

Contributions of Individual Layer 6 Pyramidal Neurons to Local Circuitry in Macaque Primary Visual Cortex

Anne K. Wiser and Edward M. Callaway

Neuroscience Program and Department of Physiology, University of Colorado School of Medicine, Denver, Colorado 80262

We have studied the contributions of individual layer 6 pyramidal neurons in macaque primary visual cortex to local cortical circuitry by intracellular labeling and analysis of the morphologies of 58 neurons. These neurons are separated based on the laminar specificity of axonal and dendritic arbors into two classes, class I and class II, and into several types within these classes. Class I neurons project axons heavily and predominantly to layer 4C, whereas class II neurons have axonal projections primarily to layers other than 4C. Only 16 of the 58 neurons in our sample (28%) project to the white matter. Class I projection neurons are found at the top and bottom of layer 6, suggesting that they project to the lateral geniculate nucleus, whereas class II projection neurons are located in the middle of

layer 6, suggesting that they project to the claustrum. The different types of class I neurons are distinguished from one another based on the sublaminar specificity of their axonal and dendritic arbors within layer 4, where they are biased toward compartments dominated by input from different functional streams. They are also distinct in their distributions within the depth of layer 6. The distinctive characteristics of the neuronal types we have identified suggest that each receives input from different sources and projects to a set of targets that is functionally appropriate. Thus, each type is likely to contribute uniquely to computations within V1 and extrinsically.

Key words: macaque; visual cortex; V1; area 17; striate cortex; local circuits; biocytin; cytochrome-oxidase

The brain is a highly organized structure the function of which is dependent on the way in which its neurons are interconnected. The visual system is no exception to this rule. Thus, an important step toward understanding how the visual cortex mediates visual perception is to know the precise anatomy of neuronal projections. Although past studies have revealed much of the detailed local circuitry of area V1 in the macaque monkey (Lund, 1973; Lund and Boothe, 1975; Lund et al., 1977; Blasdel et al., 1985; Fitzpatrick et al., 1985; Valverde, 1985; Katz et al., 1989; Lachica et al., 1992; Anderson et al., 1993; Yoshioka et al., 1994), our understanding of the contributions of individual neurons to these circuits is limited.

Here we focus on understanding the contributions of individual layer 6 pyramidal neurons in macaque V1 to visual processing. These neurons are of particular interest because they are in the unique position of being able to sample inputs both from the neurons that provide input to V1 (geniculocortical afferents) (Lund, 1988) and from those that provide output from V1 to extrastriate cortical areas (Blasdel et al., 1985; Yoshioka et al., 1994). They, in turn, project axons back to the lateral geniculate nucleus (LGN) (Fitzpatrick et al., 1994) and also locally within V1 (Blasdel et al., 1985; Yoshioka et al., 1994). Investigations of the function of layer 6 neurons in cat primary visual cortex suggest

that they contribute importantly to the generation of novel neuronal response properties that emerge in this area (Sherk and LeVay, 1983; Bolz and Gilbert, 1986; Sillito et al., 1994). However, in view of the numerous differences in the organization of primate and feline visual systems and our limited understanding of the anatomy of individual layer 6 neurons in the macaque, it is not clear how these studies might relate to layer 6 function in primates.

A number of studies have provided important insight into the contributions of layer 6 neurons to local and extrinsic circuits in the macaque. Studies based on small extracellular injections of anterograde and retrograde tracers reveal that layer 6 neurons project local axon collaterals to all of the cortical layers (Blasdel et al., 1985; Yoshioka et al., 1994). The resolution of whether these projections arise from a uniform population of neurons that each project axons throughout the cortical depth or from distinct types that each project to only a particular subset of the layers requires knowledge about the anatomy of individual neurons. Some of the earliest studies of layer 6 neurons indicated that they are in fact a diverse population. Golgi studies in adult macaque monkeys (Lund, 1973; Lund and Boothe, 1975) revealed at least five types, each with a distinct pattern of dendritic laminar specificity; axonal arbors could be visualized for only two types. Golgi studies of neonatal material resulted in better axonal labeling and the identification of eight neuronal types (Lund et al., 1977). It is not certain, however, whether the axonal projection patterns observed in neonatal animals persist postnatally, nor whether the neuronal types identified by Golgi staining represent all of the types that are present. Intracellular staining of a large population of layer 6 neurons in postnatal animals can help to resolve these issues.

Insight into the possible functional roles of the different types of layer 6 neurons also requires more detailed information about

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Correspondence should be addressed to Ed Callaway, MNL-C, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037.

Dr. Wiser's and Dr. Callaway's present address: Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.

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each type. For example, retrograde labeling studies reveal that corticogeniculate neurons are found only at the top and bottom of layer 6 (not in the middle), with those projecting to magnocellular layers being found exclusively at the bottom (Fitzpatrick et al., 1994). Also, in the cat claustral projecting neurons are found only in the middle of layer 6 (LeVay and Sherk, 1981). These observations suggest that different neuronal types might be found only at particular depths in layer 6. Information about the depth distribution of various neuronal types, therefore, could provide insight into their likely extrinsic targets. The results of Fitzpatrick et al. (1994) also suggested that only a small percentage of layer 6 cells project to the LGN. Knowledge of the percentage of layer 6 neurons that project to the white matter and their likely targets might provide support for this finding. Also, some neuronal types might lack extrinsic projections altogether, and it would be informative to identify any such types.

We therefore intracellularly labeled and reconstructed layer 6 pyramidal neurons in macaque V1 to obtain a more detailed description of visual cortical circuitry, with the eventual goal of understanding how each type contributes to the emergence of the specific visual response properties of the neurons to which it connects. Our primary aims were as follows: (1) to identify the various types of layer 6 pyramidal neuron; (2) to reveal the laminar specificity and lateral extent of the axonal and dendritic arbors of each type; (3) to determine whether particular cell types are found at particular depths in layer 6; and (4) to determine which cell types contribute input to an extrinsic target (i.e., the LGN or claustrum).

We have labeled and analyzed the axonal and dendritic arbors of 58 layer 6 pyramidal neurons. The diversity in laminar specificity of dendritic and axonal projections of these layer 6 cells leads to a separation into two classes of neurons, and several types within each class. Each neuronal type is distinctive in terms of the following: (1) its patterns of dendritic and axonal arborization; (2) its distribution within the depth of layer 6; (3) whether a subset of the neurons projects to the white matter; and (4) the depth distribution of the projection neurons (if any). We propose that each cell type serves distinct functions in the shaping of receptive fields within V1 and discuss the possibility that, in the monkey, these neurons play similar roles to those in the cat.

MATERIALS AND METHODS

Individual neurons in the primary visual cortex of macaque monkeys were intracellularly labeled in living brain slices and their axonal and dendritic arbors analyzed as described below. We labeled and analyzed 58 layer 6 pyramidal neurons from eight macaque monkeys (*Macaca radiata*) of both sexes: B8, B11, B12, B13, B14, B16, B17, and B19. The ages of the animals ranged from 2 to 9 months postnatal. We used young animals because it is much more difficult to obtain whole-cell recordings in slices prepared from older animals. The age of each animal and the number of cells it contributed to our sample are indicated in Table 1. Neurons of each type were similar in both axonal and dendritic morphology, regardless of the ages of the animals from which they were sampled. Additional neurons labeled in other layers are not described here. Examples of biocytin labeled cells are shown in the photographs in Figure 1 and after camera lucida reconstruction in Figures 2–9.

Preparation of cortical slices. We prepared 400- μ m-thick coronal slices from striate cortex using methods essentially identical to those described previously for cats (Katz, 1987; Callaway and Katz, 1992) and monkeys (Katz et al., 1989). Briefly, animals were deeply anesthetized with sodium pentobarbital (40–60 mg/kg, i.p.), and a large craniotomy was made to expose the striate cortex. The dura was removed, and a large portion of striate cortex (~2 cm wide and 2.5 cm long) was cut free with a scalpel and removed. This tissue was then placed in chilled (~2°C), oxygenated artificial CSF (ACSF; composition in mM: NaCl 124, KCl 5, KH_2PO_4 1.25, MgSO_4 2, CaCl_2 3, NaHCO_3 26, *D*-glucose 10, and kynurenic acid 1, pH

7.4). The animal was overdosed with an additional dose of sodium pentobarbital (100 mg/kg, i.p.) after removal of the cortex. After chilling for ~1 min, the cortical tissue was placed on filter paper saturated with ACSF, the pia was removed with fine forceps, and the tissue was cut into two blocks, each ~6–8 mm wide and 2 cm long, that were then sliced coronally to a thickness of 400 μ m using a specialized “egg slicer”-like device (Katz, 1987). This procedure yielded >100 slices of which 40–60 were typically stored in oxygenated interface chambers warmed to 34°C for later intracellular filling. These slices originated from positions distributed over nearly the entire exposed surface of area V1—anteriorly from the V1/V2 border to the occipital pole posteriorly, and mediolaterally from the midline to ~2.5 cm laterally. Thus, labeled neurons in our sample are not localized to any particular representation of the visual field, with the exception that regions corresponding to tissue folded beneath the exposed surface of the brain were not sampled.

Intracellular labeling. After incubating in the interface chamber for at least 1 hr, slices were transferred to a recording chamber where they were submerged in warm (34°C), oxygenated ACSF circulating at a rate of ~2 ml/min. Whole-cell recordings were obtained in either voltage-clamp or current-clamp mode using methods similar to those described previously (Blanton et al., 1989). The electrodes used had resistances of 5–15 M Ω and were filled with the following solution: 130 mM K-gluconate, 1 mM EGTA, 2 mM MgCl_2 , 0.5 mM CaCl_2 , 2.54 mM ATP, 10 mM HEPES, and 2% biocytin (Sigma, St. Louis, MO), pH 7.3. After a whole-cell recording was obtained, biocytin was iontophoresed into the cell with positive current pulses of 0.3 nA, and 33 ms duration at 10 Hz. Cells were typically filled for at least 10 min, and this procedure invariably resulted in complete filling of the axonal and dendritic arbors (see Fig. 1). Occasionally, recordings were lost after shorter periods, but the filling was often excellent despite the shorter filling duration. *Only neurons in which the entire dendritic and axonal arbor could be clearly visualized after staining are included in our analyses.* Electrode penetrations into the slice were spaced such that labeled neurons would be at least 1 mm apart laterally. After several neurons in a slice were labeled, it was returned to the interface holding chamber for ~2–6 hr. This postincubation procedure resulted in removal of nonspecific labeling at the locations of electrode penetrations, which would otherwise obscure the processes of specifically labeled neurons. After postincubation, slices were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer for 12–14 hr. The short fixation time was crucial for obtaining good quality CO staining; shorter fixation times did not result in adequate tissue preservation.

Tissue processing. Following fixation, slices were sunk in 30% sucrose in 0.1 M phosphate buffer and then sectioned on a freezing microtome to a thickness of 50–100 μ m. Sections were rinsed three times in PBS and then stained for CO by incubation in a solution containing 30 mg of cytochrome *c*, 20 mg of catalase, and 50 mg of diaminobenzidine (DAB; all from Sigma) per 100 ml of PBS, for 2–4 hr at 40–50°C (Horton, 1984). Sections were rinsed again three times in PBS and then incubated in 10% methanol and 3% H_2O_2 in PBS for 30 min. This step inactivates endogenous peroxidases commonly associated with blood vessels in the nonperfused brain tissue. The sections were rinsed again five times in PBS and then incubated 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 hr. Sections were rinsed three times in PBS, submerged in 0.1% glutaraldehyde in PBS for 4 min (to reduce background staining), and then rinsed five times in PBS. Finally, the intracellularly injected biocytin was revealed by reacting the sections in a solution containing 50 mg of DAB, 2.8 ml of 1% CoCl_2 , 2.0 ml of 1% nickel ammonium sulfate, and 10 μ l of 30% H_2O_2 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 (see Fig. 1).

