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Morphology of Physiologically Identified Retinal X and Y Axons in the Cat's Thalamus and Midbrain as Revealed by Intraaxonal Injection of Biocytin

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ABSTRACT

Prior morphological studies of individual retinal X and Y axon arbors based on intraaxonal labeling with horseradish peroxidase have been limited by restricted diffusion or transport of the label. We used biocytin instead as the intraaxonal label, and this completely delineated each of our six X and 14 Y axons, including both thalamic and midbrain arbors. Arbors in the lateral geniculate nucleus appeared generally as has been well documented previously. Interestingly, all of the labeled axons projected a branch beyond thalamus to the midbrain. Each X axon formed a terminal arbor in the pretectum, but none continued to the superior colliculus. In contrast, 11 of 14 Y axons innervated both the pretectum and the superior colliculus, one innervated only the pretectum, and two innervated only the superior colliculus. Two of the Y axons were quite unusual in that their receptive fields were located well into the hemifield ipsilateral with respect to the hemisphere into which they were injected. These axons exhibited remarkable arbors in the lateral geniculate nucleus, diffusely innervating the C-laminae and medial interlaminar nucleus, but, unlike all other X and Y arbors, they did not innervate the A-laminae at all. In addition to these qualitative observations, we analyzed a number of quantitative features of these axons in terms of numbers and distributions of terminal boutons. We found that Y arbors contained more boutons than did X arbors in both thalamus and midbrain. Also, for axons with receptive fields in the contralateral hemifield (all X and all but two Y axons), 90–95% of their boutons terminated in the lateral geniculate nucleus; the other two Y axons had more of their arbors located in midbrain.

Indexing terms: lateral geniculate nucleus, superior colliculus, pretectum, terminal arbors, intracellular labeling

Recent advances in neuroanatomical techniques have permitted the reconstruction and visualization of single axons and their individual terminal arbors (see, e.g., Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Humphrey et al., 1985a,b; Sur et al., 1987; Tamamaki et al., 1988; Uhlrich and Cucchiaro, 1992). This is achieved either through intracellular labeling of a single axon or by extracellular placement of a label that completely delineates a small enough number of axons to reconstruct individuals. When analyzed at the light microscopic level, such reconstructions offer important and unique information, including the distribution of terminal boutons from a single axon, the extent to which a single axon's projection pattern conforms to that of its parent pathway, potential differences among subtypes of projection axon within a pathway, variations in projection pattern of similar axons, and the course and branching patterns of individual axons. Electron microscopy can extend this by revealing the types of synaptic contact and postsynaptic target associated with individual axons.

One limitation to most morphological studies of single axons is that they tend to be limited to single termination

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zones, yet many axons branch to innervate multiple zones. Many studies of retinofugal projections in cats serve as an example of this limitation. Despite the fact that retinal axons commonly branch to innervate the thalamus and midbrain (Hoffmann, 1973; Fukuda and Stone, 1974; Kelly and Gilbert, 1975; Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Sawai et al., 1985; Sur et al., 1987), virtually all of these studies have focused their quantitative analyses on the terminal branches within the lateral geniculate nucleus (Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Sur et al., 1987). This is because these earlier studies used horseradish peroxidase (HRP) as an intraaxonal label, and the diffusion of HRP through the axon is limited by its large molecular size (King et al., 1989; Imai and Aoki, 1993). Thus, whereas geniculate terminations could be quantitatively analyzed, they required limited diffusion of label from the injection site in the subjacent optic tract, and branches from these axons directed towards the midbrain were not routinely and completely labeled. Interestingly, one study (Bowling and Michael, 1984) qualitatively described one retinal Y axon that branched to innervate the lateral geniculate nucleus, pretectum, and superior colliculus; another study (Sur et al., 1987) pointed out that every one of their intraaxonally labeled retinal axons, including the thinner X axons, branched in the optic tract, with one branch terminating in the lateral geniculate nucleus and the other heading towards the midbrain.

We recently discovered that the use of biocytin as an intracellular label has many advantages over HRP. The main advantage, for the purposes of the present study, has to do with the fact that the smaller biocytin molecules readily diffuse within the entire axonal arborization over distances of at least several cm, even for the thinner X axons.[1] Thus, we found that retinal axons injected in the optic tract could be reconstructed throughout their entirety in both thalamus and midbrain. This permitted us to address a number of questions for the first time. We could determine the extent to which the two main retinal axonal types, X and Y, innervate both thalamus and midbrain and how these types might differ. We could determine for the first time what midbrain sites (e.g., superior colliculus and/or pretectum) are targets of these axons. We could determine whether there is any correlation within axons between terminal patterns in thalamus and midbrain. We could estimate the entire terminal output of single axons and determine how these output numbers relate between zones. That is, does a larger arbor in thalamus imply a larger one in midbrain because some axons are very successful in developing and maintaining terminations? Conversely, does a larger arbor in thalamus imply a smaller one in midbrain because there is a sort of "conservation" of terminals that each axon supports? Perhaps there is no correlation between these arbor sizes. In the process of addressing these questions, we also applied our more sensitive labeling techniques to confirm the basic patterns described previously with HRP.

[1]The other main advantage is in electrophysiological recording. The smaller biocytin molecule means that smaller electrode tips can be used while still allowing iontophoresis. This makes them more suitable for intracellular penetration. There is also less clogging and polarization of the tips with biocytin. Finally, the biocytin solution provides a better electrolyte than do the solutions typically used with HRP, so the recording electrodes have lower resistance and less noise.

MATERIALS AND METHODS

Nine cats were used for this study. Our general techniques have been published in full elsewhere (Sur and Sherman, 1982; Tamamaki et al., 1984, 1988; Humphrey et al., 1985a,b; Sur et al., 1987) and are briefly summarized below. The cats were deeply anesthetized with intravenous injections of sodium pentobarbital (initially 15 mg/kg, with 5–10 mg supplements as needed), cannulated, intubated, and placed in a stereotaxic apparatus where they were paralyzed with 5 mg of gallamine triethiodide, artificially respired, and prepared for surgery. We continuously monitored heart rate and end-tidal CO_2, which we kept at 4% ± 0.2%. We maintained the rectal temperature at 38°C via a feedback-controlled heating blanket. Wound edges and pressure points were periodically infused with 2% lidocaine. The cats were kept anesthetized with 0.4–1% halothane in a 1:1 mixture of N_2O and O_2, and they were kept paralyzed with intravenous infusion of gallamine triethiodide (5 mg/kg/hour) and d-tubocurarine (0.35 mg/kg/hour). EEG and blood pressure were monitored. To dilate the pupils and retract the nictitating membranes, we topically applied atropine sulfate and phenylephrine hydrochloride. The corneas were covered by contact lenses chosen by slit retinoscopy to focus the retinas on a white wall or a cathode ray tube in front of the eyes. We made a craniotomy and reflected the dura to allow penetration of bipolar stimulating electrodes, which we placed across the optic chiasm at A14 and L1.0–1.5. We set their depth by maximizing the potential recorded across them and evoked by light flashed into the eyes. We used these electrodes to stimulate recorded retinal axons orthodromically. Another craniotomy was made bilaterally and centered over the anterior and lateral regions of the lateral geniculate nucleus (roughly A6 and L9) to enable penetration of the recording and labeling electrode.

Electrophysiological recording and intraaxonal labeling

The recording and iontophoretic electrode was a glass micropipette with a tip diameter < 0.5 μm. Electrodes were used to penetrate, record intracellularly from, and inject biocytin into retinal axons within the optic tract. Electrodes were filled with a solution of 6% biocytin (Sigma) dissolved in 0.5 M KCl and had an impedance of 20–40 MΩ. We aimed penetrations through the anterior and lateral regions of the lateral geniculate nucleus because the subjacent optic tract is relatively thick. Furthermore, it is easier there to locate parent trunks of retinal axons before they branch to innervate various thalamic and midbrain targets.

Abbreviations

Lam. A	lamina A of the lateral geniculate nucleus
Lam. A1	lamina A1 of the lateral geniculate nucleus
Lam. C	lamina C of the lateral geniculate nucleus
Lam. C1	lamina C1 of the lateral geniculate nucleus
LGN	lateral geniculate nucleus
MIN	medial interlaminar nucleus of the lateral geniculate nucleus
PT	pretectum
SC	superior colliculus
SGS	stratum griseum superficiale of superior colliculus
ZO	stratum zonale of superior colliculus

