/lower 3B. (b) This neuron is of the 5B-4 variety, which lacks projections to the superficial layers. The axon is profuse and localized close to the dendritic field in 5B. Descending axon trunks pass to the white matter without contributing to lamina 6. (Another example of a 5B-4 neuron is shown in Fig. 5.) Both cells are from Golgi Rapid impregnations of 3-week-old Macaca nemestrina. Scale bar = 100 μm.
Fig. 5. An example of a 5B-4 neuron whose axon shows less overlap with its dendritic field than in the example shown in Fig. 4b. Here the dendrites bridge the depth of lamina 5 and the soma lies on the 5B/6 boundary. The axon spreads broadly in lower 5B largely beyond the range of the dendrites.

Fig. 6. Two examples of the variety 5B-5 local circuit neurons. The dendrites may intrude only slightly into lamina 6 (e.g., example (a) from the perimacular representation) or they may reach from the upper boundary of 5B down to the white matter (e.g., example (b) from the peripheral field representation in the roof of the calcarine fissure). The axon forms a characteristic cascade of descending branches occupying both lamina 5 and 6 with collaterals that emphasize oblique and vertical orientations. A vertically rising axon trunk is present (see segments marked with asterisks in (a) and (b), but so far this rising, unbranched axon process has not been traced beyond upper 4Co, ceasing to impregnate within the section, probably due to myelination. Golgi Rapid impregnations from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Figure 6
Fig. 7. Two examples of local circuit neuron variety 5B-6-1. These neurons are found on the border where laminae 5B and 6 meet. They appear to be “basket” neurons and are suspected of having an unevenly distributed field of axon and dendritic processes, which are perhaps oriented along particular axes in the neuropil. Both are from Golgi Rapid impregnations of 3-week-old Macaca nemestrina. Scale bar = 100 μm.
Fig. 8. This figure and figure 9 are two examples of local circuit neuron variety 6-1. The cell bodies of these cells lie in mid to lower 6 with a dendritic field that spreads widely down through lower lamina 6 and into the white matter; an occasional dendritic process may reach into lamina 5. The axon is robust, like the spreading dendritic field, and stout trunks travel laterally away from the cell with prominently beaded collaterals. Their form suggests they belong to the "basket" neuron class. Golgi Rapid impregnations from 3-week-old Macaca nemestrina. Scale bar = 100 μm.
Fig. 9. A further example of a neuron of the 6-1 class; see Fig. 8 and legend for description. Golgi Rapid impregnation from 3-week-old Macaca nemestrina. Scale bar = 50 μm.

than background stain in the cytoplasm), lying usually but not always high in the layer near the 5-6 border. These pericellular arrays, truly "baskets" of terminals, are often also found along this border without a traceable connection to a cell of origin and it appears that the large diameter collateral trunks are already myelinating by 2–3 weeks postnatal. A stout rising axon trunk can usually be found emerging from the initial axon segment, but so far the destination of this rising axon has not been traceable, the trunks ceasing to impregnate within the section in lamina 5—again probably due to early myelination.

Variety 6-3

The 6-3 variety (Figs. 11a, 12, 13) of local circuit neuron has a robust dendritic field in lamina 6 with a 350–400 μm width at the upper margin of the layer and a narrower field of descending dendrites that reach and enter the white matter. An occasional single dendrite may extend vertically into lamina 5. The axon that arises on the pial or lateral aspect of the soma is of large diameter with prominent terminal beaded collaterals; stout trunks spread laterally, especially near the 5-6 border, which they may cross to enter lamina 5B to a limited extent. The total width of the axon arbor is 450–600 μm (which may reach its greatest width for neurons of the calcarine roof, i.e., peripheral field representation; Fig. 12). If the cell has any dendritic processes extending vertically into lamina 5, a small number of axon collaterals may travel with them to reach the upper margin of lamina 5. This neuron variety may be of the "basket" class.