Determination of laminar boundaries. Laminar boundaries were defined based on the pattern of CO staining in each section, as indicated in Figure 1. The criteria used were similar to those of Lund (1987, 1988). The most distinct laminar feature in the CO-stained tissue is layer 4C, which is the most densely stained region in the middle of the gray matter. The subdivisions of this layer, 4C α and 4C β , are not distinguished by the CO stain and, therefore, we define the upper half of layer 4C as 4C α and the lower half as 4C β . Superficial to layer 4C is a lightly stained zone, layer 4B, and a thin, darker-staining band, layer 4A. Layer 1 is a thin, CO-dense zone just beneath the pial surface. We refer to the region between layer 4A and layer 1 as layer 2/3 and further subdivide this region into layers 2/3A (the upper two-thirds) and 3B (the lower third). Layer 5 is a lightly

stained zone just below layer 4C, and it is separated from the white matter by the darker-staining layer 6. Layer 5 is divided into an upper region, layer 5A (the upper third of layer 5), and a lower region, layer 5B (the lower two-thirds of layer 5). The border between layer 6 and the white matter is marked by a sharp decrease in the intensity of CO staining. CO staining also distinguishes the blob and interblob regions in layer 2/3.

Analyses of labeled neurons. Stained sections were scanned at 100× magnification [10×, 0.5 numerical aperture (NA) objective] to locate labeled neurons, and suitably labeled cells were identified. Such cells were labeled well enough that even the most distal axonal processes could easily be seen with the 10× objective (see Fig. 1). Each neuron was then carefully scrutinized at variable magnification to determine in detail its patterns of axonal and dendritic arborization and its relation to the cortical layers and CO blob and interblob regions of layer 2/3. Detailed camera lucida reconstructions of axonal arbors were made using a 60× or 63× (1.4 NA) oil immersion objective. Crude dendritic drawings were made using a 10× or 20× objective; the thickness of dendrites and other details such as dendritic spines are not indicated in these drawings, but all dendritic branches are shown. Camera lucida drawings were photographed with a large-format camera, and photographic prints were made from the negatives. The prints were then scanned electronically using a PC-based computer system. Scale bars and hand-drawn lines indicating laminar and blob/interblob borders were replaced with computer-drawn lines at their original positions on the scanned images, using the program CorelDraw.

Although both en passant synaptic boutons and side-spine boutons could be clearly visualized in all of the labeled neurons, we have not reconstructed their distributions or analyzed them quantitatively. We do note, however, that synaptic boutons are present on all axonal branches, with the exception of the thick, unbranched recurrent collaterals rising up from class I neurons. Thus, to a rough approximation, the density of axonal branches is representative of the strength of a projection, and we refer to this characteristic as our primary determinant of projection strength.

Each neuron was also investigated to determine whether its main descending axon projected to the white matter. When the axon could be seen entering the white matter, the cell was scored as a projection neuron. Neurons were scored as nonprojecting only if the descending axon clearly ended within the plane of the slice. In no case was the determination of projection to the white matter ambiguous (i.e., we did not ever encounter descending axons leaving the plane of the slice before we could determine whether they projected to the white matter).

We calculated the depth at which the cell body of each neuron lies in layer 6 by the following: drawing with the camera lucida the bottom and top borders of layer 6 and the position of the cell body; measuring the distance between the base of the cell body and the layer 5/6 border; dividing this distance by the whole depth of layer 6; and multiplying this ratio by 100. We defined “superficial” as a depth $\leq 40\%$, “middle” as between 40 and 60%, and “deep” as $\geq 60\%$.

RESULTS

We have labeled and analyzed 58 pyramidal neurons with cell bodies in layer 6. Photographs indicating the quality of neuronal labeling that was observed for the neurons included in our analyses are shown in Figure 1. Note that even with the low-power 10× objective used to make the photographs in Figure 1*a–c*, axonal branches are clearly visible. These branches are actually even more obvious than can be shown with these black and white photographs because of the contrasting color of the CO versus biocytin labeling. Also, many out-of-focus branches can only be seen by changing the focal plane. In some cases, there is a “haze” of labeling surrounding the cell body, which presumably results from leakage during iontophoresis and/or from pressure ejection of dye before obtaining whole-cell recordings (Fig. 1*a*). Despite this haze, observation with a higher-power objective and stopped-down condenser aperture allows clear visualization of the axon emerging from the base of the cell body, unequivocally linking the reconstructed axonal arbor to the parent cell.

We have distinguished two major classes of layer 6 pyramidal neurons, which we designated as class I and class II. These classes were defined based on the laminar specificity of axonal and

dendritic arbors. Class I cells ($n = 28$) have dense axonal projections primarily within layer 4C (Fig. 1*a–c*), whereas class II cells ($n = 30$) have more widespread axonal projections. In addition, class I neurons lack dendritic branches in layer 5B (Fig. 1*a–c*), whereas class II cells do have dendritic branches in layer 5B (Fig. 1*d*). We have subdivided class I neurons into several types, again according to the laminar specificity of their processes. Class I was divided into five types: $I\alpha$ ($n = 6$), $I\beta$ ($n = 8$), $I\beta A$ ($n = 7$), I_m ($n = 4$), and I_C ($n = 3$). Although the patterns of dendritic and axonal arborization of class II neurons are quite diverse, we are not able to separate most of them into distinct groups. Only one type stands out: a type we have called IIB ($n = 6$). We will refer to the class II cells that do not belong to the type IIB as type IIA .

Class I neurons

Twenty eight of the 58 layer 6 pyramidal cells in our sample were classified as class I neurons. As shown in Figures 2–6, class I cells project heavily and predominantly to layer 4C. Each type of class I neuron is distinct in terms of the following: (1) the sublaminar specificity of axonal arbors within layer 4C; (2) the pattern of dendritic arborization; (3) the distribution of neurons within the depth of layer 6; and (4) the depth distribution of the neurons that project out of V1, if any.

Type $I\alpha$

Out of the 28 class I cells, 6 have dense axonal arbors predominantly within layer 4C α and are therefore defined as type $I\alpha$ cells (Figs. 1*a*, 2). The axonal projections of these neurons are quite focused in 4C α , with occasional branches dropping into 4C β or extending into layer 4B; but no branching is observed in these layers. Most (5/6) also have weak axonal projections within layer 6 (Fig. 2*b*). Axonal arbors extend 1–2 mm laterally within layer 4C α . These arbors appear to have a uniform distribution without any signs of periodic clustering (Fig. 2*a,b*). None of the six type $I\alpha$ neurons projects to the white matter (Fig. 2*a,b*, Table 2). Type $I\alpha$ neurons have numerous basal dendrites in layer 6 and apical dendritic branches in layers 6 and 4C α ; two of them also have dendritic branches in layer 5A (Fig. 2*a*). Four out of six have their somata in the middle of layer 6 (Fig. 2*a,b*), whereas the other two are somewhat deeper (Fig. 10, Table 2).

Type $I\beta$

Our sample includes eight type $I\beta$ neurons (Fig. 3). These cells have axonal arbors predominantly in layer 4C β (Fig. 3*a,b*), and half (4/8) also send weaker projections to their own layer, layer 6 (Fig. 3*b*). Although some axonal branches can extend into the bottom of layer 4C α (Fig. 3*b*), they clearly do not extend to the top of layer 4C. These cells have a more limited lateral axonal spread than the type $I\alpha$ neurons; they never extend more than 1 mm laterally and can be as narrow as 360 μ m. These arbors also do not form periodic clusters. Both apical and basal dendritic branches lie in layer 6, and the apical dendrite also branches in layers 5A and 4C β (Fig. 3). In two cases, the apical dendrite extends up to layer 4A. Type $I\beta$ neurons can be found throughout the depth of layer 6 (Figs. 10, 11, Table 2), and three out of eight project to the white matter (Table 2). None of four neurons with somata near the middle of layer 6 (depths of 35–75%) projects to the white matter, whereas three of four located more superficially or deeper do project to the white matter. Thus, there appears to be a tendency for the projecting cells to be situated at the edges of layer 6, but not in the middle (see Fig. 11).