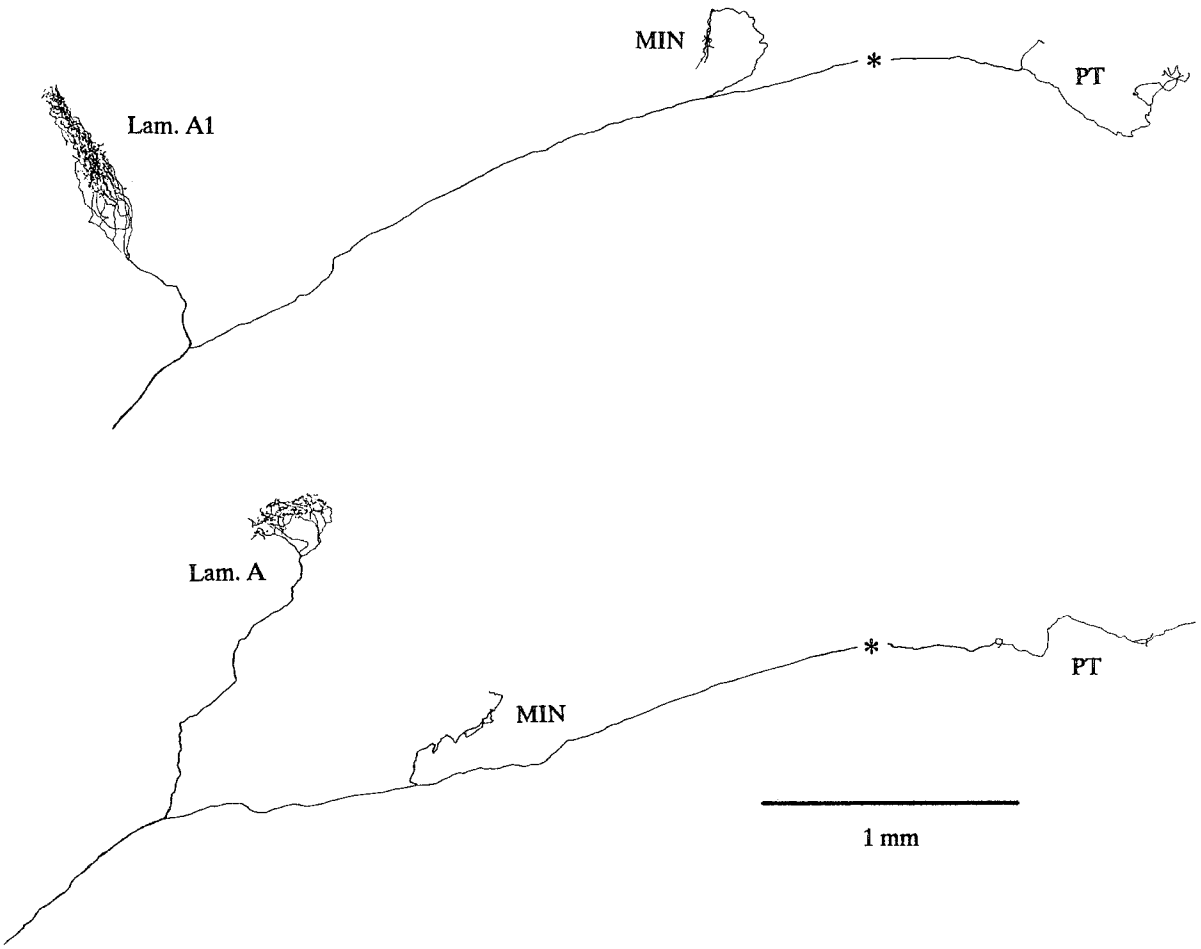


Fig. 1. Reconstructions, in the coronal plane, of retinal X axons that innervated the lateral geniculate nucleus and pretectum. The asterisks in each reconstruction indicate a gap not shown as each axon coursed through the brachium of the superior colliculus without branching. **Top:** Axon originating from the ipsilateral eye with receptive field (azimuth, elevation) of +20°, −2.0°. **Bottom:** Axon originating from the contralateral eye with receptive field of +2.0°, −10°.

We first recorded extracellularly from a retinofugal axon, plotted its receptive field with small visual stimuli, noted the eye that drove it, and determined various receptive-field characteristics, such as the center type (i.e., on or off), the linearity of spatial and temporal summation in response to a sinusoidal grating stimulus, the strength of the surround response and antagonism, the tonicity of the center response, and the responsiveness to large, fast-moving targets. The axon's response latency to optic chiasm stimulation was also measured. These various response properties were used to identify each axon as X or Y.

We then attempted to impale the axon by a brief mechanical shock to the stereotaxic apparatus. This was verified by a large (> 50 mV) drop in the DC voltage and large action potentials (> 30 mV in amplitude). The axon's physiological properties were quickly rechecked to ensure that we were inside the same axon that we studied extracellularly. Biocytin solution in the glass micropipette was injected by applying nitrogen gas pressure (Tamamaki et al., 1984, 1988). We gradually increased nitrogen gas pressure from 0 to 3 kg/cm^2 for several seconds. Ejection of the solution into the axon was monitored via electrode impedance (Tamamaki et al., 1988): the ejection of the solution was accompanied by more than a 20% drop in electrode impedance.

Tissue preparation

After a 20–36 hour survival period, the animal was given a lethal, intravenous injection of sodium pentobarbital and transcardially perfused with saline and aldehydes (4% paraformaldehyde, 0.5% glutaraldehyde, and 0.1 M phosphate buffer at pH 7.4). We removed the brain and postfixed it overnight with the same fixative solution, and we then immersed it in 20% sucrose with phosphate buffer for 2 days. Serial frozen sections were taken at a thickness of 40 μm in the coronal plane, and these were immersed in phosphate buffered saline with 0.3 Triton-X 100 for 2 days at 4°C. Retinas were also removed in two experiments, and these were postfixed. We applied the "ABC" reaction (Vector stain kit, following the enclosed instructions), using cobalt-intensified diaminobenzidine, to detect biocytin in the sections and retinas. Some sections were counterstained with cresyl violet. We usually injected several axons per animal, but always ensured that each had receptive field properties making it easy, based on ocular dominance and

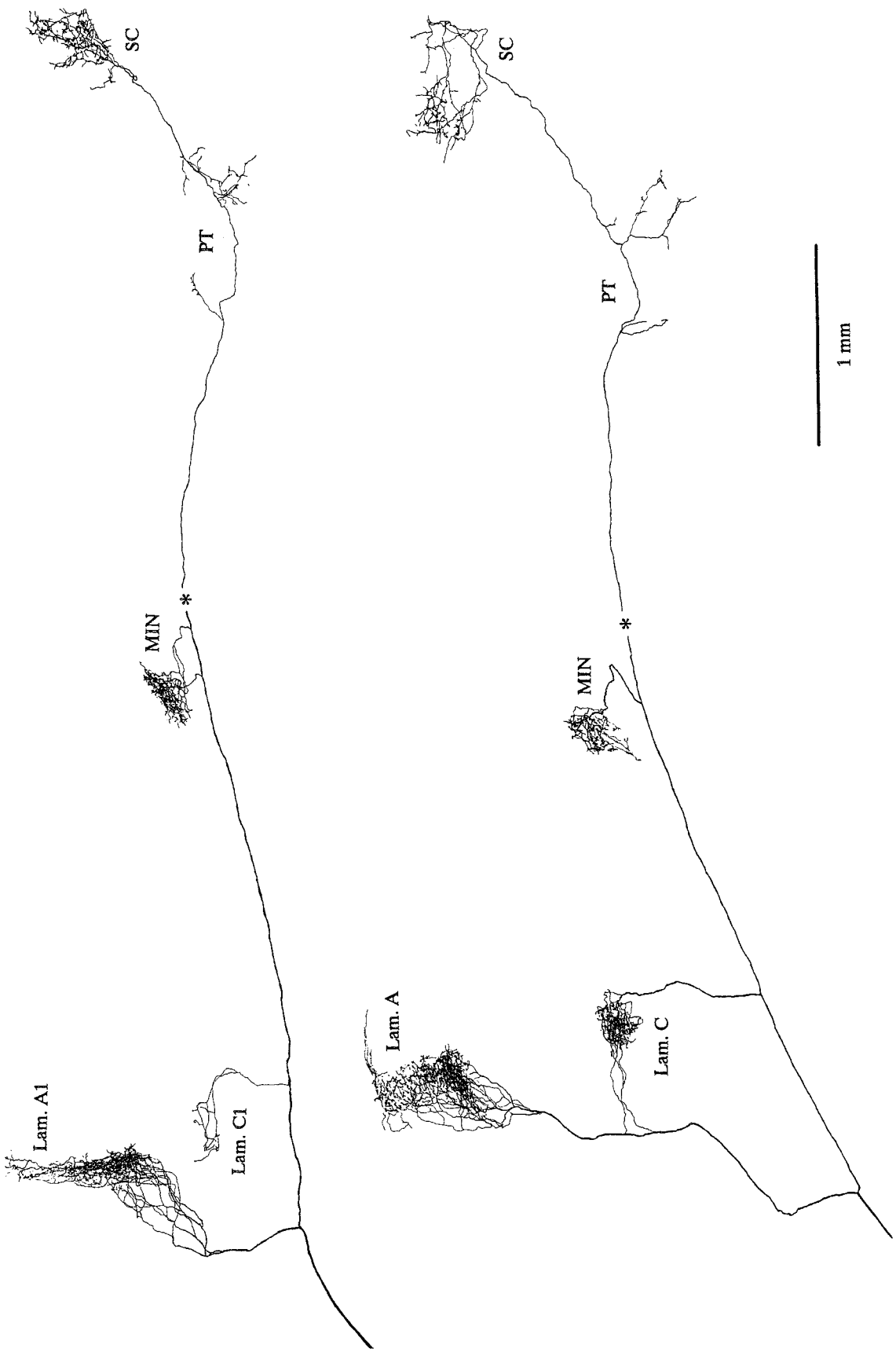


Fig. 2. Reconstructions, in the coronal plane, of Y axons that innervated the lateral geniculate nucleus, pretectum, and superior colliculus; conventions as in Figure 1. **Top:** Axon originating from the ipsilateral eye with receptive field of +17°, −7.5°. **Bottom:** Axon originating from the contralateral eye with receptive field of +3.5°, −3.0°

retinotopic mapping, to identify the recovered arbor with the recorded axon (cf. Sur et al., 1987).

Axon reconstructions

We used a camera lucida attached to a microscope with a ×40 or ×100 oil immersion objective for our detailed morphological analysis. Biocytin-filled axons were reconstructed in their entirety and in three dimensions by tracing them through serial sections. Synaptic boutons were noted in terminal arbors and plotted separately.

RESULTS

We successfully impaled, intracellularly labeled, and recovered 20 retinal axons (6 X and 14 Y), and these form the basis of this report. One of the X axons was too lightly labeled to obtain detailed quantitative data concerning distribution of its terminal boutons in the pretectum, although qualitatively it seemed like the other X axons and clearly innervated the same targets. All of the other X and Y axons were quite darkly labeled throughout and could easily be reconstructed through multiple sections. Thus, we limited quantitative analysis to 5 X and 14 Y axons. We encountered no retinal W axons. The electrophysiological properties of the labeled X and Y axons were similar to those described previously (see, e.g., Bowling and Michael, 1984; Sur et al., 1987), and these, in turn, are indistinguishable from other retinal X and Y cells reported in the literature (Enroth-Cugell and Robson, 1966; Cleland et al., 1971; Cleland and Levick, 1974; Hochstein and Shapley, 1976a,b; Bullier and Norton, 1979; Sherman and Spear, 1982; Rodieck and Brening, 1983; Sherman, 1985). Therefore, these physiological properties are not discussed in detail here.