Variety 6-4

The 6-4 variety (Figs. 14, 15) may comprise several different cell types, but we cannot yet be confident of an accurate description of each, so we grouped them as a single 6-4 variety. In general these cells have a narrow (150 μm) vertically extended bifurcated (double bouquet) form of dendritic field with a prominent bunch of dendrites reaching...
Fig. 10. (a) The 6-2 local circuit neuron variety has its soma in upper lamina 6 and a descending array of rather tortuous dendrites, many of which end with forked tips, like the earlier illustrated chandelier neurons (see Fig. 3). These neurons have very prominently beaded axons, which form an arbor local to the dendrites as well as stout trunks that travel for long distances laterally, particularly in upper lamina 6. Pericellular clusters of terminals are given off from these trunks and often the cell bodies and initial dendritic segments of large pyramidal neurons can be seen as dark shadows within these terminal clusters. Often the pericellular clusters occur in isolation, emerging from portions of the stout trunks, and it is probable that the axon trunks are already myelinated in the early postnatal period, making it difficult to trace the connections between cell and terminal axon arrays. In (b) several isolated pericellular clusters are drawn for comparison. These 6-2 neurons also appear to have rising axon trunks (see asterisk in (a)), but these have ceased to impregnate within lamina 5 in material examined so far. (Another example is seen in Fig. 11b). Golgi Rapid impregnations from 3-week-old Macaca nemestrina. Scale bar = 50 \( \mu \)m.

[Image of neuron diagram]
Fig. 11. (a) This neuron and those of Figs. 12 and 13 belong to the variety 6-3. These neurons have well-developed dendritic fields in lamina 6, which widen in lateral extent near the top of lamina 6. An occasional dendrite may extend into lamina 5. The axon is of large diameter with prominent beaded terminal collaterals that again spread most widely in upper lamina 6. (b) This neuron is of the 6-2 variety (see also Fig. 10) and shows the characteristic forked tips at the ends of its dendrites. Whereas this cell did not have laterally spreading trunks that could be followed to their terminal collaterals, the local axon enclosed a large cell soma lying lower in layer 6 amongst a tangle of the cell's dendrites. The rising axon trunk ceased to impregnate within the section thickness (see asterisk) as has been found typical of this cell type in our material. Both cells are from Golgi Rapid impregnations in 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Fig. 12. An example of the 6-3 variety of neuron from the roof of the calcarine fissure (i.e., peripheral field representation). The dendrites and axons of these neurons may reach their maximum lateral spread, particularly at the 5/6 border when lying in the calcarine fissure. (See Figs. 11a and 13 for other examples.) Golgi Rapid impregnation from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
the upper border of lamina 5 as well as descending dendrites reaching the white matter (Figs. 14a–c, 15). The axon differs in character from that of the 6-3 variety, being of finer diameter and having smaller terminal boutons; it forms a dense local field in layer 6, a little wider than the dendritic field (250–300 μm); a slender rising axon process provides minimal input to layer 5. Figures 14c and 15 show two deviations from this typical form: Figure 15 shows a neuron with a somewhat wider spread of axon and dendrites within layer 6, and Figure 14c illustrates a neuron with an axon that favors the 5-6 border zone rather than being largely confined to layer 6. For the moment we place these neurons in the 6-4 group.

Variety 6-5

These 6-5 cells (Fig. 16) strongly resemble the 5-5 variety in axon form, although their dendritic arbor is narrower (250–300 μm) and emphasizes a distribution in layer 6. The axon forms a cascade of vertical and oblique collaterals in both laminae 5B and layer 6 and has a lateral spread of at least 500 μm. It is uncertain if this 6-5 variety ever contributes any axon processes to layers above lamina 5.

Variety 6-6

The 6-6 variety (Figs. 17–20) certainly contains different cell types, but for the present they are grouped together in terms of their common feature of providing rising axon projections to one or more of the laminae closely associated with thalamic inputs—4C, 4A, 5A—as well as having a local axon arbor within lamina 6. Most but not all have two or more long vertically oriented dendrites that regularly reach the top of 4Cα and even extend as far as 4A; they also have a more numerous set of shorter basal dendrites that reach down to the white matter. The axons of the cells of this group can differ significantly from one another in their diameter; some (see, for instance, Figs. 18, 19a) are robust, with large diameter, beaded terminal collaterals, whereas others are exceptionally fine and difficult to follow (Figs. 17, 20a. In terms of distribution, whereas their axon arbors can include upper 4Cβ and 5A, the lower 4Cβ region between these two divisions seems to be omitted, as is layer 4B, and no arbors have been traced above lowermost lamina 3B. In general, neurons whose axons that provide collaterals to mid 4C (upper 4Cβ and lower 4Cα; Figs. 17, 19b) also have collaterals in 5A and may add a terminal arbor in the 4A region; their diameter is generally fine; we have called this type (i). The axons that favor 4Cα, in particular upper 4Cα, seem of larger diameter and although occasional collaterals are seen in 5A, collaterals in this region are not prominent (Figs. 18, 19a, 20); we have called these neurons type (ii). The axon arbors in lamina 6 of the neurons with rising projections to 4Cα often (but not always) resemble those of the “basket” axon class.