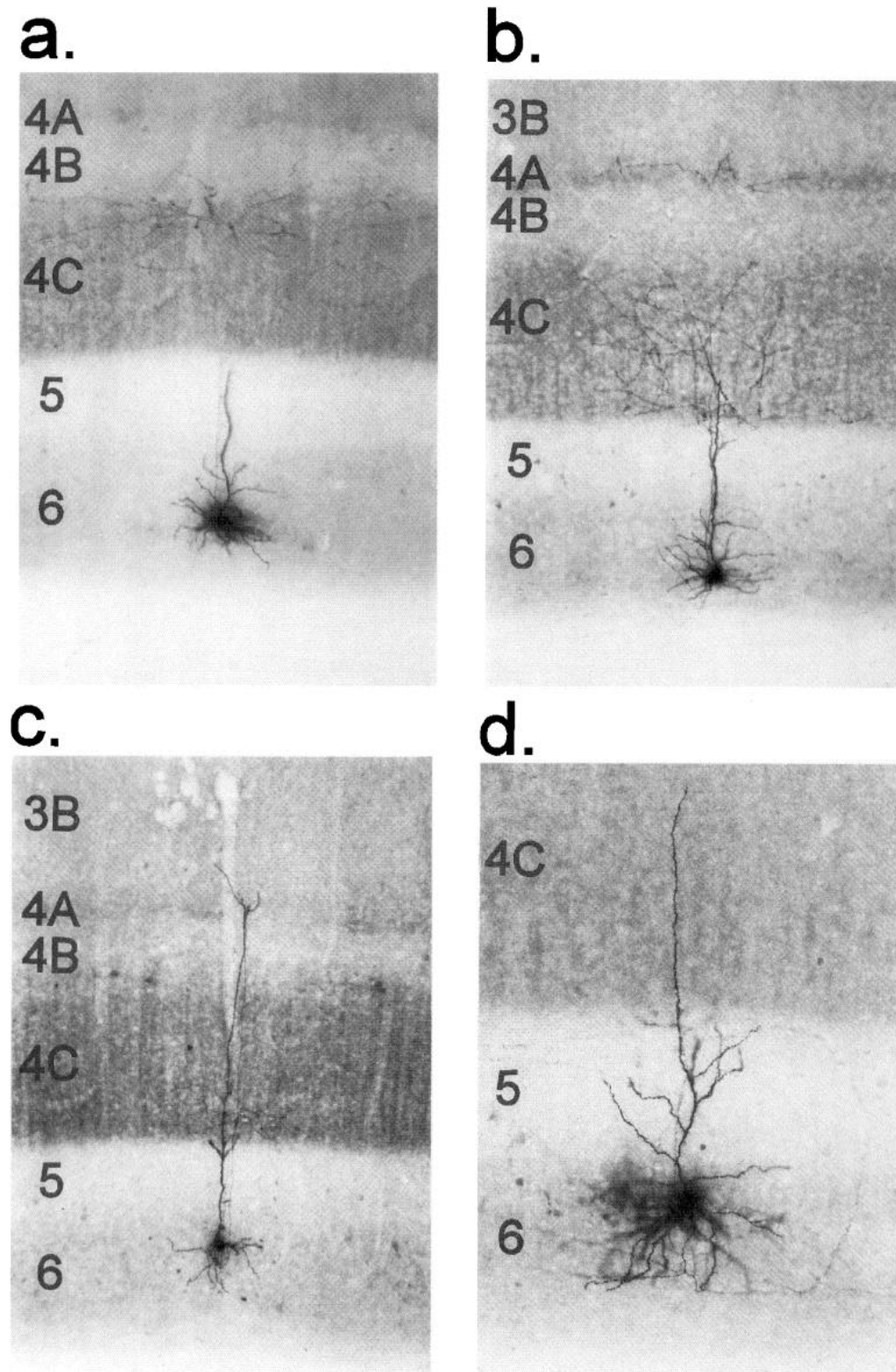


Figure 1. Photographs of processed tissue sections containing biocytin-filled layer 6 neurons. The cortical layers revealed by cytochrome oxidase staining are identified at the *left side* of each photograph. The dendritic arbor appears thicker than the axonal arbor. *a* illustrates a type Ia neuron. The basal dendrites are restricted to layer 6. The apical dendrite has a few branches in layer 6, ascends unbranched in layer 5 where it leaves the plane of this section, and then reappears and branches at the top of layer 4C (4C α). The axon branches in layer 6, ascends unbranched in layer 5 where it also leaves the plane of this section to reappear along with numerous branches in layer 4C α (4C). A reconstruction of this neuron is shown in Figure 2*b*. Shown in *b* is a type IC neuron with its cell body in deep layer 6. The apical dendrite of this cell has a few branches in layers 6 and 5A (5) and leaves the plane of this section in 4C β (4C). The axon has a few branches in layer 6, and the main descending axon extends into the white matter. Ascending axonal collaterals branch throughout layer 4C and in layer 4A. *c*, Photograph of a type I β A neuron. The cell body is in superficial layer 6. The basal dendrites are restricted to layer 6. The apical dendrite branches in layers 5A (5), 4C β (4C), and 4A; it does not extend above layer 3B. A majority of axonal branches are present in other sections of the slice where this neuron was labeled, but branches in layer 4C β (4C) are visible. The camera lucida reconstruction of this neuron is shown in Figure 4*a*. *d*, A typical type IIA neuron with its apical dendrite branching throughout layer 5 and extending unbranched into layer 4C.

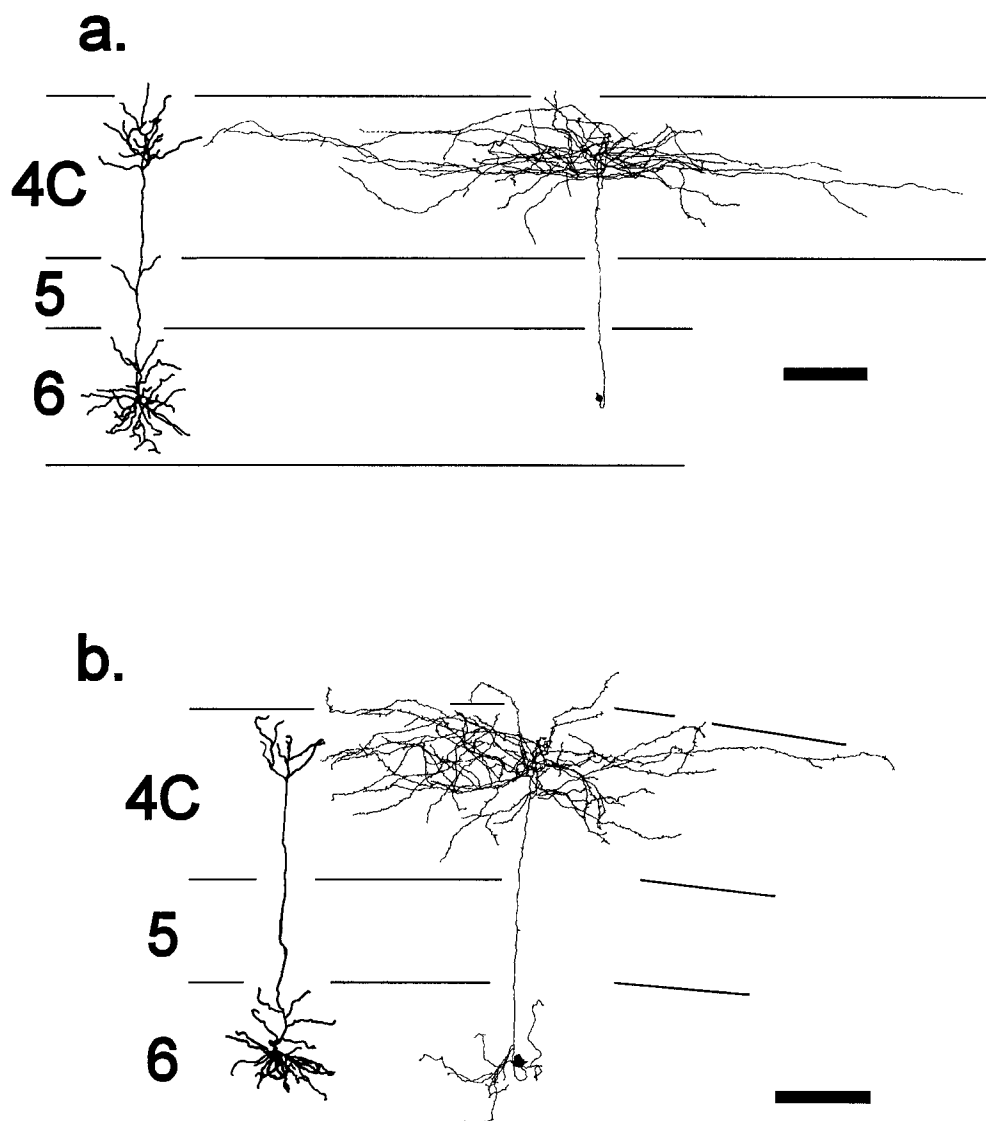


Figure 2. Camera lucida reconstructions of type Ia neurons. Axonal arbors (fine lines) are illustrated in their actual positions and originate from the cell body as indicated (filled figures). Dendrites (in bold) are shown at their actual height and size but are offset to the side to avoid obscuring the axonal arbor. Horizontal lines indicate the laminar boundaries of the cortical layers identified by labels on the left side. Slanted lines (to the right of *b*) indicate laminar boundaries as the tissue curves. The cell bodies of the neurons shown in *a* and *b* are in the middle of layer 6. Both neurons project axonal branches predominantly to layer 4C and, hence, belong to class I. More specifically, they project to layer 4C α and, therefore, belong to the type Ia (see Results). The neuron shown in *b* also projects to its layer of origin (layer 6). As illustrated in *a* and *b*, type Ia cells do not project to the white matter. The apical dendrites of type Ia cells branch in layer 4C α (4C). The neuron illustrated in *a* also has dendritic branches in layer 5A (5). The basal dendrites and additional apical dendritic branches are in layer 6. A photograph of the neuron illustrated in *b* is shown in Figure 1*a*. Scale bars, 200 μ m.

Type I β A

We labeled seven type I β A cells (Figs. 1*c*, 4). These cells have axonal arbors similar to type I β cells, except that they also project an ascending collateral to layer 4A (and sometimes to layer 2/3, 3 of 6 cells) where it branches (Fig. 4*b*). The lateral axonal spread of I β A neurons in layer 4C is similar to the type I β , ranging from \sim 300 μ m to 1.1 mm. In layer 4A, it can be as narrow as 170 μ m and as wide as 1.5 mm (Fig. 4*b*).

The dendritic pattern of the type I β A cells is similar to the type I β , except that the apical dendrite of the type I β A cells in our sample always extends to layer 4A (and occasionally to layer 3B) where it branches. All type I β A cells have somata in upper layer 6 (Figs. 10, 11, Table 2), further distinguishing them from the type I β cells. The three type I β A cells located most superficially in layer 6 project to the white matter, whereas the four with somata closer to the middle of layer 6 do not (Fig. 11, Table 2).

Type Im

We labeled 4 class I cells that, instead of having axonal arbors focused in layer 4C α or 4C β , project heavily to the middle of layer 4C, with few branches at the edges of the layer (Fig. 5). We called these cells type Im. The lateral spread of their axons is compara-

ble in extent with type I β and I β A cells, but less variable (range, 560–775 μ m). The axonal arbors lack any signs of periodic clustering. Unlike many class I cells, none of the type Im neurons in our sample has axonal branches in their own layer.

All type Im cells have apical and basal dendritic branches in layer 6, and apical dendrites that extend into layer 4C and branch in layer 5A. Two out of four cells also have apical dendritic branches in the middle of 4C (Fig. 5). The apical dendrite can extend as high as layer 4B (Fig. 5*a*). Most type Im cells (3/4) have somata in the middle of layer 6 (one was slightly above the middle, Fig. 10, Table 2), and none projects to the white matter (Table 2).

Type IC

Our sample includes three type IC neurons (Figs. 1*b*, 6). These cells project axons predominantly to layer 4C and throughout the depth of this layer without any preference for any subdivision of 4C (Fig. 6*a,b*). Like type I β A cells, they also project axons to layer 4A (Fig. 6*b*) and sometimes 3B (Fig. 6*a*). The lateral spread of axonal arbors is centered around 1 mm in a tight range: 900 μ m to 1.1 mm with no indications of periodic clusters. Type IC cells have basal dendrites in layer 6 and their apical dendrite branches in layers 6 and 5A, and occasionally in layer 4C or 4A. Type IC