Qualitative analysis of labeled axons

The intracellular biocytin technique produced much more extensive labeling of the retinal axons than has been reported previously (Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Sur et al., 1987), presumably because prior studies used horseradish peroxidase (HRP) as the intracellular tracer for labeling (see opening paragraphs). Every one of our labeled axons not only innervated the lateral geniculate nucleus but also innervated the pretectum and/or superior colliculus. As was noted in the opening paragraphs, there have been previous hints from HRP labeling that at least some retinal axons may branch to innervate both thalamus and midbrain (Bowling and Michael, 1984; Sur et al., 1987). The better biocytin labeling allows us to deduce for the first time that all of these retinal axons indeed innervate the midbrain. Also, with the exception of one axon (see above), the labeling was quite dark, even within the finest terminals, and there was no sign of fading of the label as the terminal arbors were reached. This suggests that we were able to visualize complete retinofugal terminal arbors for these axons.

The labeled axons were traced back from the injection site towards the retina, and to the optic chiasm in some cases. We found no branching throughout these proximal regions, and no terminations were found in the suprachiasmatic nucleus. It thus appears that all branching occurs distal to the injection site. For this reason, we did not include proximal portions in our detailed reconstructions.

In previous studies (Sur and Sherman, 1982; Sur et al., 1987) from this laboratory in which HRP was used as the intraaxonal label, excellent retrograde labeling of retinal ganglion cells was achieved. In the present study, we removed and processed retinas after two experiments (see Materials and Methods) involving at least ten labeled axons. We found no retrogradely labeled retinal ganglion cells. Thus, despite the excellent orthograde labeling seen after intraaxonal injection of biocytin and the retrograde labeling often seen after extracellular biocytin injections (King et al., 1989; unpublished observations), it appears that biocytin does not transport well retrogradely when placed inside an axon, although, as noted above, we could trace the axons retrogradely for a limited distance.

Figures 1 and 2 illustrate camera lucida reconstructions of representative axons from our material. Figure 1 shows two X axons, one projecting ipsilaterally (top) and the other, contralaterally (bottom). Figure 2 shows analogous examples of Y axons, also projecting ipsilaterally (top) and contralaterally (bottom). These reconstructions represent the coronal plane, and the considerable third dimension along the rostrocaudal axis is collapsed (see figure legends for details). Each of the axons branched in the optic tract. One or more of the branches ascended to innervate the lateral geniculate nucleus, both the laminated portion (i.e., the A- and C-laminae) as well as the medial interlaminar nucleus, while a final branch continued medially and posteriorly. This final branch for the X axons terminated exclusively in the pretectum (see Fig. 1). In the Y axons shown in Figure 2, the final branch innervated the pretectum and the superior colliculus. In our material, every one of the six X axons innervated the lateral geniculate nucleus plus the pretectum but not the superior colliculus. Every one of the 14 Y axons also innervated the lateral geniculate nucleus and midbrain, but, in the midbrain, 11 of them innervated both pretectum and superior colliculus, one innervated only the pretectum, and the final two innervated only the superior colliculus (see also below). We can also conclude from the dark labeling of these axons at their terminals that no X or Y axon innervates other retinofugal targets, such as the ventral division of the lateral geniculate nucleus or the accessory optic nucleus. Finally, the branching pattern within the optic tract was as originally described by Sur et al. (1987): Retinal Y axons formed two thick branches, each as thick as the parent axon, one branch innervating the lateral geniculate nucleus and the other innervating the midbrain; retinal X axons formed one medium-caliber branch, as thick as the parent axon, to innervate the lateral geniculate nucleus and one exceedingly thin branch to innervate the midbrain.

Terminal arbors in the lateral geniculate nucleus. With few exceptions, noted below, the morphology of the X and Y terminal arbors in the lateral geniculate nucleus is essentially as described previously from HRP labeling studies (Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Sur et al., 1987). Figure 3 shows representative photomicrographs of a Y axon to illustrate this point. Boutons of X arbors tended to occur in grape-like clusters with prominent gaps between clusters, whereas those of Y axons were more diffusely distributed throughout the terminal arbor. All X axons from the contralateral retina innervated geniculate lamina A, and those from the ipsilateral retina innervated lamina A1. Retinal Y axons from the contralateral retina targeted laminae A and C, whereas those from the ipsilateral retina innervated laminae A1 and C1. The projection to lamina C1 by Y axons was very sparse, but it was clearly present in all four Y axons from the ipsilateral

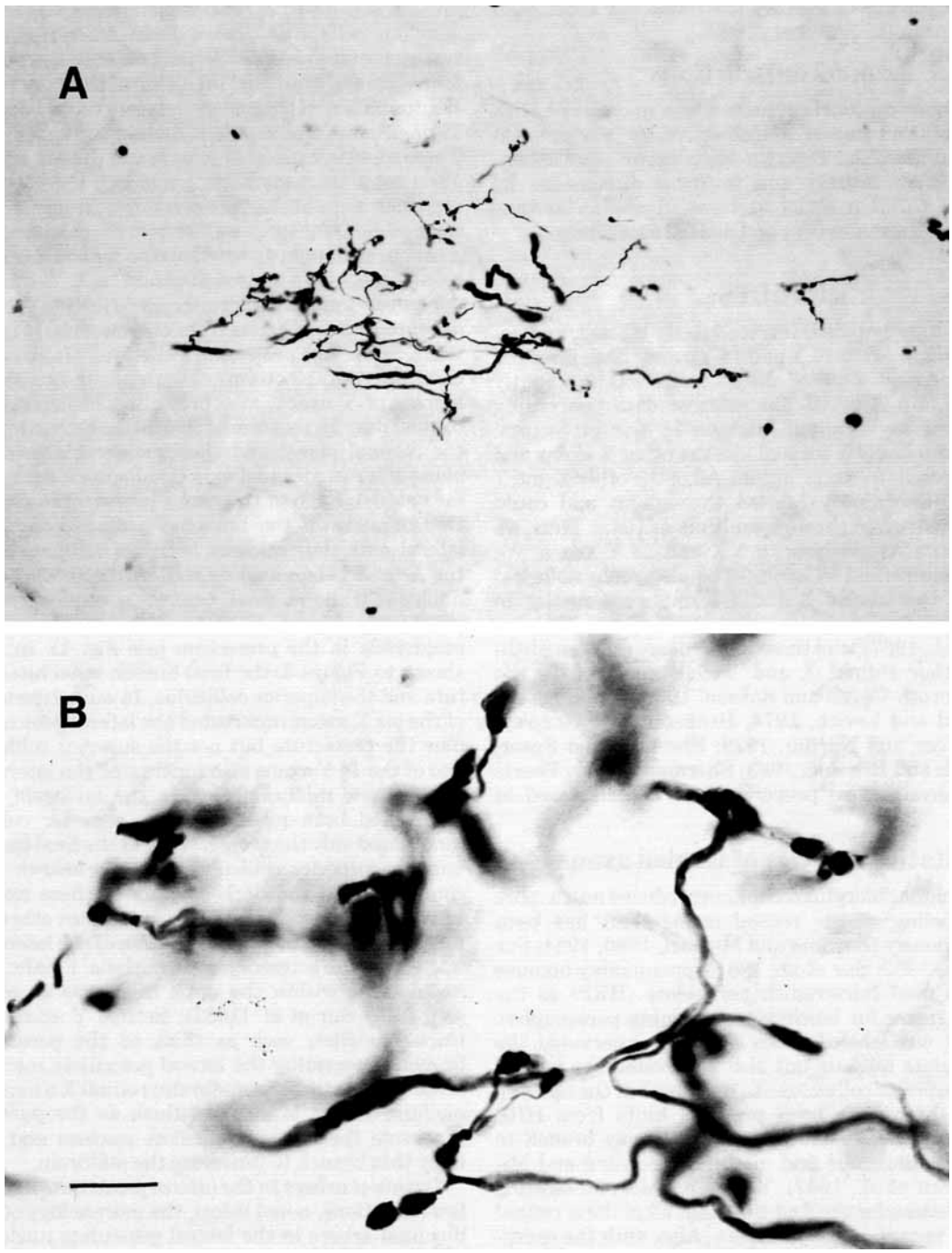


Fig. 3. **A,B:** Photomicrographs of part of a terminal arbor from a labeled retinal Y axon. The axon originated from the contralateral eye, and the view is located in lamina C of the lateral geniculate nucleus. A: Lower power view. B: Higher power view. Scale bar = 10 μm for B, 50 μm for A.

retina. This represents one difference from that of previous studies, where Y axon innervation of laminae C1 was rarely observed (Bowling and Michael, 1984) or not seen at all (Sur et al., 1987). Every Y axon and five of the X axons projected to the medial intralaminar nucleus, with the Y axons providing a larger innervation (see below under Quantitative analysis of labeled axons). Because we found little new to report regarding retinogeniculate arbors, the rest of this report focuses on midbrain arbors.

Terminal arbors in the pretectum. As was noted above, every labeled retinal axon projected a branch towards the midbrain. In the pretectum, axon collaterals formed relatively sparse, unelaborated terminal arbors with large boutons. These boutons occurred mostly en passant. Figure 4 shows a photomicrograph from a typical retinal arbor, in this case from a Y axon, in the pretectum.