Comments on local circuit neurons of 6

Attention was paid to neurons lying below layer 6 in the white matter. In general their axons were hard to follow and incomplete. However, we feel that those neurons we have so far found in our tissue, whose somata and dendrites lay mainly within the white matter, are not of a special class or single morphology. Rather, they appear to resemble the varieties we have already illustrated and seem to have been somewhat distorted by their position with the predominantly horizontally oriented fiber layer.

DISCUSSION

It is clear from this examination of the local circuit neurons of laminae 5B and 6 that classes that have been described previously in cerebral cortex, e.g., “basket” neurons
Fig. 14. Parts (a) and (b) show typical examples of local circuit neuron variety 6-4. The bitufted dendritic field spreads equally into laminae 5 and 6, whereas the axon forms a prolific local field within layer 6 with only minimal input to layer 5. Part (c) shows one variation in the 6-4 form where the axon arbor favors the 5-6 border region in its terminal field rather than being confined largely to layer 6 (See another example in Fig. 15). All are Golgi Rapid impregnations from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Fig. 15. A further example of a neuron of variety 6-4. This neuron is somewhat atypical in the wider lateral spread of axon and dendrites in mid to upper lamina 6. The axon has been drawn separately for clarity. (Other examples are seen in Fig. 14a–c.) Golgi Rapid impregnation from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Fig. 16. This neuron is of the 6-5 variety. The axon and dendrites have been drawn separately for clarity. Note the strong resemblance of the axon to that of the 5-5 variety (see Fig. 6). Whereas the dendrites are largely confined to lamina 6, the axon forms a cascade of vertical and oblique branches in both 5B and 6. We have not yet found a clear axon contribution to more superficial layers from this cell type. Golgi Rapid impregnation from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Both appear to belong-has an axon projection from single sive lateral spread of their axons. Another example is the outreaches in lateral extent that of the latter. It is possible the 5B-2 variety, but the axon spread of the former far bor both laminae 5B and 6; but even for these neurons it is clear that their dendrites do not always sample the 5B and 6 laminae equally. From the preceding description of local circuit neurons, we believe it to be clearly illustrated that laminae 5B and 6 have their own populations of local circuit neurons. Whereas neurons of the same class can occur in both laminae, comparing the varieties of the same class present in laminae 5B and 6, they are found to have distinct differences in their interlaminar axonal projections and should therefore differ in the functional outcome of their activity in terms of visual processing within area VI. Only one cell class—to which the varieties 5B-5 and 6-5 both appear to belong—has an axon projection from single neurons that includes within significant portions of its arbor both laminae 5B and 6; but even for these neurons it is clear that their dendrites do not always sample the 5B and 6 laminae equally.

Whereas the dendritic and axonal fields of all but one variety (5B-6-1) of the 5B and 6 local circuit neurons bridge the depth of at least their primary lamina, the lateral extent of their axon and dendrites within the lamina varies considerably between different varieties. In this regard, it is found that lateral dendritic spread of individual neurons is not a reliable indicator of the extent of their axonal spread; for instance, the pericellular "basket" neurons (variety 6-2) have quite narrow dendritic fields but very extensive lateral spread of their axons. Another example is the 5B-1 variety, which has much the same dendritic spread as the 5B-2 variety, but the axon spread of the former far outreaches in lateral extent that of the latter. It is possible that the dimensions of axonal spreads we see in the Golgi material are relatively accurate and relate to other aspects of the scaling of events in the striate cortex. For instance, the largest "basket" cells have axonal spreads in the order of 800–1000 μm; this distance is close to the sum of 2 ocular dominance column widths (i.e., a right eye-left eye doublet or a distance that enables columns of the same ocular dominance to interact). A second order of magnitude seen commonly for axon spread is around 450–500 μm, which would be closer to a single ocular dominance column width or perhaps linking a right eye-left eye pair. The narrowest axonal spread (200–250 μm) seems to be that of the chandelier neurons of lamina 5B (variety 5-3). The different scale of axon spread between the basket neurons and chandelier neurons, both of which are supposed to exert inhibitory control on pyramidal neurons (the chandelier axons contacting cell body and dendritic initial segments), suggests a quite different lateral scale of relationship between each class and the postsynaptic pyramidal neuron populations. Each chandelier neuron would presumably control a small closely adjacent group of neurons, whereas the basket neurons would each contribute to the control of a rather widely scattered group of postsynaptic neurons.