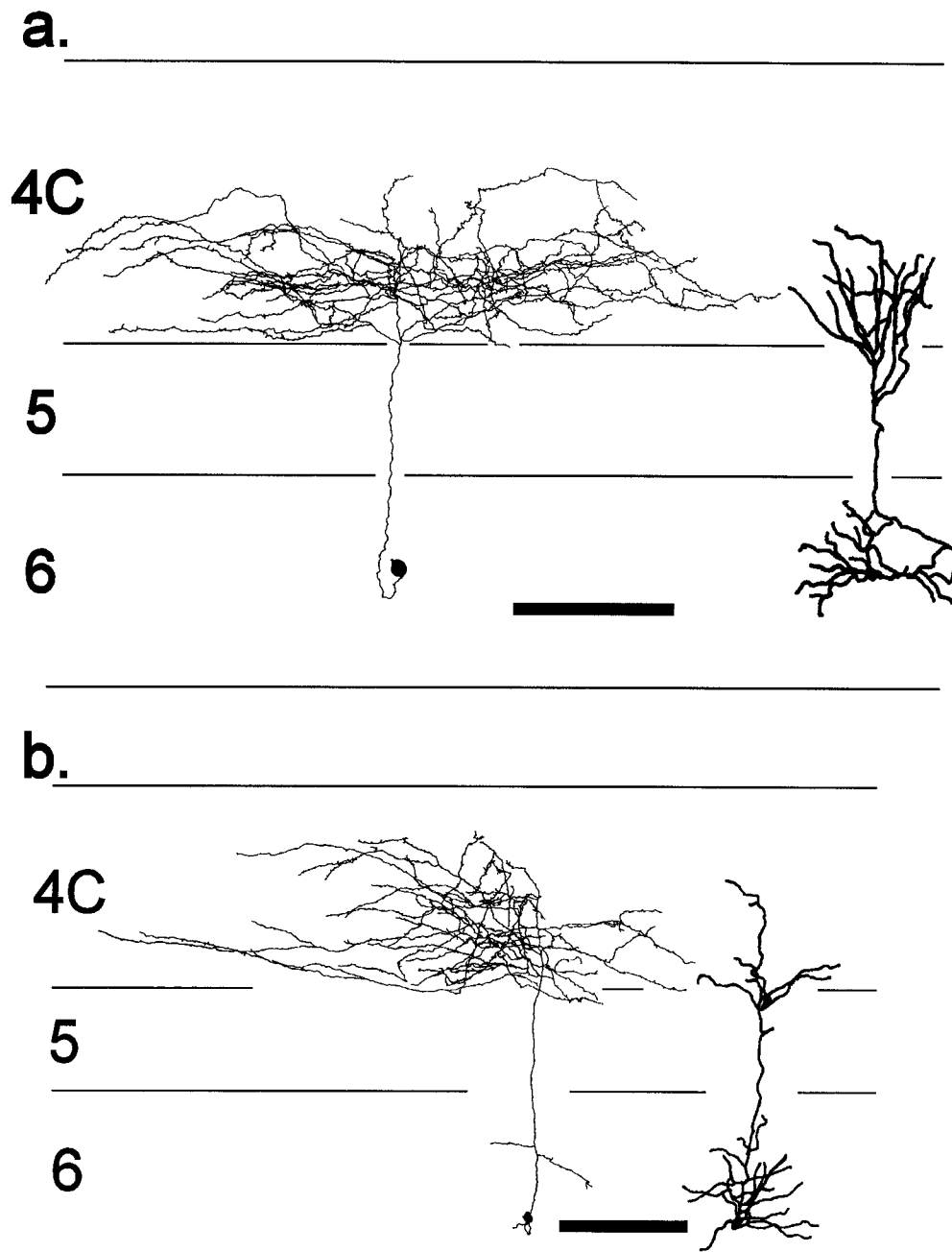


Figure 3. Camera lucida reconstructions of type I β neurons. Both neurons (*a*, *b*) project axonal branches predominantly to layer 4C β (4C) and, therefore, belong to the type I β (see Results). The neuron shown in *b* also has weak axonal projections to layer 6. The neuron shown in *a* has its cell body in the middle of layer 6. The soma of the cell shown in *b* is in deep layer 6. These two neurons do not project to the white matter, but others of the same type do. Dendritic branches of both cells arborize in layers 6, 5A (5), and 4C β (4C). See legend of Figure 2 for conventions. Scale bars, 200 μ m.

cells are distinguished further from type I β and I β A cells in that all are found deep in layer 6 (Fig. 10) and project to the white matter (Table 2).

Relationship of class I neurons to layer 5

Overall, only 3 of 28 class I cells have dendritic branches in layer 5B. One type I β neuron has three dendritic branches in layer 5B, one type I β A cell has two, and another has one branch in this sublayer (Fig. 4*b*). This contrasts sharply with class II cells (see below).

Class II neurons

Class II neurons lack a preference of axonal arbors for the main LGN recipient layer, layer 4C (Figs. 7–9). Also, these neurons always have dendritic branches in layer 5B (Figs. 1*d*, 7–9). Thirty out of 58 layer 6 cells fall into this class. Most class II cells (24/30) project axons to layer 2/3, but none has an obvious preference for

CO blobs or interblobs. There is no apparent difference in the density of axon collaterals or branches in blobs versus interblobs.

Type IIA

Type IIA cells ($n = 24$) are the most diverse type of layer 6 neuron. Some have relatively sparse axonal arbors that form branches throughout the cortical depth (Fig. 7*a,b*). Most, however, have more extensive axonal arbors within the infragranular layers (Fig. 8*a,b*). We do not separate these into distinct types, however, because they appear to represent extremes along a continuum. Some type IIA cells have lateral axonal branches in layers 6 and 5, and also project to the superficial layers (Fig. 8*b*); others project laterally (up to 2.55 mm) within layer 6 and sometimes 5, but do not project into more superficial layers (Fig. 8*a*). Long-distance collaterals in layer 6 can form periodic clusters of axonal branches that are spaced ~ 700 μ m apart (Fig. 8).

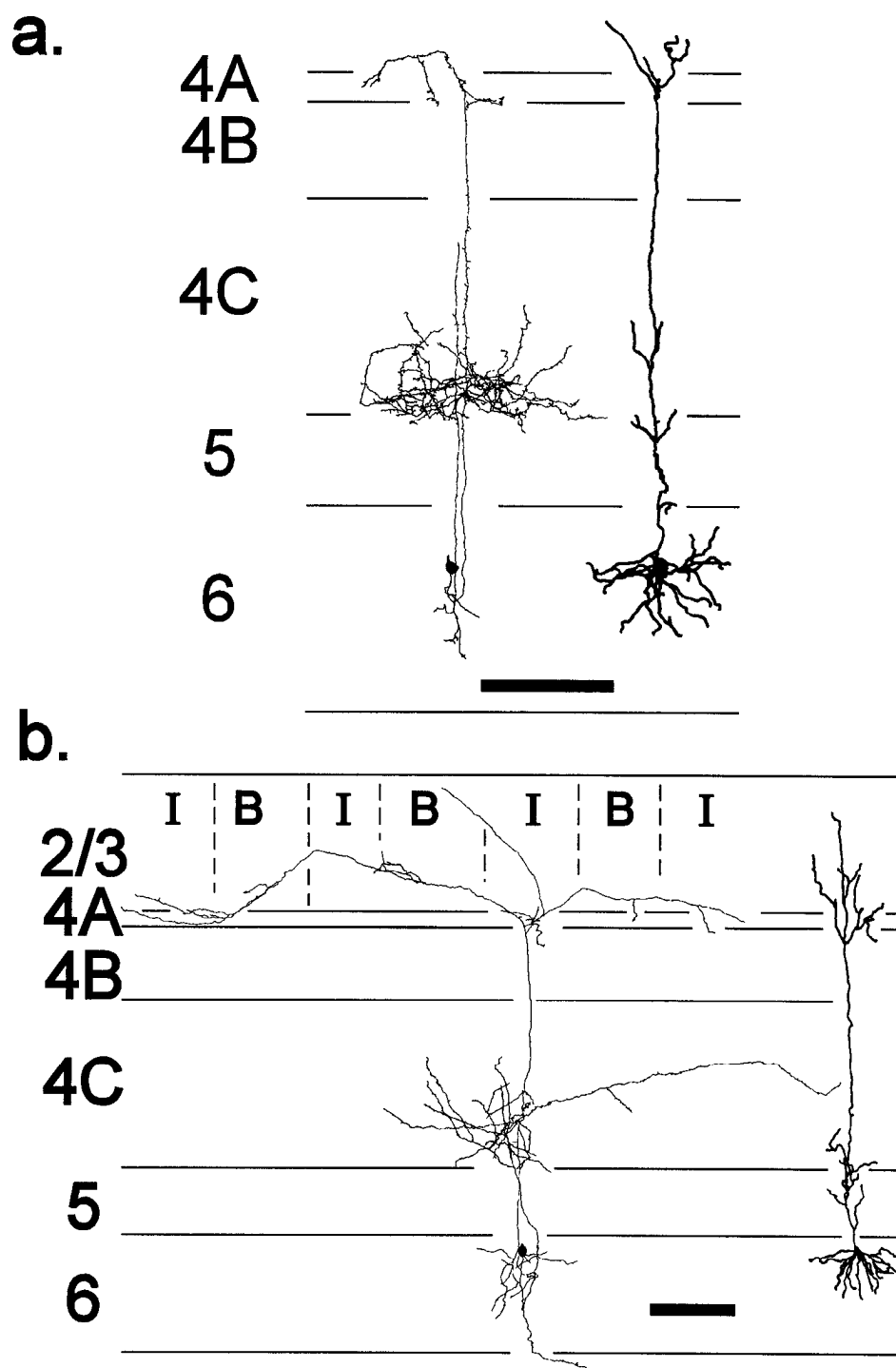


Figure 4. Camera lucida reconstructions of type IβA neurons. Both neurons (*a*, *b*) project axonal branches predominantly to layers 4Cβ (4C) and 4A and, therefore, belong to the type IβA (see Results). Both neurons also have axonal branches in their layer of origin (layer 6). The neuron shown in *b* has its cell body in superficial layer 6, whereas the soma of the neuron in *a* is closer to the middle of layer 6. The cell shown in *b* is a projection neuron. It has axonal branches in layer 2/3 that have no apparent specificity for blobs or interblobs. [Vertical dashed lines in *b* indicate transitions between blob (B) and interblob (I) compartments in layer 2/3.] For both cells (*a*, *b*), basal dendrites are in layer 6 and the apical dendrite branches in layers 5A (5), 4Cβ (4C), 4A, and 3B (2/3). The neuron shown in *b* also has two apical dendritic branches in layer 5B, which is unusual for class I neurons. A photograph of the neuron in *a* is shown in Figure 1c. See previous figures for other conventions. Scale bars, 200 μm.

In addition to apical and basal dendrites in layer 6, type IIA cells have apical dendritic branches throughout layer 5 or in 5B (Figs. 7, 8). The apical dendrites extend to varying levels of layer 4 and occasionally extend to layer 2/3, but only rarely branch above layer 5A (Fig. 8*b*). Their somata can be found throughout the depth of layer 6 (Figs. 10, 11, Table 2). Seven out of 24 project to the white matter (Table 2). Unlike class I projection neurons, the type IIA projection neurons have cell bodies located toward the middle of layer 6 (Fig. 11).

As we have noted, we could not easily sort type IIA cells into distinct categories without making arbitrary distinctions. One possible exception is a neuron with a dense axonal projection in layer

4A (Fig. 7*a*). The axonal branches of this cell within layer 4A are clearly focused on the regions beneath interblobs. However, we have only recognized one neuron with this morphology.