Each of the X axons in our sample was labeled in the pretectum, five of the six quite darkly (see above), and

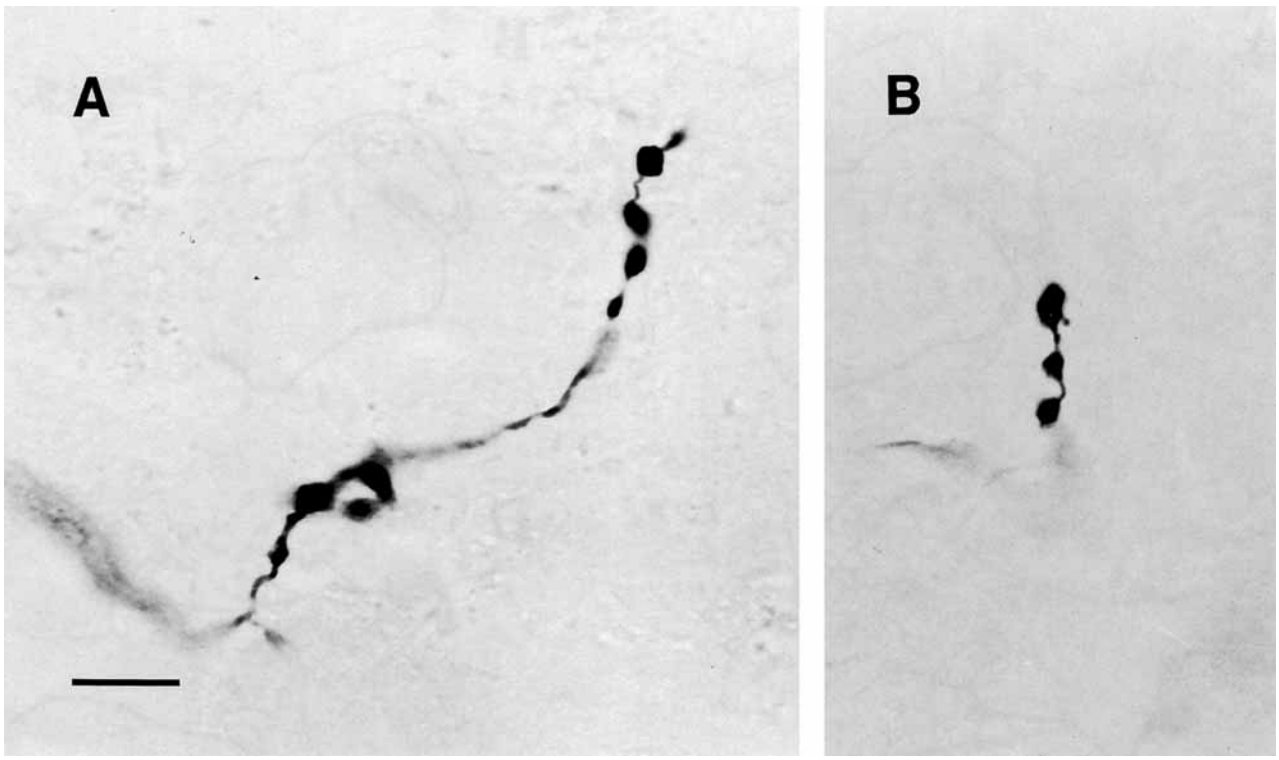


Fig. 4. **A,B:** Two photomicrographs from nearby sections of terminal boutons in the pretectum from a retinal axon, in this case a Y axon. Scale bar = 10 μm.

terminated there; none continued toward the superior colliculus. Figure 5 illustrates the pretectal terminal fields of four retinal X axons. In each example, the axon emerged from the optic tract and entered the pretectum, where it branched sparingly and produced a small number of boutons (see below under Quantitative analysis of labeled axons). The precise boundaries of the pretectal nuclei are unclear in our tissue and are thus not marked on the illustrations. However, most or all of the terminal boutons in the pretectum appear to fall within the nucleus of the optic tract. Figure 6 illustrates the pretectal terminal arbors of three Y axons. As was noted above, 12 of the 14 Y axons innervated the pretectum via axon collaterals as they projected toward the superior colliculus; the top and bottom axons of Figure 6 exemplify this pattern. However, the middle axon in Figure 6 illustrates the only Y axon of our sample that innervated the pretectum without continuing to the superior colliculus. Two additional Y axons (not illustrated) did not form axon collaterals in the pretectum as they projected to the superior colliculus. Each of these three Y axons, one innervating pretectum but not superior colliculus and two innervating superior colliculus but not pretectum, was quite darkly labeled, and we are reasonably confident that our failure to detect innervation of both midbrain targets is not artifactual.

The boutons of both X and Y axons in the pretectum tended to be located in two or three small clusters. Because the retinal axons travel mediocaudally through this region, the clusters are located at different levels both mediolaterally and rostrocaudally in the pretectum. The rostrocaudal extent of the pretectal axons is thus lost in the reconstructions illustrated in Figures 5 and 6. Figures 7 and 8 represent an attempt to illustrate both this clustering and the complete extent of retinal arbors in the pretectum. Figure 7, which portrays the pretectal arbors of two Y axons that are different from those illustrated in Figure 6, shows the individual clusters. As shown, individual Y axons may innervate the pretectum throughout its rostrocaudal extent, from rostral levels near the lateral geniculate nucleus to caudal levels bordering the superior colliculus. Figure 8 illustrates two additional Y axons that innervated much of the rostrocaudal extent of the pretectum. However, these axons terminate very medially in the pretectum, unlike the other examples that we have illustrated. It is interesting that the receptive fields of the two axons in Figure 8 were located in the upper visual field, while the receptive fields of all of the other illustrated axons were located in the lower visual field. These results are consistent with reports that the pretectum is retinotopically organized along its mediolateral extent for visual field elevation (Graybiel and Berson, 1980; Koontz et al., 1985; Kubota et al., 1987); the upper visual field is represented medially, and the lower visual field is represented laterally.

Figure 9 illustrates a dorsal view of two or three labeled terminal arbors from three separate experiments. Each of the axons displays one to three separated terminal clusters. Note that the clusters from the axons, especially those in Figure 9C, line up to form diagonal bands of terminals. It is interesting that these bands are similar in orientation and separation to those described in the pretectum following tracer injections in the eye (Hoffmann et al., 1984; Koontz et al., 1985).

All of the axons illustrated in Figure 9 have ventrally located visual receptive fields, and all of these axons are

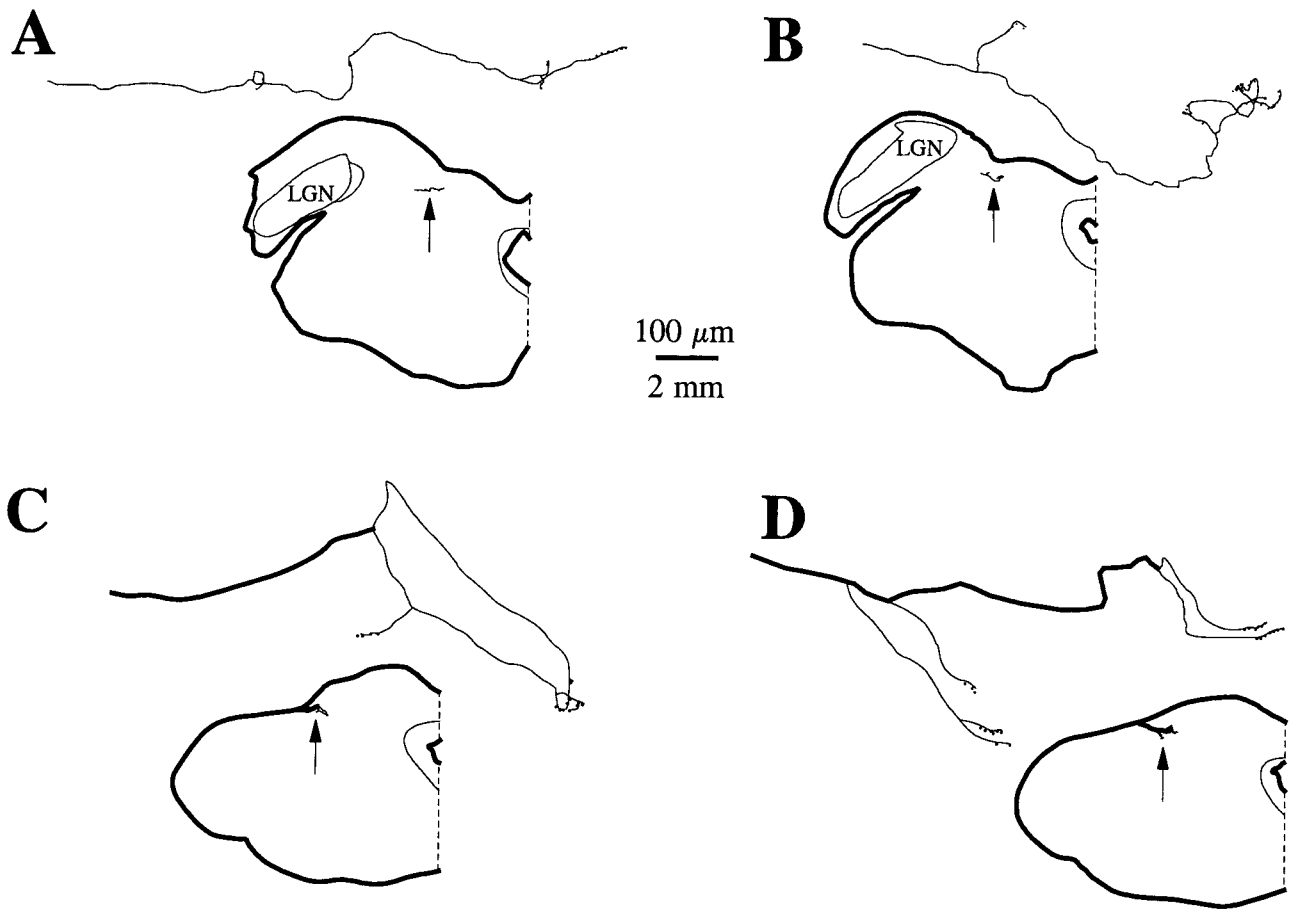


Fig. 5. Reconstructions of terminal arbors in the pretectum of four X axons. Each reconstruction is shown in the coronal plane and includes a higher power reconstruction of the terminal arbor (scale = 100 μm) next to a lower power reconstruction (scale = 2 mm) to illustrate the location of the arbor in the pretectum. **A,C:** Axons from the contralateral eye. The receptive fields were +2.0°, −10°for A and +17°, −19°for C. **B,D:** Axons from the ipsilateral eye. The receptive fields were +20°, −2.0°for B and 0°, −19.5°for C.

located in a relatively lateral position in the pretectum. This is consistent with the known retinotopic organization of the pretectum (Graybiel and Berson, 1980; Koontz et al., 1985; Kubota et al., 1987). Because there are two or three arbors in each experiment shown in Figure 9, we can further analyze the retinotopy of this projection. When we do, we see that the retinotopic organization for visual elevation in the pretectum is not very precise. In Figure 9A, the arbor with the lowest visual receptive field is located most medially, along with an arbor whose visual receptive field was located 20° higher. The third arbor, whose receptive field was located at an elevation between the first two arbors, is positioned more laterally. In Figure 9B, the expected relationship exists, in that the axon with the higher receptive field is located in a more medial position. However, in Figure 9C, the reverse occurs. The axon with the most medial arbor had the lowest receptive field location, whereas the axon with the most lateral arbor had the highest receptive field elevation.