The extent of the local circuit neuron axon arbors should be seen much better in neurons that have been well filled intracellularly in vivo with HRP. It will be interesting to see if a reasonable sample of such data for these local circuit neuron populations can be generated, but for the moment we can accept the Golgi data only as providing minimal estimates of axon spread. Another question, also probably best answered in HRP filling studies, is whether over longer distances specifically orientated trajectories or clustering of terminals occurs in the local circuit neuron axon fields. Of course, we have no evidence here of such a contact preference or that cells we have said resemble basket neurons are indeed of a common class.

An important issue is whether the axonal and dendritic processes of these various local circuit neurons do indeed recognize laminar or sublaminar boundaries in cortical depth, i.e., can be broken down as we have suggested into varietal laminar subgroups. A parcelization of territory would suggest that the neurons are particularly concerned with functional events occurring within particular spatial limits in the cortical neuropil. Since we know that the physiological response properties of neurons in laminae 5B and 6 differ (Hubel and Wiesel, '68; Livingstone and Hubel, '84; Orban et al., '86; Hawken et al., '87), restriction of the processes of individual neurons to one or other of these territories suggests that they might be part of the substrate for generating the particular response properties seen in each laminar division. From the preceding description of local circuit neurons, we believe it to be clearly illustrated that laminae 5B and 6 have their own populations of local circuit neurons. Whereas neurons of the same class can occur in both laminae, comparing the varieties of the same class present in laminae 5B and 6, they are found to have distinct differences in their interlaminar axonal projections and should therefore differ in the functional outcome of their activity in terms of visual processing within area VI. Only one cell class—to which the varieties 5B-5 and 6-5 both appear to belong—has an axon projection from single neurons that includes within significant portions of its arbor both laminae 5B and 6; but even for these neurons it is clear that their dendrites do not always sample the 5B and 6 laminae equally.

Fig. 17. This neuron and that shown in Fig. 19b are of the variety 6-4, type (i). The 6-6 variety is distinguished by having axon projections to one or more divisions of 4C, and sometimes the arbor includes 4A, lowermost 3B, and 5A. The type (i) illustrated here has a fine caliber axon that includes in its terminal distribution laminae 5A, upper 4C, and lower 4C. The type (i) 6-6 neuron shown in Figure 19b illustrates the finding that the axon of this variety also may include the 4A/3B region in its arbor. The dendrites of the 6-6 neurons form a bifurcated array with the upper tuft reaching as far as the terminal axon array (i.e., well into 4C or even to 4A). Golgi impregnation from 5-week-old Macacus nemestrinus. Scale bar = 100 μm.