Type IIB

We labeled six cells in the bottom of layer 6 that have a distinctive dendritic morphology and project axons heavily to layer 4B (Fig. 9*a,b*). They also project to both the blobs and interblobs throughout the depth of layer 2/3. Five out of six cells also project axons to layers 5 and 6 (see Fig. 9*b* for counterexample); these cells rarely have axonal branches in layer 4C (see Fig. 9*b*). The lateral extent of these neurons is greatest in layers 2–4B and is between

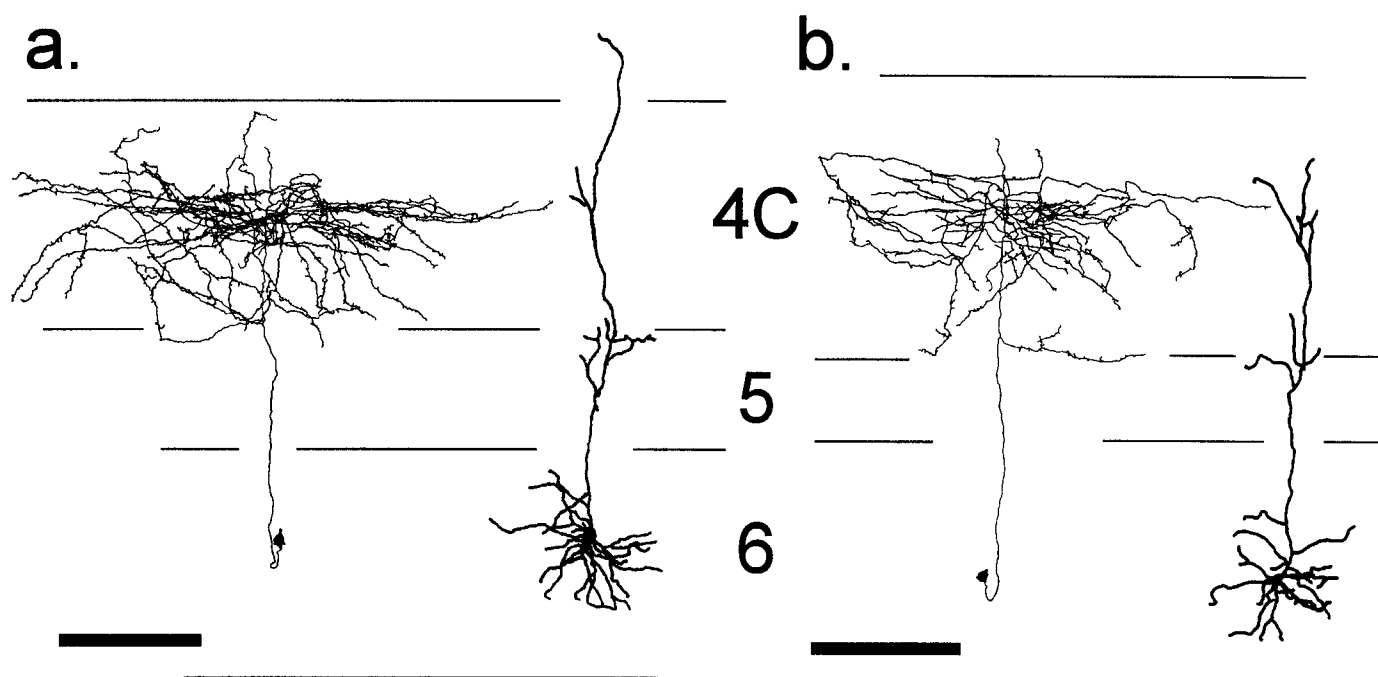


Figure 5. Camera lucida reconstructions of type Im neurons. The layers are identified by *number* in the space between the two drawings. Neurons shown in *a* and *b* have their soma in the middle of layer 6. They have their densest network of axonal branches in the middle of 4C. They do not project to their own layer (6) or to the white matter. The apical dendrites of both cells branch in layer 5A (5) and in the middle of layer 4C. See previous figures for conventions. Scale bars, 200 μ m.

1 and 2 mm. Type IIB dendritic arbors do not extend above layer 5. Instead, the apical dendrite gives rise to numerous oblique branches throughout layer 5. These cells are deep in layer 6 (Fig. 10, Table 2) and do not send an axonal branch into the white matter (Table 2).

DISCUSSION

Summary

Layer 6 pyramidal neurons in macaque V1 make up a diverse population that can be separated into two classes and several types within these classes (see Fig. 12 for summary). Class I neurons have dense axonal arbors in layer 4C and lack dendritic branches in layer 5B, whereas the axonal arbors of class II neurons are focused on layers other than 4C and they have extensive dendritic arbors in layer 5B.

Type I α neurons have axonal and dendritic arbors that are specific for layer 4C α . Type I β axonal arbors are specific for layer 4C β , and their dendritic arbors are specific for 4C β and sometimes 4A. Type I β A axonal and dendritic arbors are specific for layers 4C β and 4A. Type Im neurons have axonal and sometimes dendritic branches specific for the middle third of layer 4C (not the edges). Type IC neurons project axons throughout the depth of layer 4C and to layer 4A. The lateral spread of axonal arbors within layer 4C is about twice as extensive for type I α neurons than for the remaining four class I types (up to 2 mm vs up to 1 mm).

Class II is subdivided into two types, type IIA and type IIB. Type IIA neurons can project axons to any cortical layer, but most project predominantly to the infragranular layers. Projections within layer 6 can be long and clustered. Type IIB neurons have dense axonal projections to layers 2–4B, 5, and 6, but avoid layer 4C. They have extensive dendritic branches in layer 5 but no higher. Although most class II neurons project axons to layer 2/3,

there is no evidence for selectivity for CO blobs versus interblobs in this layer.

Most layer 6 pyramids are nonprojection neurons. Only a subset of types I β , I β A, IC, and IIA projects to the white matter. All type I α , Im, and IIB neurons in our sample lack projections to the white matter.

Technical considerations

We are confident that all of the axonal and dendritic processes of neurons in our sample were detected, except those extending beyond the plane of the parent brain slice. Thus, we have no doubt that all of the projections we have observed exist and arise from the cells we identified. We cannot rule out the possibility that additional projections or cell types also exist. For example, we have not encountered a Meynert cell, probably because of their low density (perhaps 25 cells/mm²; Winfield et al., 1981). We also cannot rule out sampling biases because, for example, certain cell types might be more difficult to record from.

It is important to note that the neurons in our sample are from young postnatal monkeys. We feel that their morphologies represent those in adults. First, 23 of 58 neurons in our sample are from animals >8 months old, and all animals were at least 2 months old. Because laminar specificity of axonal arbors, ocular dominance columns, and specificity of circuitry for CO blobs all develop prenatally in macaque monkeys (Lund et al., 1977; Callaway, 1993; Horton and Hocking, 1995), it is unlikely that there are dramatic changes in laminar specificity after the eighth month. Second, maturation would be expected to result in increased specificity of local circuits. However, we observe a high degree of specificity even in the youngest cells. Finally, although our sample comes from animals ranging in age from 2 to 9 months, each

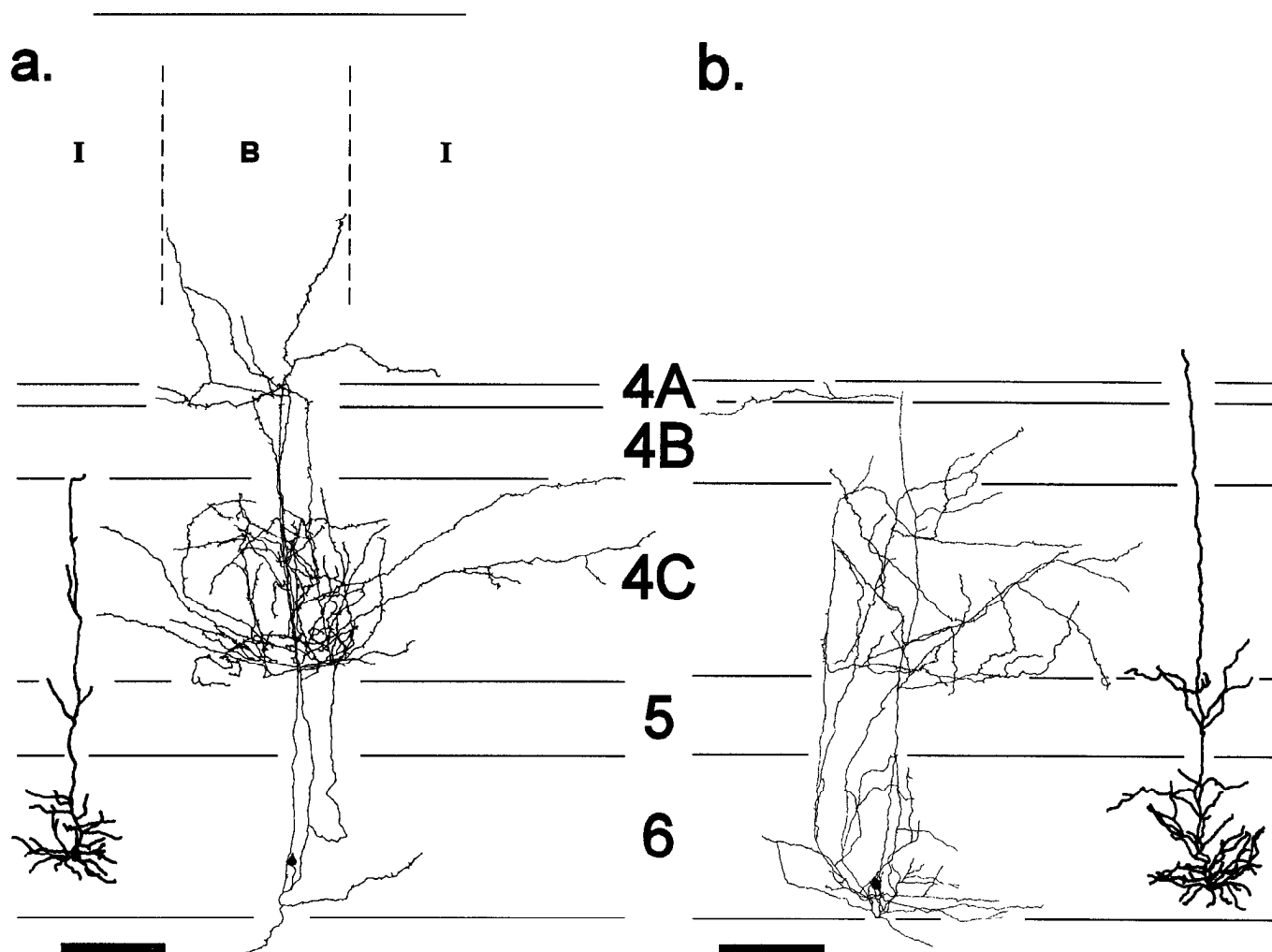


Figure 6. Camera lucida reconstructions of type IC neurons. These neurons (*a*, *b*) project dense axonal branches throughout layer 4C and, therefore, belong to the type IC. They also project to layers 4A and 6. The neuron in *a* also projects to layer 3B (not shown), predominantly but not exclusively to the blob compartment situated directly above its cell body. Neurons shown in *a* and *b* have their cell body deep in layer 6, and they are projection neurons. Both neurons have basal dendrites in layer 6 and an apical dendrite that extends through layer 4C and branches in layers 6 and 5A (5). Cell *a* also has apical dendritic branches in layer 4C. See previous figures for conventions. Scale bars, 200 μ m.

neuronal type, except Im, was observed in both 2- to 3-month-old and 8- to 9-month-old animals (type Im was observed only in the two oldest animals; see Table 1). There are no apparent differences between these age groups in the degree of specificity of axonal arbors or any other characteristics of the neurons. Such invariance would not be expected over this span of >5 months if the neurons were undergoing developmental changes in the parameters we analyzed.

Class I neurons

About half of the layer 6 pyramidal neurons are class I neurons. These have dense axonal projections predominantly to layer 4C. This is not surprising because both Golgi studies and extracellular tracer injections have revealed strong projections from layer 6 to this layer (Lund and Boothe, 1975; Lund et al., 1977; Fitzpatrick et al., 1985; Yoshioka et al., 1994).

Our observations suggest that class I neurons belong to distinct types such as those we have defined. The distinct differences in laminar specificity of axonal arbors within layer 4C allowed unambiguous classification of all 28 class I neurons;

there are no cells with intermediate levels of specificity. Also, none of the types was defined merely to accommodate cells that did not fit into another group; at least three neurons are included in each type. Laminar specificity of axonal arbors is also correlated with other distinguishing features that are consistent within each population. In particular, each type has a distinct pattern of dendritic arborization and a distinct depth distribution. Nevertheless, we cannot rule out the possibility that intermediate forms or other cell types exist but were not detected in our sample.