We uncovered no organization related to visual field azimuth. For example, the receptive field azimuths of the axons in Figure 10 varied widely, but there is no obvious position of the termination that reflects this. Rather, the terminals from axons of differing receptive field azimuths seem simply to line up in a terminal band, and their position in the band is determined roughly by receptive field elevation. Figure 10 further illustrates the lack of precise retinotopic organization in the pretectum by comparing this in the horizontal plane for two Y axons labeled in the same experiment. The axon with the receptive field location of +17° elevation and −7.5° azimuth is the same one as shown in the coronal plane in the right half of Figure 7. Note that these axons of Figure 10, which have quite different receptive field locations, terminate in discrete arbors in the superior colliculus with loci in keeping with retinotopic organization there (Feldon et al., 1970), yet their arbors in the pretectum are diffuse and substantially overlapped.

The third dimension in the pretectum, depth from the pial surface, also fails to relate systematically either to any receptive field locations of the axons or to their functional characteristics. Figure 11 illustrates this for the same two Y axons as were labeled in Figure 10, but in the coronal plane. These two Y axons, which enter the pretectum at different depths, terminate in substantially overlapped terminal

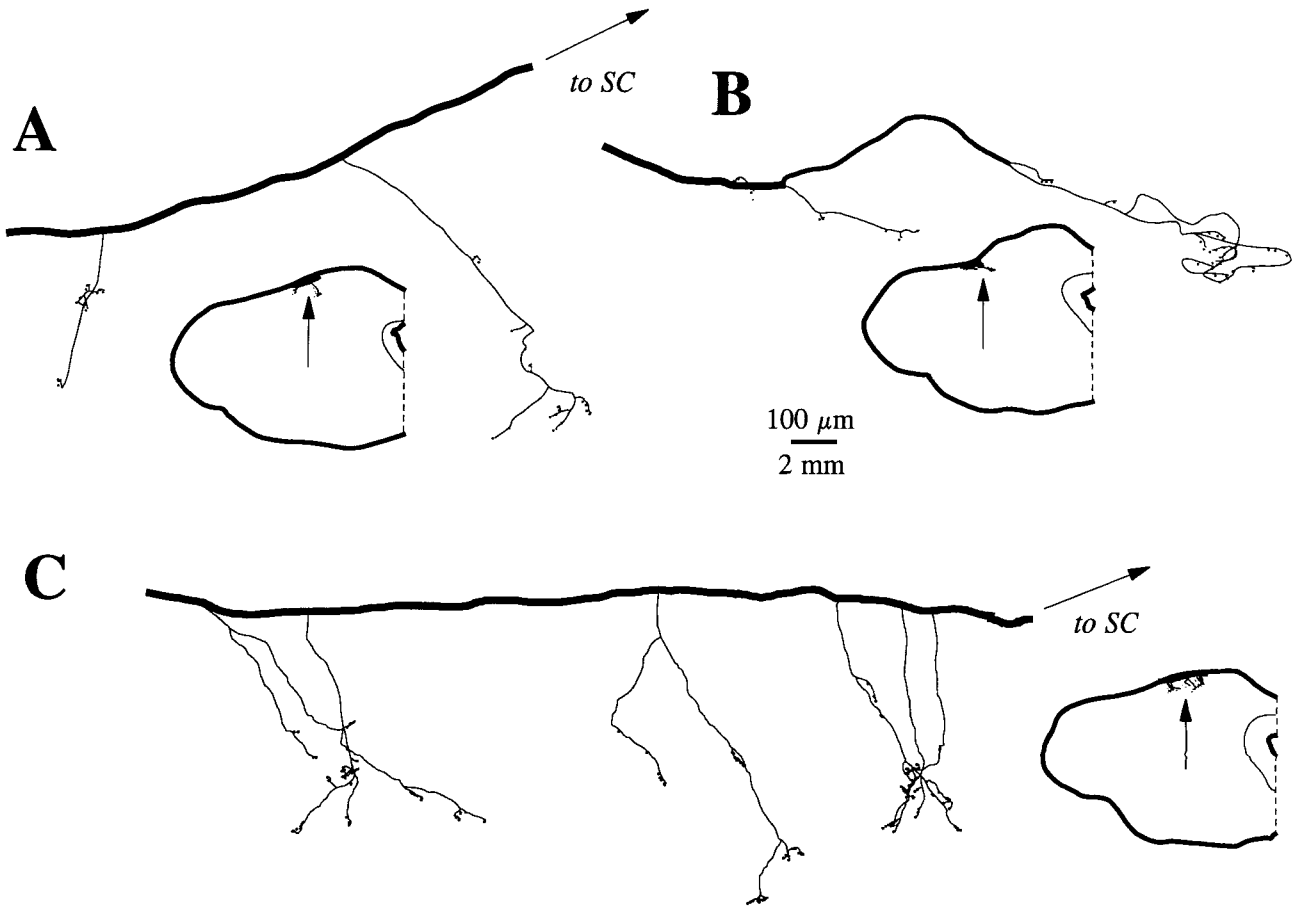


Fig. 6. Reconstructions of terminal arbors in the pretectum of three Y axons; conventions as in Figure 5. **A:** Axon from the contralateral eye with receptive field located in the hemifield contralateral (+35°, +38°) to the recording site. **B:** Axon from the ipsilateral eye with receptive field located in the hemifield contralateral (+10°, −1.0°) to the recording site. **C:** Axon from the contralateral eye with receptive field located in the hemifield ipsilateral (−12°, −3.0°) to the recording site.

arbors. This is best illustrated by the actual bouton distributions shown in the bottom half of Figure 11.

Terminal arbors in the superior colliculus. As was noted above, none of the retinal X axons that innervated the lateral geniculate nucleus innervated the superior colliculus, but 13 of the 14 retinal Y axons did so. After entering the superior colliculus, the Y axons ran parallel to the elevation lines of the retinotopic map within the superior colliculus, and they formed collaterals that branched to form the terminal arbor (see Fig. 10). Figure 12 shows a photomicrograph through two of these arbors. Boutons within these arbors were located predominantly en passant, although smaller boutons occasionally appeared at the end of short stalks (Fig. 12C). The terminal arbors themselves displayed considerable heterogeneity: Some arbors terminated in a simple, rod-like shape (see, e.g., Fig. 12A), while others innervated the superior colliculus with a more extensive arbor (see, e.g., Fig. 12B). Figures 13 and 14 illustrate representative Y arbors in the superior colliculus from the contralateral retina (Fig. 13) and ipsilateral retina (Fig. 14). Arbors from the contralateral retina varied more. They included the smallest and most extensive terminal arbors that we have observed in the superior colliculus.

The primary laminar target of all 13 Y arbors that innervated the superior colliculus was the stratum griseum superficiale, mainly in the ventral half of this lamina, and six of these extended additional arbor branches ventrally into the dorsal portion of the stratum opticum. Examples of such arbors are shown in Figures 13E–G and 14B,C. Four other axons terminated at least partly in the dorsal half of the stratum griseum superficiale, and examples of these are found in Figures 13A,B,D (axons that formed the smallest collicular arbors in our sample) and 14A,B.

The position of the arbor was estimated from the contour of the reconstructed superior colliculus and superimposed on the collicular map as determined by Feldon et al. (1970). Within the limits of our methodology, we detected no discrepancy between the published retinotopic maps and our reconstructions. Also, as expected, the location of the arbors of individual axons in both the lateral geniculate nucleus and superior colliculus occupied the same location in terms of retinotopic maps of the two structures.

Terminal arbors representing the ipsilateral hemifield. Prior anatomical (Guillery et al., 1980; Rowe and Dreher, 1982) and physiological (Sanderson and Sherman, 1971; Lee et al., 1984) studies showed a small but significant