Local circuit neurons of lamina 5B

The local circuit neurons of 5B (see Fig. 21) include in their axon targets projections to the superficial layers, with an emphasis on terminals in the 3A-2 laminar division. It is already known that pyramidal neurons of layer 5B have recurrent axon projections to the 3A-2 region (Blasdel et al., '75) and small injections of HRP into lamina 5B show the strongest axonal projection from the layer to be the same 3A-2 division of the superficial layers (Blasdel et al., '85). In regard to retrograde labeling, injections of HRP made in the 3A-2 laminar division result in heavy cell body labeling in lamina 5B, whereas injections into the 3B-4A division give most retrogradely labeled neurons in lamina 5A. A similar pattern is seen with injections of [3H] GABA into the upper layers (Somogyi et al., '83). We have seen in our first study of local circuit neurons (Lund, '87) that the 5A local circuit neurons do not emphasize a projection to the 3A-2 division but have either an even distribution through the superficial layers including layer 1, or more prominently emphasize projections to 3B and 1. The coincidences in projection patterns of pyramidal and local circuit neuron projections for neurons in 5B and 5A, and the difference in relationship between the 5A and 5B laminae and the upper and lower regions of the superficial zone of the cortex, indicate a clear lockstep relationship of inhibitory and excitatory circuits. Physiological differences between...
Fig. 18. The neuron illustrated belongs to the 6-6 variety and is of type (ii). The axon projection to upper 4Ca is robust as are the local collaterals in lamina 6. This neuron lies in the roof of the calcarine fissure. The axon projection to 4Ca is the particular feature of the 6-6, type (ii) neurons, and a robust spreading arbor in lamina 6 is seen in many type (ii) cells. (See also Fig. 19a, and two neurons with axons of finer caliber projecting to 4Ca—also placed in the 6-6 type (ii) group—are shown in Fig. 20). Golgi rapid impregnation from 3-week-old Macaca nemestrina. Scale bar = 50 μm.
Fig. 19. (a) An example of a variety 6-6 neuron of type (ii) where the axon projects to 4Co and has a robust spreading arbor in lamina 6. The asterisk marks an axon trunk that failed to impregnate, whereas the other branch of the same rising axon was traceable to an incomplete arbor in 4Co. (Another example is shown in Fig. 18.) (b) A 6-6 neuron of type (ii). This neuron has fine caliber axon projections to 5A, upper 4Cs, 4Co, and the 4A/3B region, as well as an arbor within lamina 6. (Another example is shown in Fig. 17.) Golgi Rapid impregnations from 3-week-old Macaca nemestrina. Scale bar = 100 μm.
Fig. 20. Two examples of variety 6-6, type (ii) neurons with axon projections to lamina 4Co. Part (a) is from the outer operculum of area 17, in the perimacular representation, whereas (b) is from the calcarine roof peripheral field representation. (See Fig. 18 and 19a for other examples.) Golgi Rapid impregnations from 3-week-old Macaca nemestrina. Scale bar = 100 μm.
the 5A-5B region or between the 2-3A and 3B-4A regions have not yet been clearly defined, but one would certainly expect differences in function, given the very different circuitry that each region engages in.

For those 5B local circuit neurons whose bilaminar axon distribution to 5B and 2-3A matches that of the 5B pyramidal neuron basal and apical dendritic fields, it might be suggested that the correlation indicates a relationship; for instance, the local circuit neurons may contribute input to the two parts of the dendrite field of the 5B pyramidal neurons. However, the relative lateral spreads of the local axon arbors within 5B and in 2-3A can be quite different for single axons; sometimes the 5B arbor is narrowly confined and the superficial arbor wide and sometimes it is the reverse, with laterally wide spreading arbor in 5B and narrow field in 2-3A. The branching pattern of the 5B axon field can also be quite differently structured from the lamina 3A-2 field of the same axon. For instance, variety 5B-1 has an axon field within 5B that resembles that typical of "basket" neurons; such axons have been described as contacting primarily cell bodies and dendritic initial segments; in the case of these 5B-1 neurons the spreading axon would presumably make rather limited contact to each of rather widely separated postsynaptic neurons. The superficial axon arbor of the same axon is, however, confined to a quite narrow but dense, cone-shaped arbor that seems spatially suited to contact processes in a tight cluster, perhaps apical dendritic terminal arbors. Since the descending axon trunks of single layer 2-3A pyramidal neurons can give off collaterals that spread quite widely in 5B, another suggestion might be that the 5B-1 local circuit neurons are reciprocally related to tight clusters of 2-3A pyramidal neurons and to the widely spread axon target sites in 5B of these same 2-3A neurons.