Within class I, we find three types of neurons that project specifically to either layer 4C α or 4C β : types I α , I β , and I β A. These layers are dominated by a single functional stream [magnocellular (M) or parvocellular (P); see Lund, 1988], and these cell types are therefore likely to be involved in stream-specific computations. Two other cell types, Im and IC, have axonal arbors that are not restricted to 4C α or 4C β . These neurons could mediate cross-talk between the M and P streams. Furthermore, type Im cells, which project specifically

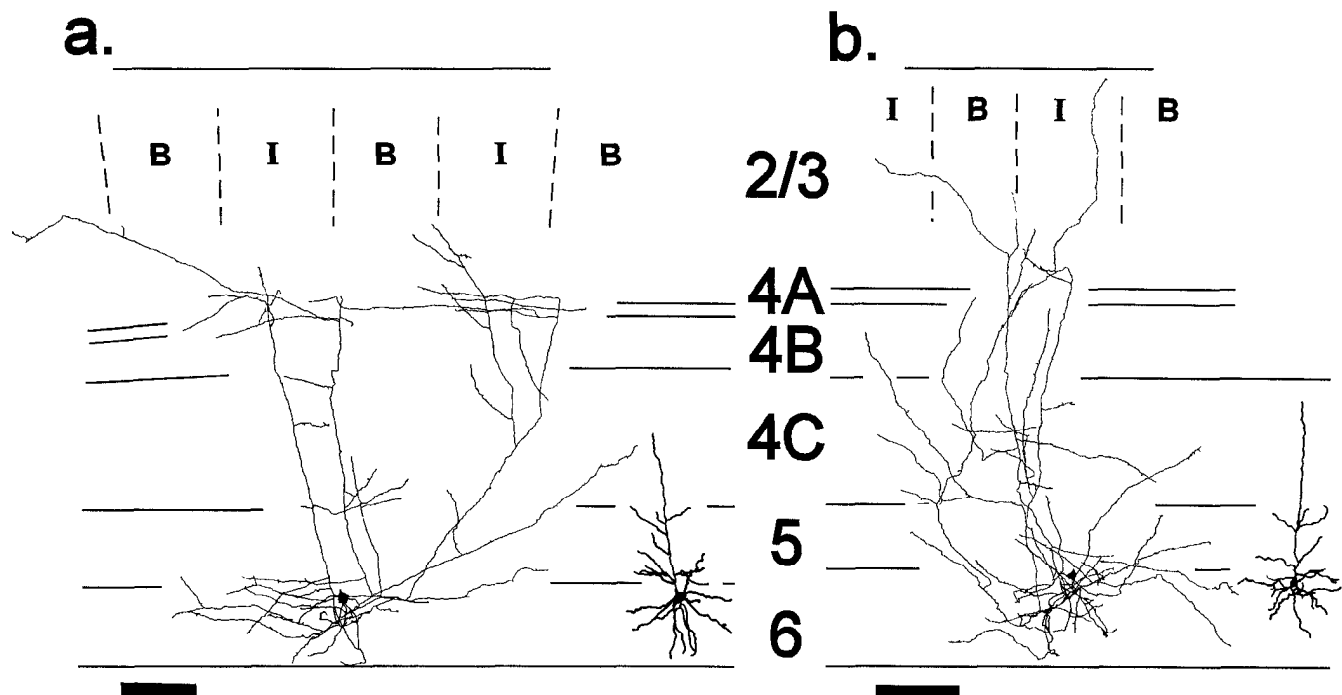


Figure 7. Camera lucida reconstructions of type IIA neurons. These neurons have widespread axonal projections that do not specifically target layer 4C and apical dendritic branches in layer 5B (5); they therefore belong to class II. They lack the features characteristic of type IIB neurons and, therefore, belong to type IIA (see Results). Both cells have heavy axonal projections to layer 6 and weaker projections throughout the cortical depth. The neuron illustrated in *a* shows a particular affinity for layer 4A, where clusters of axons are located directly beneath interblobs. The cell bodies of both neurons are superficial in layer 6. Like a majority of type IIA neurons, these are nonprojection neurons. The apical dendrites of these cells extend into layer 4C and (unlike class I cells) branch throughout layer 5. See previous figures for conventions. Scale bars, 200 μ m.

to mid-layer 4C, might reflect the specialization of mid-4C for computations requiring convergent M and P input (Mates and Lund, 1983; Blasdel and Fitzpatrick, 1984; Yoshioka et al., 1994).

Each of the five types of class I neuron has a distinct laminar pattern of dendritic arborization that is strikingly similar to the pattern of axonal arborization. This observation suggests that class I neurons provide input onto neurons with which they share common sources of geniculate input. In addition, these neurons might receive input from the same neurons to which they project. Thus, they appear to provide feedback to layer 4C. The functional significance of this is considered below.

Based on Golgi-stained prenatal material, Lund et al. (1977) defined four types of layer 6 pyramidal neurons (8 including "variants"). These would all be characterized as class I neurons using our criteria. However, only two of our five types of class I neurons, types Ia and I β A, clearly correspond to types described in these studies. Five of the eight types identified by Lund et al. (1977) are not present in our postnatal sample, suggesting that they represent immature forms, that there were errors in laminar identification (see Lund, 1988), or that they exist postnatally but were not detected in our sample. Our types I β and Im do not correspond to any of those described by Lund et al. (1977), nor do the class II neurons. A type I β A neuron has been filled intracellularly by Anderson et al. (1993).

Previous studies did not reveal which types project to extrinsic targets or their laminar distributions. With intracellular labeling, however, we identified a clear relationship between the depth of class I cells in layer 6 and whether they project to the white matter.

Because LGN-projecting neurons and class I projection neurons are both found primarily in the upper and lower thirds of layer 6 (Fitzpatrick et al., 1994), we suggest that class I projection neurons target the LGN. The type Ia and Im cells in our sample do not project to the white matter and are found in midlayer 6. The three type IC cells in our sample all project to the white matter and have somata deep in layer 6. For the type I β and I β A cells, superficial and deep cells tend to project to the white matter, whereas cells in midlayer 6 are usually nonprojecting. Thus, the LGN is likely to be targeted by types I β , I β A, and IC, but may not receive input from types Ia or Im.

Furthermore, neurons projecting to magnocellular layers of the LGN are found almost exclusively at the bottom of layer 6 (Fitzpatrick et al., 1994). These could be type I β or IC neurons, but we suggest that type IC neurons are more likely because type I β cells restrict their local projections to P-recipient layer 4C β . Neurons projecting to parvocellular LGN are found at the top and bottom of layer 6 (Fitzpatrick et al., 1994). We suggest that type I β A projection neurons, found only at the top of layer 6, along with superficial type I β neurons, make up the superficial neurons targeting parvocellular LGN. Type I β and/or IC could make up the deeper population. Finally, it is possible that the type IC neurons (and perhaps also the deep type I β cells) project to both M and P layers of the LGN.

Class II neurons

Class II is better described as a continuum of cells with varying axonal and dendritic morphologies than as a series of distinct types. Only type IIB neurons compose a distinct type of class II

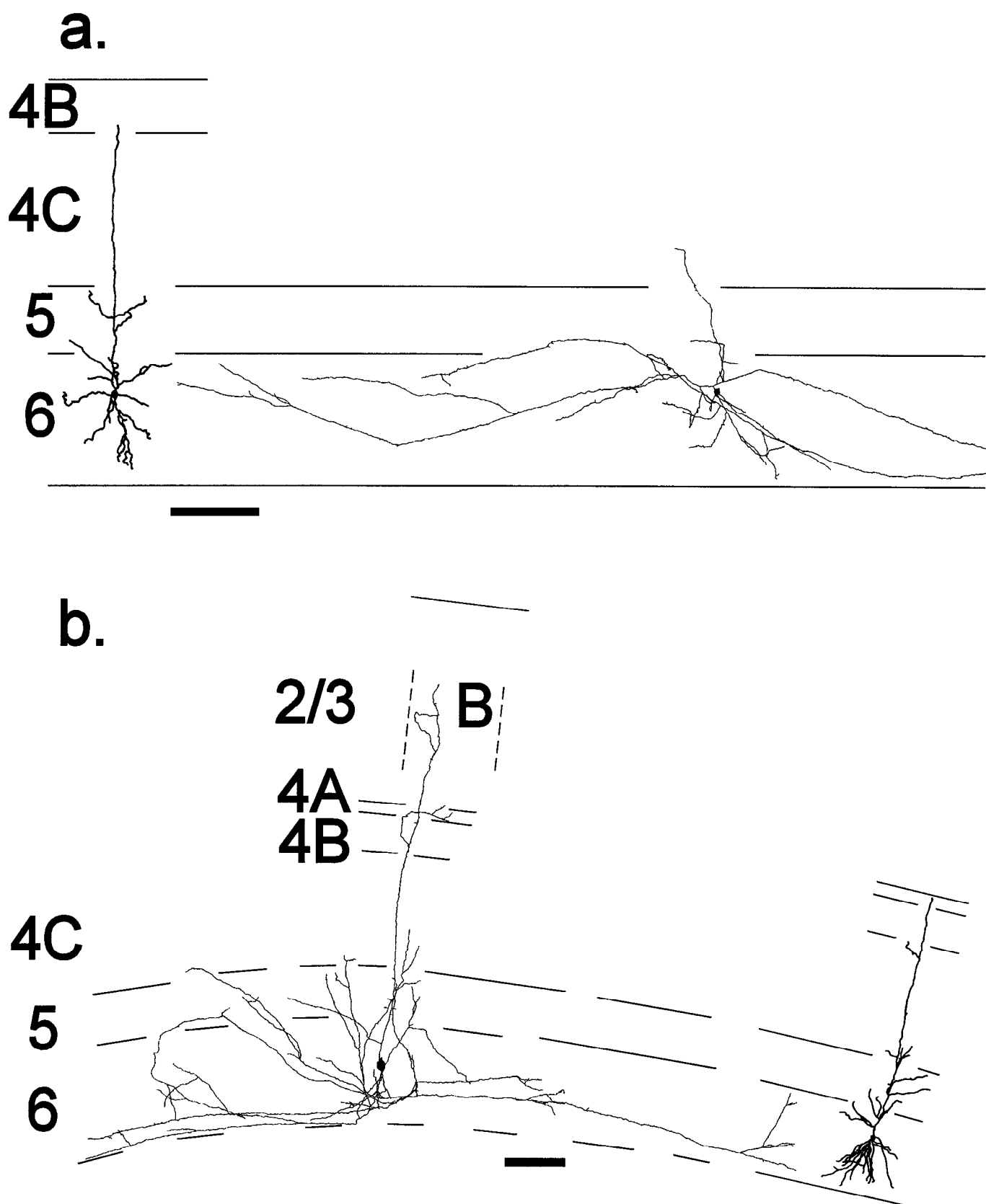


Figure 8. Camera lucida reconstructions of type IIA neurons. Both neurons (*a*, *b*) have long, patchy axonal projections in layer 6. The neuron illustrated in *b* also has a strong axonal projection to layer 5 and weaker projections to layer 4A and to a blob in layer 2/3. The cell body of the neuron shown in *a* is in superficial layer 6, whereas the one belonging to the neuron shown in *b* is closer to the middle of layer 6. Neither neuron projects to the white matter. The apical dendrite of neuron *a* extends to layer 4B and branches in layer 5. The apical dendrite of *b* extends to layer 4A and branches once in layer 4Cα (4C) and throughout layer 5. See previous figures for conventions. Scale bars, 200 μm.