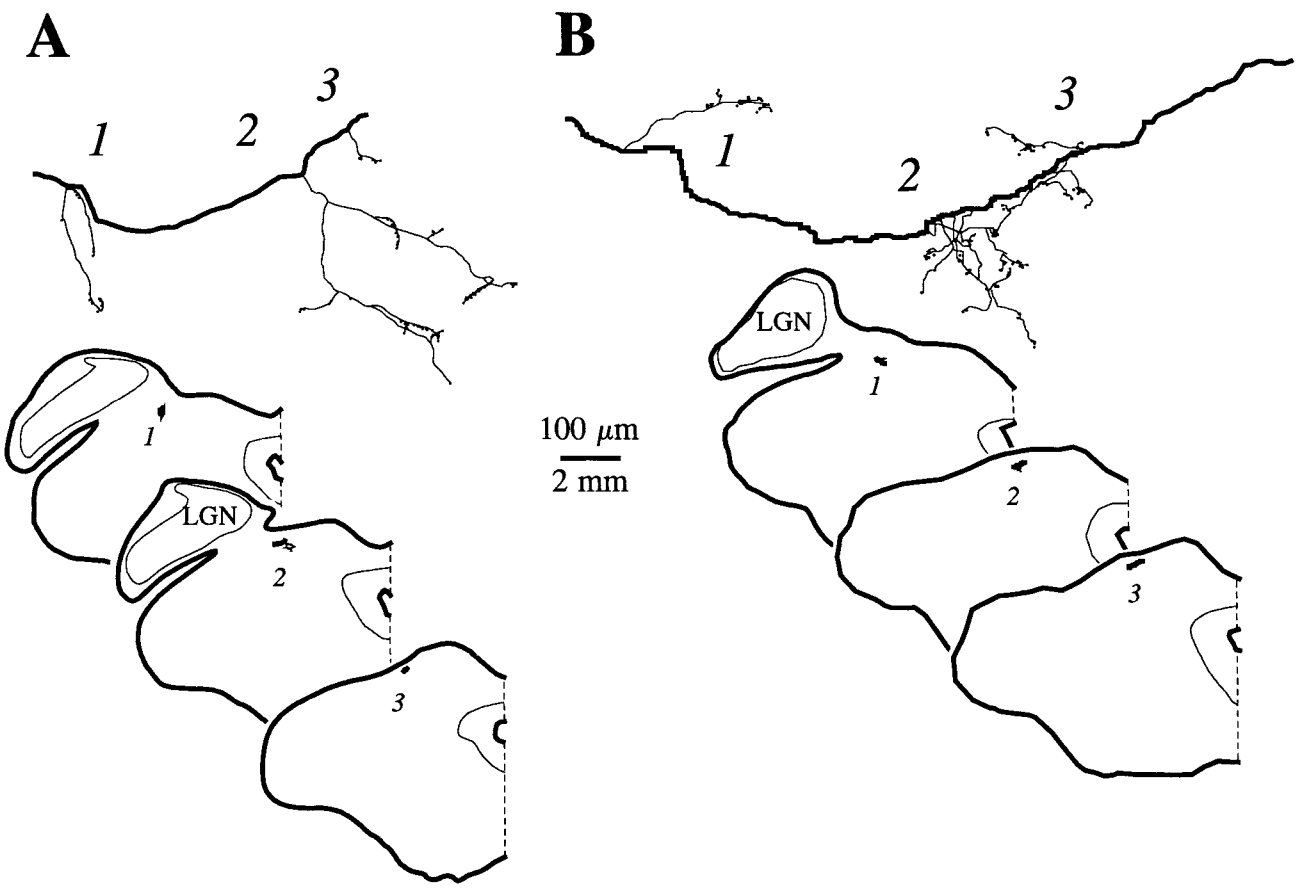


Fig. 7. Reconstructions of terminal arbors in the pretectum of two Y axons; conventions as in Figure 5. Each axon gives rise to three terminal clusters. The lower power drawings indicate the coronal plane at which each cluster is located, and these are indicated by numbers. The top reconstruction in each lower power reconstruction is the most rostral. **A:** Axon from the contralateral eye with receptive field of +3.5°, −3.0°. **B:** Axon from the ipsilateral eye with receptive field of +17°, −7.5°.

retinofugal projection that crosses in the optic chiasm yet emanates from ganglion cells scattered throughout the entire temporal retina. This projection thus represents the temporal retina or nasal hemifield contralaterally. However, previous studies of individually labeled retinal axons failed to sample such axons (Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Sur et al., 1987). We did manage in the present study to label two such retinofugal axons, both Y. While the small sample precludes any definitive description of contralaterally projecting ganglion cells from the temporal retina, it seems worthwhile to describe these axons since they have never been described before.

Figure 15 shows that these axons exhibited a morphology markedly different from that of other retinal Y axons (e.g., compare with Fig. 2). Their densest projection in the thalamus terminated in the medial interlaminar nucleus. They sparsely and rather diffusely innervated the C-laminae, and, most strikingly, they failed to enter the A-laminae at all. Both of these axons were well labeled and could be traced to the superior colliculus, so the unusual morphology in the lateral geniculate nucleus cannot be attributed to incomplete labeling.

Several additional features of these axons bear elaboration. As noted in the preceding paragraph, the projection to the C-laminae is diffuse; in fact, it is surprisingly unrestricted. The terminal arbors of the two axons extended sparsely across 670 μm and 1,850 μm of horizontal extent, respectively, which is three and seven times wider than that seen in the most extensive of our other retinogeniculate Y arbors (again, compare with the Y axons illustrated in Fig. 2). Also, the projection in the C-laminae was not restricted to a single lamina. The example in Figure 15A spans the full dorsal-ventral extent of the C-laminae. As shown in the photomicrograph of Figure 16, this span includes lamina C3. This is particularly notable; although from the contralateral retina, these axons innervate all the C-laminae, including laminae C1, which is supposed to be innervated only from the ipsilateral retinae (Hickey and Guillery, 1974), and lamina C3, which is supposed to be devoid of retinal input (Hickey and Guillery, 1974; Torrealba et al., 1981). Presumably, the projection represented by these axons was too sparse to be distinguished from background labeling in prior anatomical tracing studies of the retinogeniculate pathway.

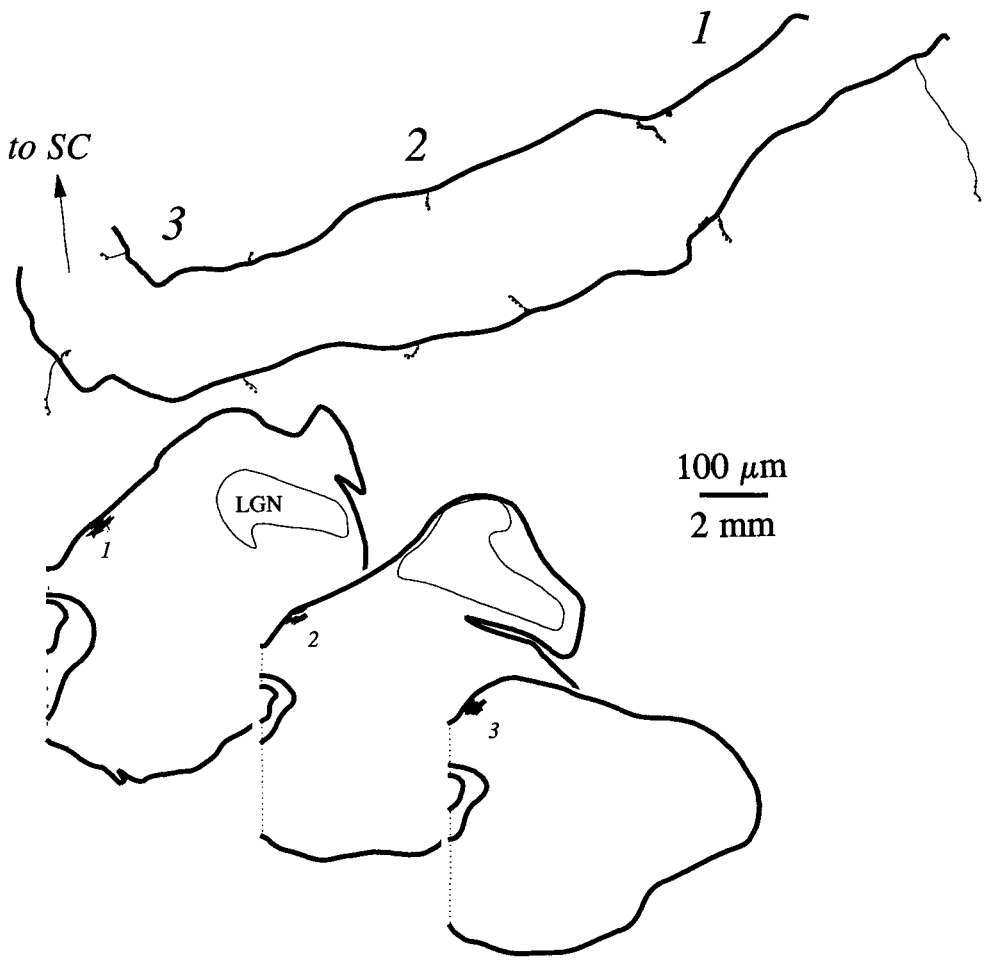


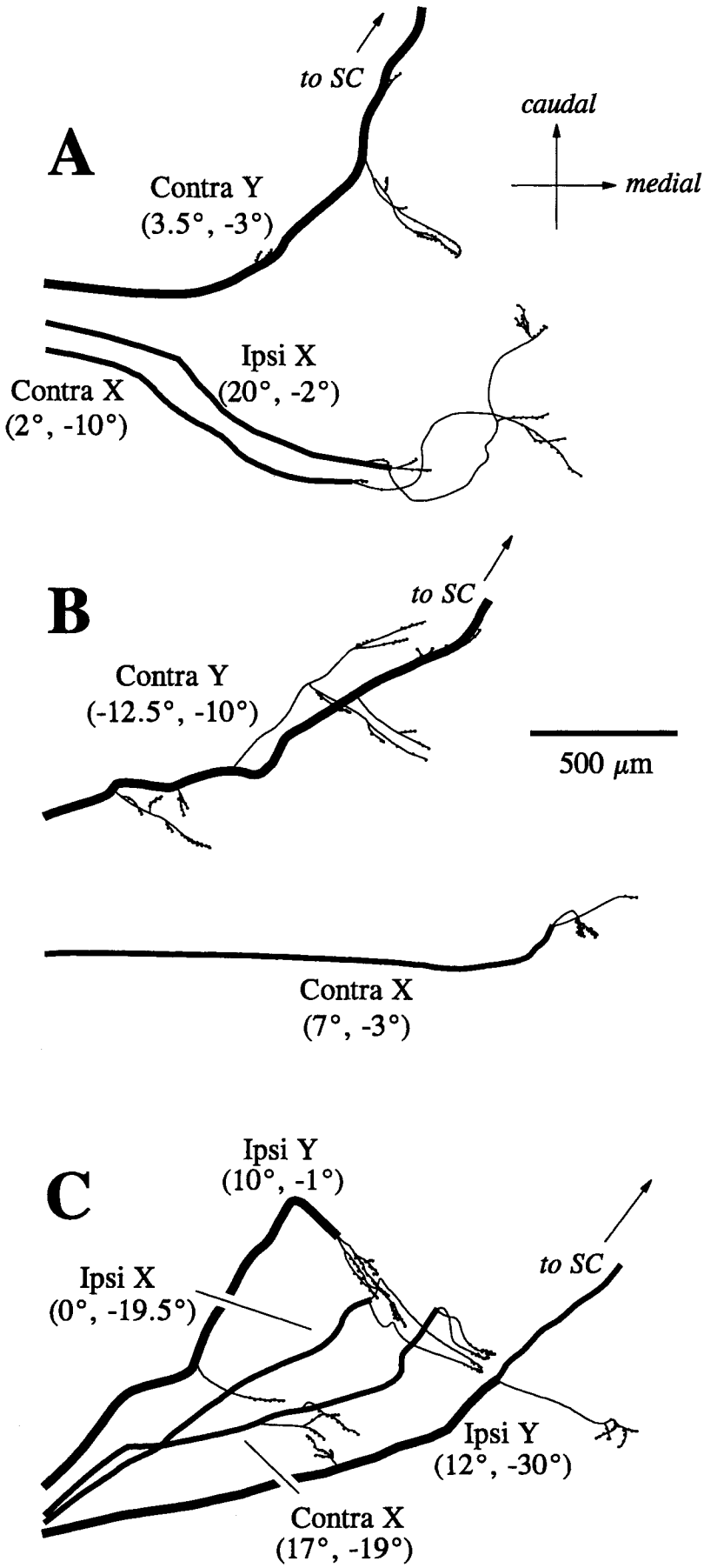
Fig. 8. Reconstructions of terminal arbors in the pretectum of two Y axons; conventions as in Figures 5 and 7. The upper axon had a receptive field of +35°, +38°, and the lower one had a receptive field of +22°, +31°.