So far we have not traced any clear-cut axon projections from 5B local circuit neurons to divisions of 4C or to 4B. The investigation of interlaminar connections using small HRP injections (Blasdel et al., '85) showed a clear projection from 4B to 5, which did not appear to be strongly reciprocated. Injections into 5B produced a coarse fiber population rising into and spreading laterally within 4Co and a more narrowly focused light terminal labeling in 4B; for the moment we are unable to explain these HRP-labeled projections to divisions of 4 on the basis of our Golgi-impregnated neuron projections. However, if any of the local circuit neurons we have described within 5B were to be suspected of projections to 4Co, particularly its upper region, it might be the 5B-5 variety whose coarse rising trunks have never been traced to their superficial terminal zone (but seem to lie higher than mid 4Co since rising axon trunks have been traced at least this far without branches) and whose local axon arbor, and often the dendritic field, extend down through layer 6, perhaps therefore justifying an arbor in divisions of lamina 4.

Several varieties of the 5B local circuit neurons regularly have axon trunks that descend and enter the white matter, perhaps providing efferent projections from V1. In our earlier study of the local circuit neurons of laminae 5A and 4C, no axon trunks had been found to pass from these neurons into the white matter; this had not surprised us for even the excitatory spine-bearing neurons of these layers do not appear to project out of the region. The relative frequency of apparent efferent projections from the local circuit neurons of lamina 5B does surprise us, although efferent projections from local circuit neurons have been reported previously, even by us (Lund et al., '79). Since lamina 5B contains pyramidal neurons projecting to mainly subcortical destinations (e.g., superior colliculus, pulvinar, and pons), it is possible that these same projections are made by both local circuit (perhaps inhibitory) neurons as well as pyramidal neurons (presumed to be excitatory). Double labeling studies with retrograde markers and GAD or GABA antibodies could be used to investigate this question. We have not detected obvious efferent axon trunks from the local circuit neurons of lamina 6, though perhaps their profuse axon fields with axon collaterals that routinely reach into the adjacent white matter may have disguised such a contribution. Interestingly, we are finding that local circuit neurons of the superficial layers, in at least the 3A-2 region, also contribute axons to the white matter.

**Local circuit neurons of lamina 6**

The local circuit neuron varieties we have seen in lamina 6 (see Fig. 22) have some differences in class compared to those seen in layer 5B. For instance, we have regularly found chandelier neurons in 5B but not in lamina 6, and we have found pericellular "basket" neurons in 6 but not in 5B. We do not know if this finding is real or if it is due to the vagaries of sampling and staining of Golgi material, but it is certainly worth watching out for examples of these neurons in future studies of the deeper layers. Marin-Padilla ('87) also failed to find chandelier cells in layer 6 of human striate cortex, although they were present in layer 5 and in 4Co upward, as we have also found in the monkey. In the cat, however, at least the characteristic axon arbors are seen in lamina 6, although sometimes they arise as interlaminar projections from neurons in the superficial layers (Lund et al., '79; Fairen and Valverde, '80). It is also worth noticing the similarity in dendritic form of the chandelier and pericellular basket neuron, despite the marked difference in axon morphology. The forked distal dendritic tips of both these varieties of neuron may conceivably be a device for increasing dendritic surface area distally, which might in turn allow a greater number of synapses distally, thereby increasing the efficiency of inputs placed farther from the soma and spike generating site.