Table 1. Distribution of cell types and their depths

Name	Age	Layer 6 cells	Depth distribution			Type distribution						
			Superficial	Middle	Deep	I α	I β	I β A	Im	IC	IIA	IIB
B13	2 months	1	0	0	1	0	0	0	0	0	1	0
B12	3 months	2	0	1	1	1	1	0	0	0	0	0
B16	3 months	10	0	5	5	1	1	1	0	1	4	2
B19	3.5 months	7	5	1	1	0	0	1	0	0	6	0
B17	4.5 months	12	2	4	6	3	0	1	0	1	6	1
B14	5 months	3	0	0	3	0	1	0	0	0	1	1
B8	8 months	18	8	6	4	1	4	3	3	1	4	2
B11	9 months	5	3	2	0	0	1	1	1	0	2	0

Column 1 lists the identifying number for each animal used in this study. Column 2 indicates the age of each of the eight animals from which our data were obtained. Column 3 indicates the numbers of individual layer 6 cells sampled from each animal. The subsequent three columns indicate the total number of neurons shown in column 3, broken down into the three depth bins defined in Materials and Methods. The seven remaining columns break down the cells from each animal into the types we have identified.

cell. The remaining cells (type IIA) vary considerably in their morphology.

Many class II neurons project axons quite far laterally in layer 6, in agreement with the observations of Blasdel et al. (1985). A majority of class II neurons also project to layer 5, consistent with

retrograde labeling reported by Fitzpatrick et al. (1985). Class II cells do not project strongly to layer 4C, and some avoid this layer altogether. This was surprising because previous studies have not revealed such neurons. Also, class II cells can have sparse or even dense projections in layer 4B. This projection was not detected by

Table 2. Catalog of all layer 6 neurons sampled, organized by type

Cell type	Name	Depth	WM	Cell type	Name	Depth	WM
I α	B17.15.2.1	Middle	No	IIA	B8.22.1.4	Superficial	No
	B8.24.3.1	Middle	No		B19.15.1.1	Superficial	No
	B12.14.2.2	Middle	No		B8.22.2.1	Superficial	No
	B16.15.1.2	Middle	No		B19.12.2.2	Superficial	No
	B17.13.2.1	Deep	No		B17.16.1.2	Superficial	No
	B17.11.1.1	Deep	No		B11.14.3.1	Superficial	No
I β	B8.22.1.1	Superficial	No		B8.13.1.1	Superficial	Yes
	B11.4.3.1	Superficial	Yes		B19.14.2.2	Superficial	No
	B8.27.3.2	Superficial	Yes		B19.13.1.1	Superficial	No
	B8.27.2.1	Middle	No		B11.14.4.1	Middle	No
	B16.12.2.1	Middle	No		B17.12.2.2	Middle	No
	B14.12.3.1	Deep	No		B16.11.2.2	Middle	No
	B12.12.2.1	Deep	No		B19.14.2.3	Middle	No
	B8.25.2.1	Deep	Yes		B8.11.2.1	Middle	Yes
					B16.11.1.1	Middle	Yes
I β A	B19.12.2.1	Superficial	Yes		B17.15.1.2	Middle	No
	B8.21.3.2	Superficial	Yes		B17.12.1.1	Middle	No
	B8.13.1.2	Superficial	Yes		B16.16.1.2	Deep	Yes
	B17.16.1.1	Superficial	No		B16.16.1.1	Deep	No
	B11.15.3.1	Superficial	No		B14.12.1.1	Deep	Yes
	B8.26.1.2	Middle	No		B19.14.2.1	Deep	No
	B16.12.2.3	Middle	No		B17.14.2.1	Deep	Yes
					B13.12.1.1	Deep	No
Im	B8.27.3.1	Superficial	No		B17.11.2.1	Deep	Yes
	B8.11.2.2	Middle	No	IIB	B16.15.1.1	Deep	No
	B11.12.4.1	Middle	No		B16.16.2.1	Deep	No
	B8.28.3.1	Middle	No		B14.11.4.1	Deep	No
IC	B8.26.1.1	Deep	Yes		B8.21.3.1	Deep	No
	B16.15.2.1	Deep	Yes		B8.2.2.1	Deep	No
	B17.12.2.1	Deep	Yes		B17.15.1.1	Deep	No

The identifying number of each cell is indicated in the first column. The letter and number before the first dot correspond to the animal identified (see Table 1). For each cell, its depth and whether it projects to the white matter (WM) are also indicated.

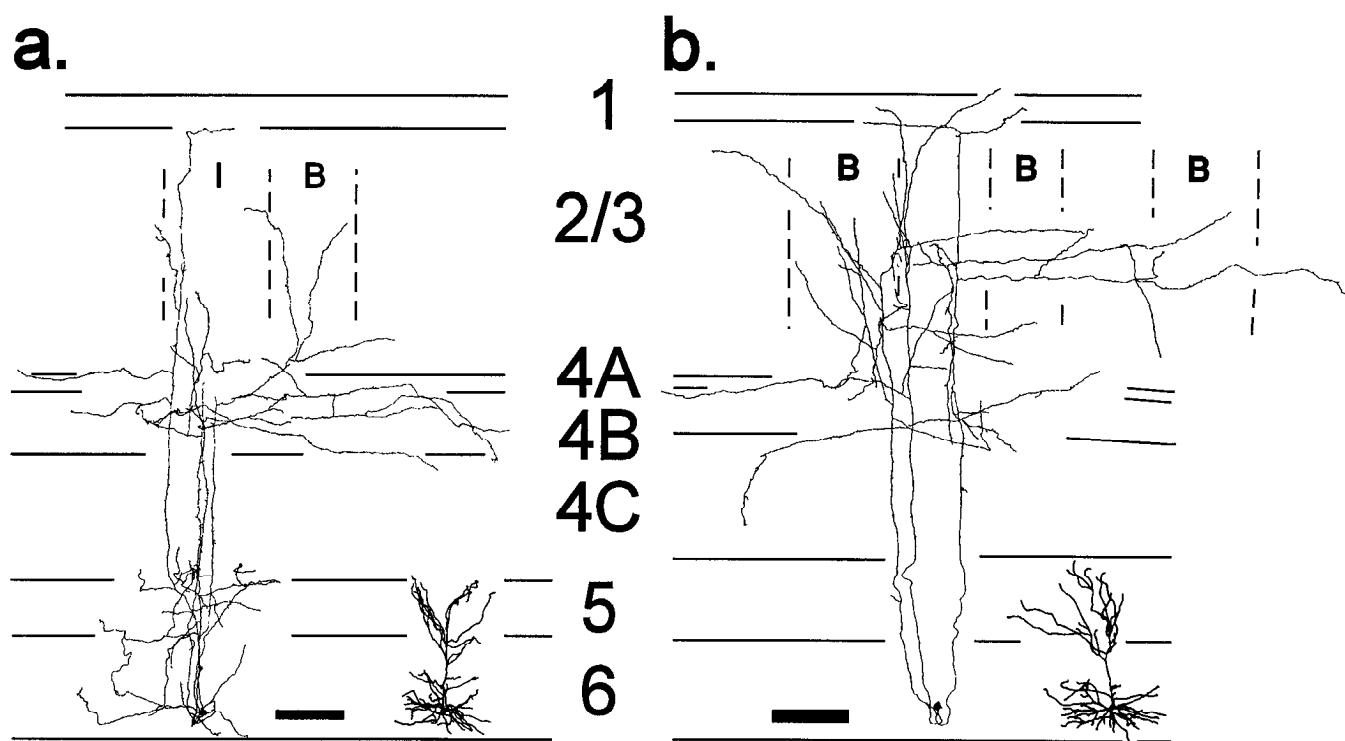


Figure 9. Camera lucida reconstructions of type IIB neurons. These neurons have widespread axonal projections that avoid layer 4C. Within layer 4, axonal branching occurs primarily in layer 4B. Axons of both neurons also project to blobs and interblobs of layer 2/3 and reach layer 1. The cell in *a* also projects to layers 5 and 6. The apical dendrites of these cells do not extend past layer 5 where they branch profusely. The somata of these cells are in deep layer 6. These are nonprojection neurons. See previous figures for conventions. Scale bars, 200 μ m.

Blasdel et al. (1985), but retrograde labeling in layer 6 after layer 4B injections (Yoshioka et al., 1994) suggested its existence. Most class II cells project to layer 2/3, with no specificity for blob or interblob regions. They, along with some type I β A and IC neurons, are probably responsible for layer 2/3 projections observed after anterograde tracer injections in layer 6 (Blasdel et al., 1985).

Type IIA cells can be found throughout the depth of layer 6, but the 27% (7/26) that project to the white matter are located predominantly in midlayer 6. Thus, they probably do not target the LGN (see Fitzpatrick et al., 1994). Because claustral projecting cells are found in midlayer 6 in the cat (LeVay and Sherk, 1981), we suggest this is the likely target of type IIA projection

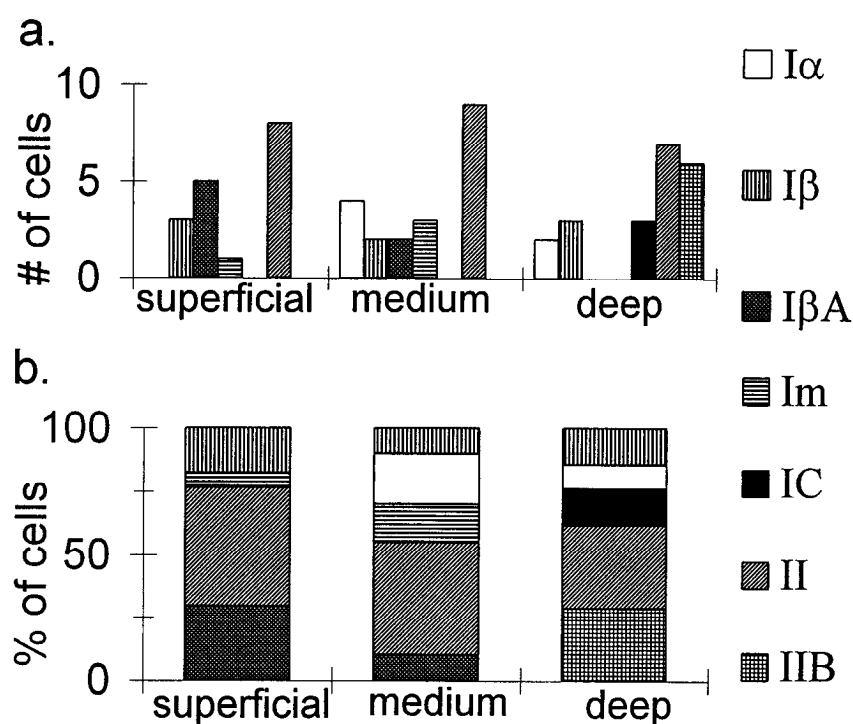


Figure 10. Depth distribution of the different types of layer 6 neurons. *a*, Bar graph of the number of cells belonging to different types versus the depth of the cell body in layer 6 (superficial, medium, or deep). *b*, Bar graph of the percentage of cells of each type found at a particular depth in layer 6. For both graphs, each type of layer 6 neuron is represented by a different fill pattern (see legend on the right).