Quantitative analysis of labeled axons

Our ability to visualize the entire terminal arbor of individual axons allows us to test certain features related to the number of terminal boutons. As was noted above, we were forced to omit one X axon from these quantitative analyses involving pretectum. Unless otherwise stated, we also limited our statistical analyses of Y axons to the 12 with receptive fields in the contralateral hemifield, since the aforementioned two mapping the ipsilateral hemifield seem to obey quite different rules of organization. However, for completeness, we include the Y axons representing the ipsilateral hemifield in the remaining graphs.

Overall extent of retinofugal arbors. We can extend earlier observations that Y axons produce more boutons in the lateral geniculate nucleus than do X axons (Bowling and Michael, 1984; Sur et al. 1987). We confirmed this for the lateral geniculate nucleus, where our sample of X axons produced 640 ± 162 (mean ± S.D., here and throughout) boutons and Y axons produced 2,183 ± 580 boutons ($P < 0.001$ on a Mann-Whitney U test). Within the lateral geniculate nucleus, Y arbors contributed more boutons to both the A-laminae and medial interlaminar nucleus (556 ± 212 vs. 1,341 ± 531, respectively; $P < 0.001$ on a Mann-Whitney U test for comparisons of both A-laminae and medial interlaminar nucleus). Also, every Y axon and none of the X axons provided some innervation of the C-laminae, although rare X axons have previously been described that project a small number of boutons to the C-laminae (Sur and Sherman, 1982; Sur et al., 1987).

We found the same difference in the midbrain arbors (i.e., total of pretectal and collicular arbors), where the X axons produced 32 ± 9 boutons and the Y axons produced 337 ± 212 boutons ($P < 0.001$ on a Mann-Whitney U test). On average, the 5 X axons analyzed produced 667 ± 173 boutons overall (i.e., the total of thalamic and midbrain boutons) compared to the 2,514 ± 695 produced by the Y axons ($P < 0.001$ on a Mann-Whitney U test). Finally, X axons terminated with a smaller percentage of their arbors (in terms of numbers of boutons) in the midbrain than did Y axons (5.1% ± 1.9% vs. 12.5% ± 6.8%; $P < 0.01$ on a Mann-Whitney U test). Figure 17 shows a weak, positive correlation ($r = +0.52$; $P < 0.02$) between the number of boutons in the lateral geniculate nucleus and midbrain when all axons are pooled, but there is no correlation for


A
to SC
caudal
medial
Contra Y
(3.5°, -3°)
Ipsi X
(20°, -2°)
Contra X
(2°, -10°)
B
to SC
Contra Y
(-12.5°, -10°)
500 μm
Contra X
(7°, -3°)
C
Ipsi Y
(10°, -1°)
Ipsi X
(0°, -19.5°)
to SC
Ipsi Y
(12°, -30°)
Contra X
(17°, -19°)

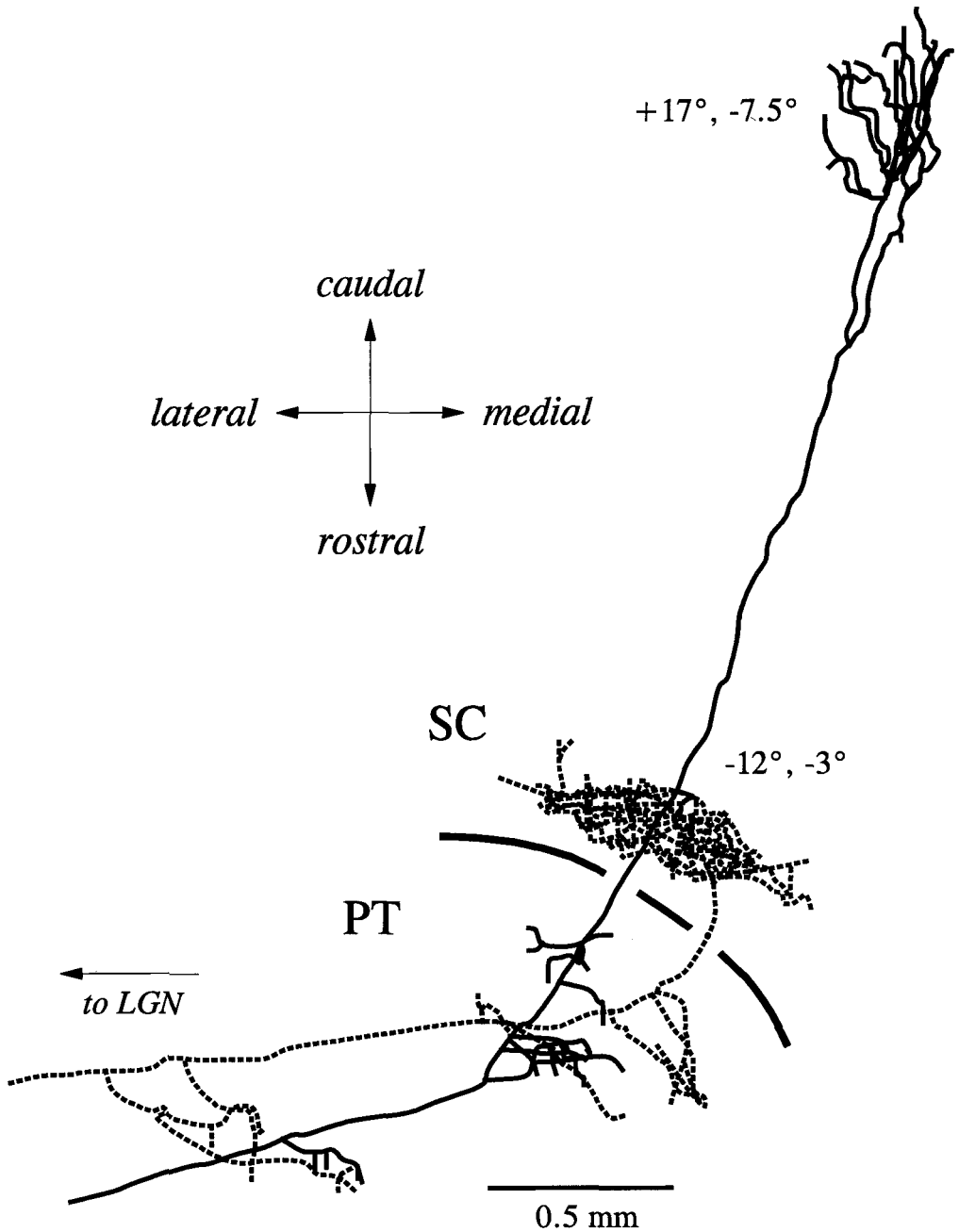


Fig. 10. Reconstructions, in the horizontal plane, of two Y axon arbors in the pretectum and superior colliculus. The arbors in the superior colliculus are widely spaced, but the arbors in the pretectum overlap considerably.

either subset of X or Y axons ($P > 0.1$ for either correlation). It is not clear if the lack of correlation for the separate X and Y subpopulations is due to their small numbers or to other factors.

Fig. 9. **A–C:** Results from three separate experiments in which two or three retinal axons were labeled and reconstructed in the pretectum. The view shown is a horizontal plane. The receptive field coordinates and axon type (X or Y) are shown for each axon. Note that the location of the arbors do not exhibit a precise retinotopic organization.

Relationships with eccentricity. In their previous study of retinogeniculate arbors, Sur et al. (1987) found that the size of Y arbors in terms of bouton numbers decreased with receptive field eccentricity, whereas X arbors showed no clear relationship between these variables. In the present study, we confirmed this observation: X arbors in the lateral geniculate nucleus showed no relationship with eccentricity ($r = +0.08$; $P > 0.1$), whereas Y arbors did ($r = -0.85$; $P < 0.001$), and the difference between correlations is statistically significant ($P < 0.001$). We were