For the local circuit neurons in lamina 6, there is much more evident intrusion of dendritic and axonal processes into lamina 5B than is seen to occur in the other direction, from neurons of 5B into lamina 6. However, only those neurons whose axons project above lamina 5 (variety 6-6) have dendrites crossing the 5-4 boundary. The axon projections of this variety of neurons distribute in the thalamic recipient regions of 4C and 4A as well as to 5A and lower 3B, and therefore mimic those of the 5A-2 group of local circuit neurons. We have elsewhere (Lund, '88) pointed out that the local circuit neurons of laminae 6, 5A, divisions of 4C and 4A all closely interrelate with each other in their patterns of axon and dendritic distribution. Since the pyramidal neurons of lamina 6 also have apical dendritic processes and recurrent axon relays to these same more superficial laminae (Lund and Boothe, '75; Lund et al., '77) and the spiny stellate neurons of 4C have axon collaterals that innervate 3B/4A, 5A, and 6, it is reasonable to suspect that these layers are in constant and close intercommunication in both excitatory and inhibitory fashion. One possible omission from the local circuit neuron axon projections to 4C is a projection from layer 6 neurons to the lower half of 4C9. We may simply have missed impregnating such
Fig. 21. Summary diagram illustrating the varieties of local circuit neurons described for lamina 5B and the 5B-6 border. Pyramidal neurons of the 5B lamina can either lack a well-developed apical dendrite or may have an apical dendrite with poorly developed (the smaller pyramids) or strongly developed (large pyramids) arbor in laminae 3A/2 and 1. The pyramids have recurrent axon collaterals that also show terminal arbors in the 3A/2 region (Lund and Boothe, '75). The 5B-1 and 6B-2 local circuit neuron axons can be seen to replicate this laminar pattern of dendritic and axonal distribution of the pyramidal neurons of the layer.
Pyramidal neurons of lamina 6 show recurrent axon projections and apical dendritic arborizations in divisions of 4C, in 4A/3B, and in 5A. The 6-6 (i-ii) local circuit neuron axon projections can be seen to replicate at least some of these laminar patterns of pyramidal neuron dendritic and axon distribution. Varieties of local circuit neurons in lamina 5A also mimic these projections (see variety 5-2 in Lund, '87, and Fig. 4a in this study).
cells but so far none have been identified that provide a clear and dense projection to this region. Interrelations between layers 5A and 5B may be present for 5B neurons merely in terms of the small degree of overlap into 5A made by the processes of many 5B local circuit neurons. However, for some varieties of 5A neuron this interrelation may be more carefully structured. For instance, the spread of 5A-3 (Lund, '87) chandelier dendrite through the depth of 5B suggests these neurons are accepting synaptic input within 5B even though their axon distribution favors lamina 5A. In addition, the 5A-2 neurons described here and in our earlier report (Lund, '87) can have a reasonable portion of their descending dendrites passing through layer 5B before entering layer 6 with the possibility that they may accept some input from the 5B region.

The special orientation of one variety of neurons (5B:6-1) along the junctional border of laminae 5B and 6 suggests this region to have a special function. We know that the majority of the neurons projecting to cortical area MT, including the largest pyramidal neurons of the striate cortex (which have been shown by Fries et al. ('85) to send collateral axon projections to both MT and the colliculus) have somata in upper lamina 6 and on the border with lamina 5B. The pericellular basket axons have the majority of their contacts on large pyramidal neurons in this same upper 6-5B border region. It is also known that the property of direction selectivity is especially emphasized in cortical area MT and therefore perhaps a quality shown by at least the largest pyramidal neurons whose somata lie at the border region if not other smaller cells in the same region. Since, in the monkey, neurons of layer 6, rather than those of layer 5, have been found to show direction selective properties (Hubel and Wiesel, '69; Dow, '74; Livingstone and Hubel, '84; Orban et al., '86; Hawken et al., '88), there is some uncertainty in our minds over the relationship of local circuit neuron varieties and the property of direction selectivity. If inhibitory circuits are involved, the border 5B:6-1 basket neurons may be good candidates and the pericellular basket neurons (6-2) are strategically placed for such activity. However, other basket cells of layer 6 also emphasize axon contributions to the upper portion of 6 and it is impossible to say at the moment which of these circuits may contribute to this important property. We earlier (Lund, '87) pointed out that laminae 4C has "basket" neurons (~6 variety) that contribute laterally wide spreading arbors in upper 4C, 4B, and 4A; this region is the only zone, other than lamina 6, of monkey striate cortex showing direction selective properties. These local circuit basket neurons of 4C are the only candidate so far described for long lateral inhibitory connections in this upper zone and they somewhat resemble the 5B:6-1 variety except their dendrites are less laterally extended.

It should be remembered that throughout this study we have been describing immature neurons. Further maturational changes will certainly occur in both axons and dendrites. For the axons, the number and size of terminal boutons and their synaptic contacts are known to change with age (Lund et al., '77; Mates and Lund, '83). The size of the intralaminar terminal arbors may also change. However, we suspect that the pattern of interlaminar projections and the choice of postsynaptic target is established early in development and then remains consistent into adulthood. The dendritic arbors may alter in the richness of their branching or total length and the spicules and spines that are a common feature of these immature interneurons are greatly reduced in number or entirely lost during maturation (Mores, '69; Marin-Padilla, '72; Lund et al., '77). Further study is needed, however, to determine what precise maturational changes may occur for specific neuron types.

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LOCAL CIRCUIT NEURONS OF MONKEY VISUAL CORTEX