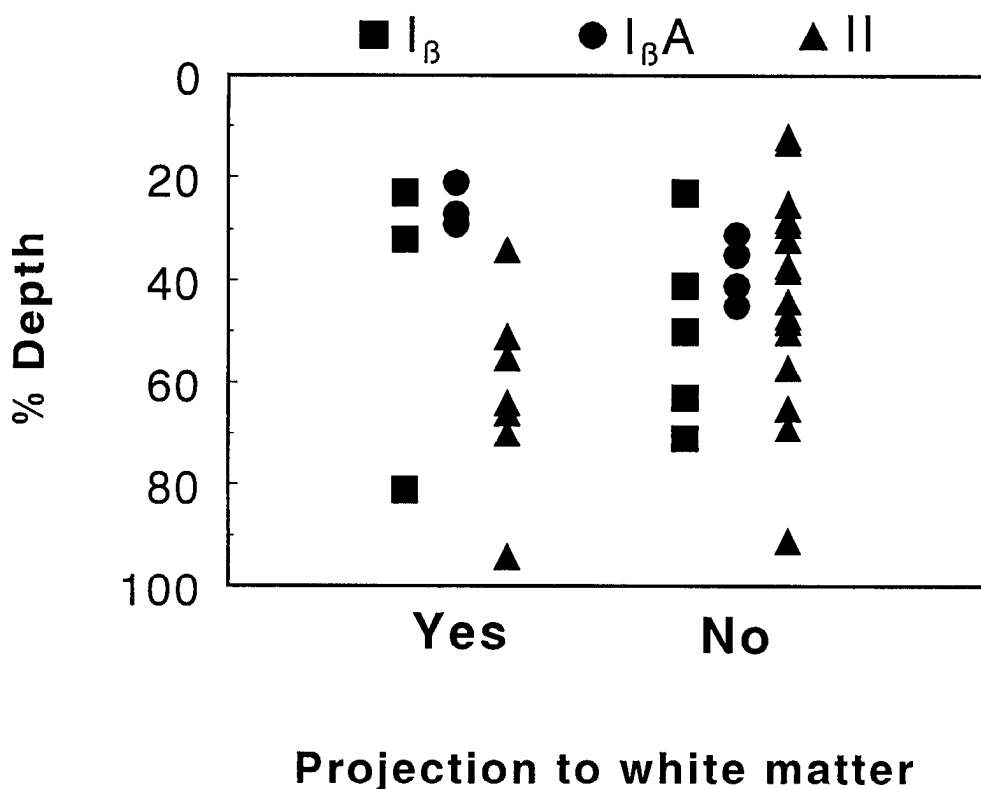


Figure 11. Depth distribution of projection and nonprojection neurons belonging to types containing both categories. This scatter plot shows the depths of individual layer 6 cells of types I_{β} (squares), $I_{\beta A}$ (circles), and II (triangles) grouped according to whether they project to the white matter (Yes) or not (No). Types I_{α} , I_m , and I_{IB} are not included because none project to the white matter. Type I_C cells are not included because all three in our sample project to the white matter and are located deep in layer 6. For type I_{β} , the projection neurons are superficial or deep, whereas nonprojection neurons are predominantly in midlayer 6. For type $I_{\beta A}$, the most superficial neurons also project to the white matter, whereas slightly deeper cells toward the middle of layer 6 do not. For type II, projection neurons are predominantly in the middle of layer 6, but nonprojection cells are found at all depths.

neurons. This possibility is supported by the similarity between type II neurons and claustral-projecting neurons in the cat; both project axons predominantly to the infragranular layers (Katz, 1987).

Functional roles of different neuron types

The differences between neuronal types suggest that they play different roles in shaping the response properties of V1 neurons. In considering our results, we have made a number of observations that suggest that layer 6 neurons in primates are specialized for functions that have been proposed based on results from cats (Hubel and Wiesel, 1965; Sherk and LeVay, 1983; Bolz and Gilbert, 1986; Sillito et al., 1994). We have therefore adapted these to the macaque monkey.

Role of class I cells

We propose that class I neurons contribute to the emergence of orientation selectivity by synchronizing the activity of layer 4C cells with circular receptive fields. This is analogous to the role proposed for the projection from layer 6 to the LGN in the cat (Sillito et al., 1994). In support of this notion, Sillito et al. have reported (Society for Neuroscience Meeting, 1994) that synchronous activation of layer 4C neurons is generated with long bars of light but not spots. Furthermore, inactivation of infragranular layers reduces the orientation selectivity of superficial neurons in Galago monkeys (Allison et al., 1995). Because the only orientation-selective neurons (those activated by bars but not spots) in V1 that project strongly to layer 4C are class I, layer 6 neurons, we propose that they are responsible for generation of synchrony in layer 4C.

If in the monkey the required synchrony can be generated in layer 4C, a strong corticogeniculate projection comparable with that in the cat may not be required. Consistent with this possibility, Fitzpatrick et al. (1994) estimate that only 13% of layer 6

neurons project to the LGN. Nine of our 58 cells are class I projection neurons. If we assume that these all project to the LGN and that type II projection neurons do not (see above), we obtain a value of 16%.

This hypothesis leads to testable predictions. Class I neurons should have short receptive fields (see below), and the positive feedback role implies that they should connect to spiny neurons. Also, axonal projections within layer 4C might be elongated along a topographic axis that corresponds to the orientation selectivity of the layer 6 neurons.

Role of class II cells

We hypothesize that class II neurons are involved in end-stopping, either via a cortico-claustral-cortical loop (Sherk and LeVay, 1983) or via local circuits (Hubel and Wiesel, 1965; Bolz and Gilbert, 1986). Regardless of how the effect is mediated, each model requires involvement of layer 6 neurons with long receptive fields. We suggest that class II neurons are likely to have long receptive fields. These could result from input from long-distance projections originating in layers 5 and 2–4B (Blasdel et al., 1985) onto the extensive dendritic branches of class II neurons in layer 5B [see Bolz and Gilbert (1989) for discussion of the generation of long receptive fields]. This hypothesis is also supported by the likelihood that type II neurons project to the claustrum (see above) and the observation that claustral-projecting cells in the cat have long receptive fields (Grieve and Sillito, 1995). Also, the intrinsic projections of class II neurons are focused on layers containing orientation selective neurons; connections to such cells are important for the local circuit version of the hypothesis (Bolz and Gilbert, 1986) (see also below).

The hypothesis that class II neurons are involved in end-stopping leads to testable predictions. First, class II cells

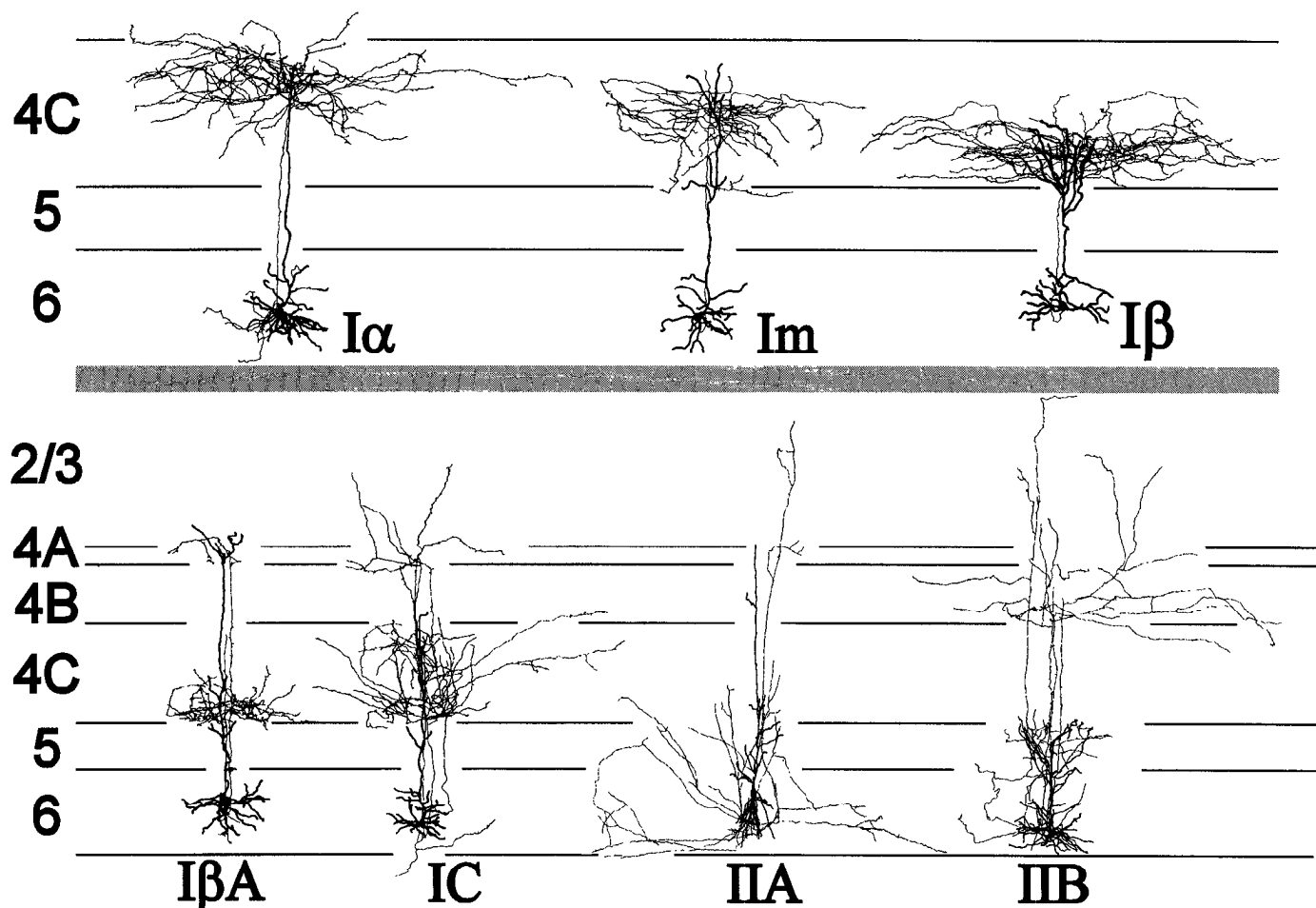


Figure 12. Summary diagram of the seven types of layer 6 neurons identified. The neuronal sketches are modified (simplified) camera lucida drawings. The type of each cell is indicated *beneath* or at the *bottom right* of each sketch. Layers are indicated by *numbering* at the *left side* of the figure. The *top* of the figure shows schematic representations of the three types of class I neurons that do not project above layer 4C. The *bottom* shows schematics of the four types of layer 6 neurons that project axons more superficially. The top and bottom of the figure are separated by a *thick gray line*, which also delimits the boundary between layer 6 and the white matter for the top portion, and the boundary between layer 2/3 and layer 1 for the bottom portion. Dendritic arbors (*bold lines*) are indicated at their actual positions, superimposed over the axonal arbors (*finer lines*).

should have long receptive fields. Second, they should project to the claustrum. Although our results suggest this is the case, more direct approaches could be used. Finally, the local projections of class II neurons should be onto inhibitory interneurons. This could be tested by electron microscopic analyses of the neurons that receive input from class II cells, or by functional analyses of the sources of input to inhibitory interneurons using photostimulation (Callaway and Katz, 1993; Dalva and Katz, 1994).

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