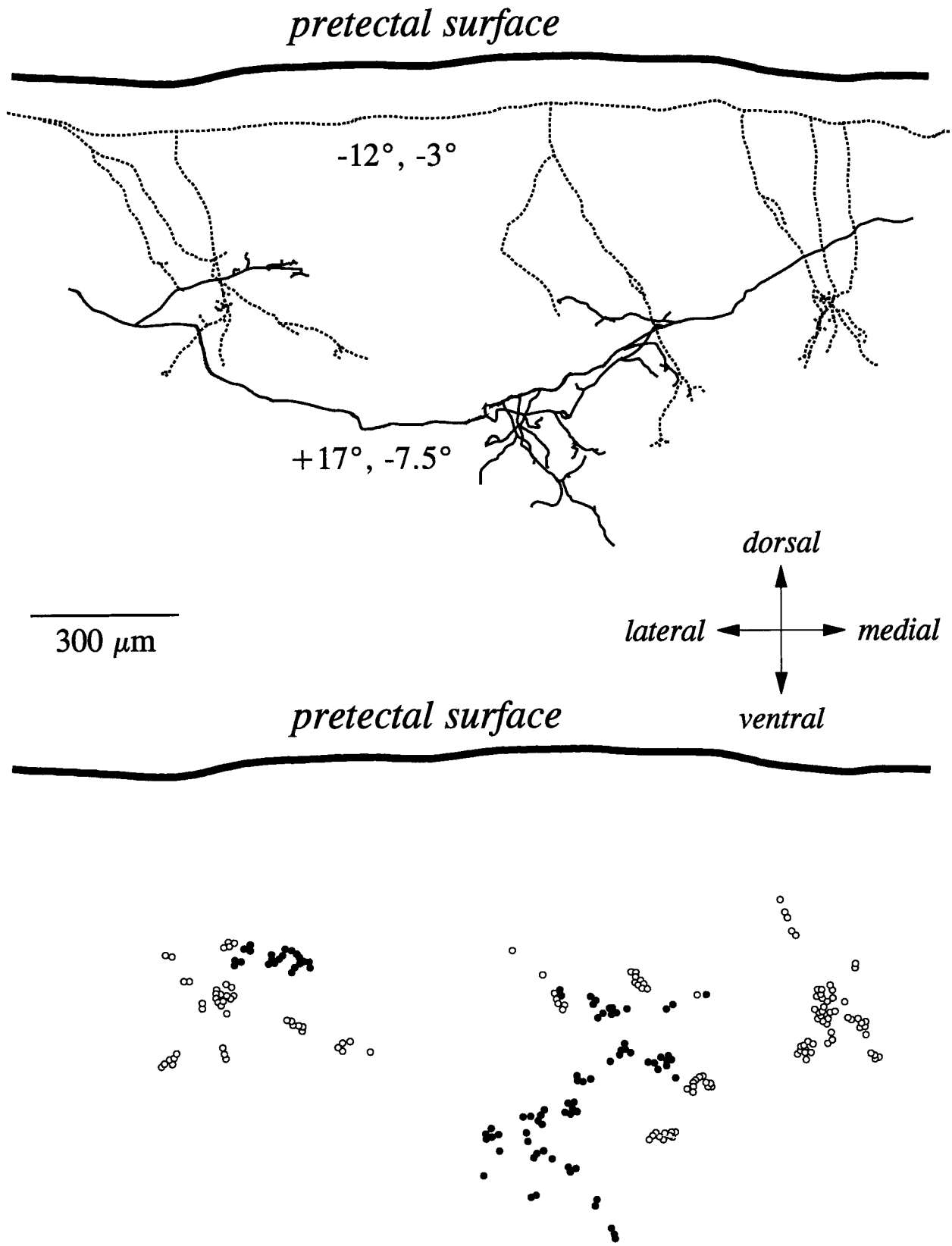


Fig. 11. Reconstruction, in the coronal plane, of the pretectal arbors of the same two Y axon arbors that are illustrated in Figure 10. **Top:** The illustration reconstructs the arbors relative to the pretectal surface. **Bottom:** The illustration shows the location of the boutons produced by these axons. The solid circles correspond to the boutons from the axon represented by the solid line, while the open circles indicate the boutons corresponding to the axon represented by the dashed line. Again, despite disparate receptive field coordinates (especially in visual field azimuth), the two bouton distributions overlap considerably throughout the depth of the pretectum.

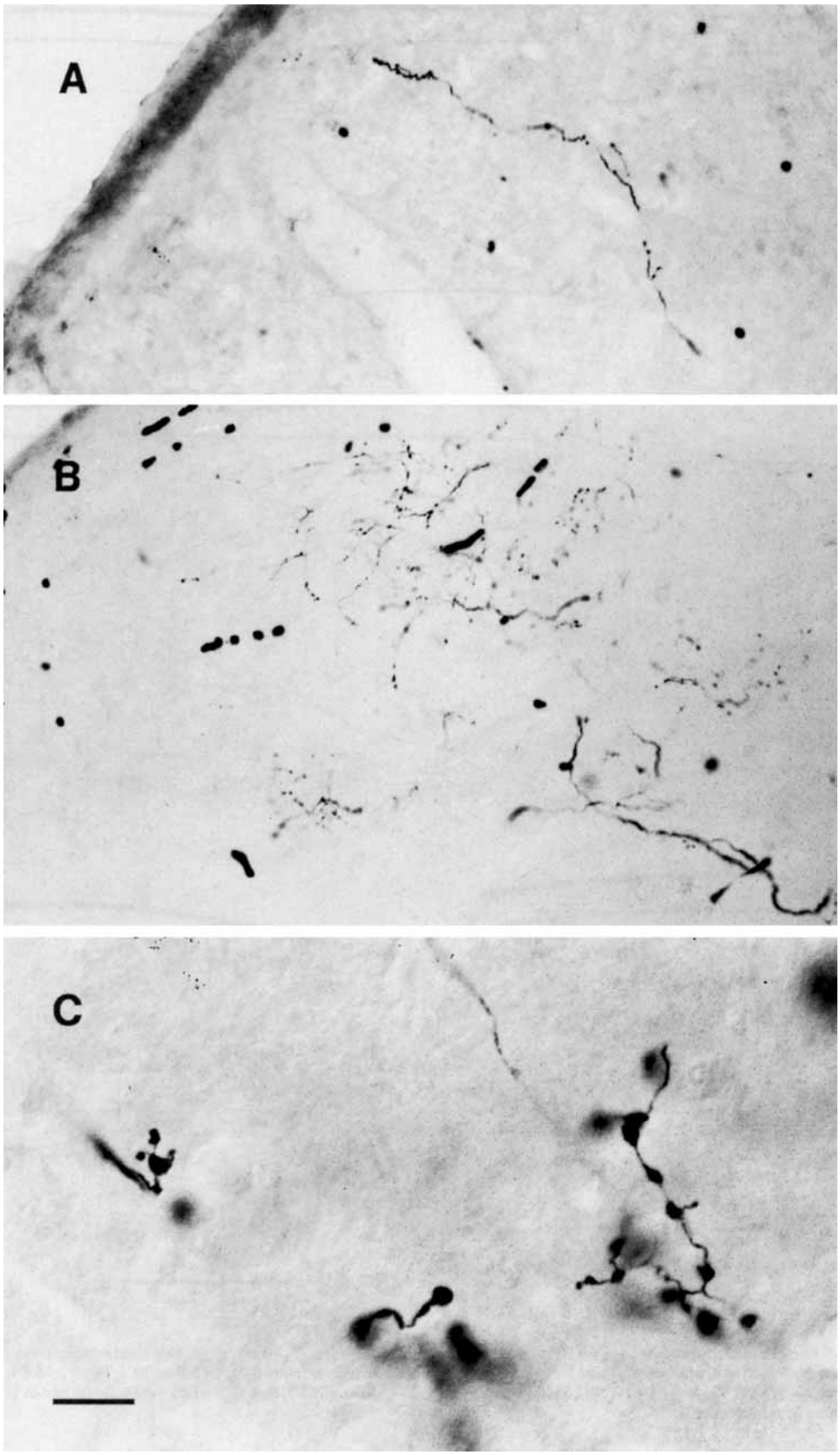


Fig. 12. Photomicrographs of Y axon terminal arbors in the superior colliculus. **A:** A rod-like terminal arbor from a contralateral axon. **B:** Lower power view of a more extensive terminal arbor from a contralateral axon. **C:** Higher power view of the arbor in B showing boutons mostly en passant with occasional smaller boutons appended. Scale bar = 10 μm in C, 50 μm in A,B.

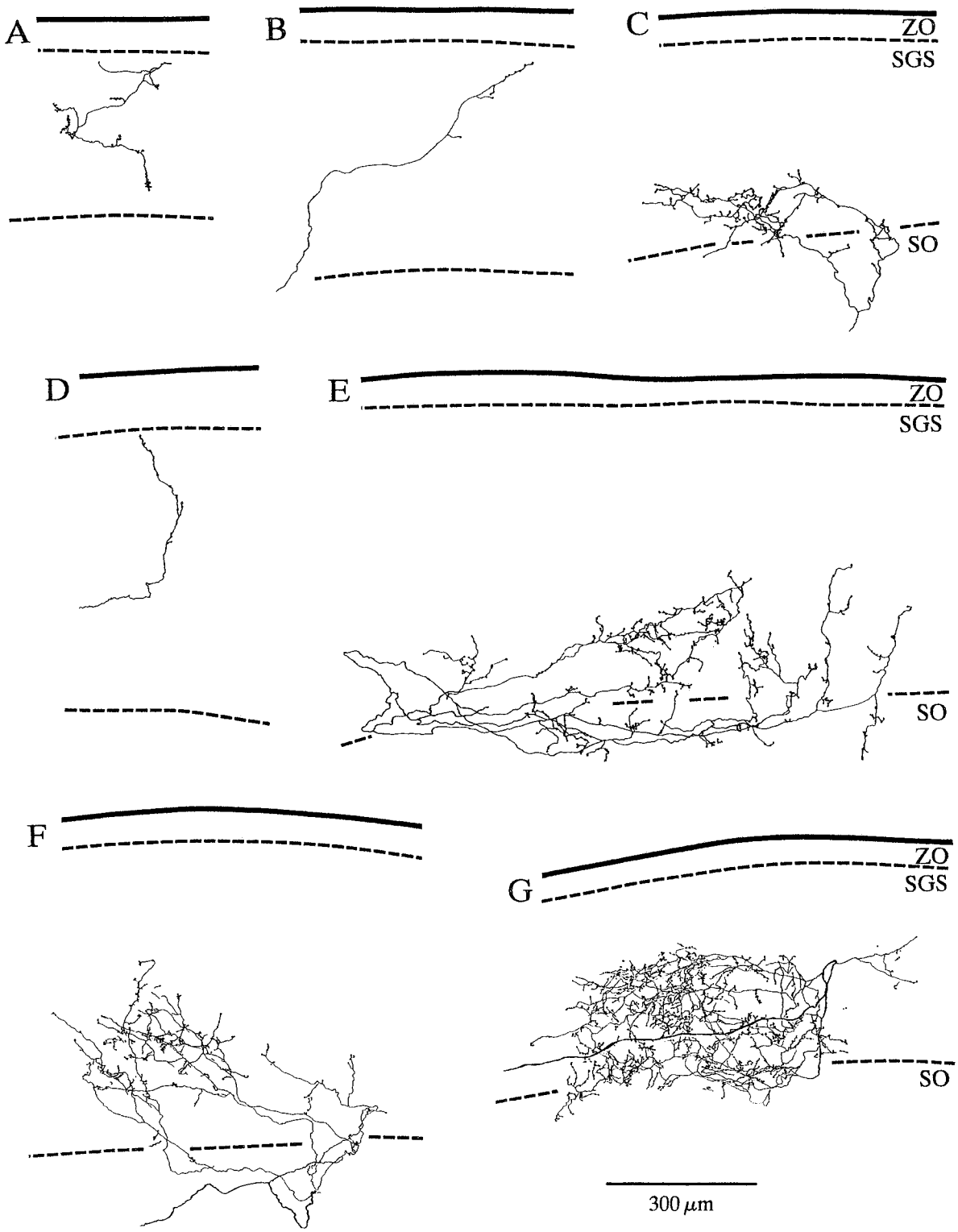
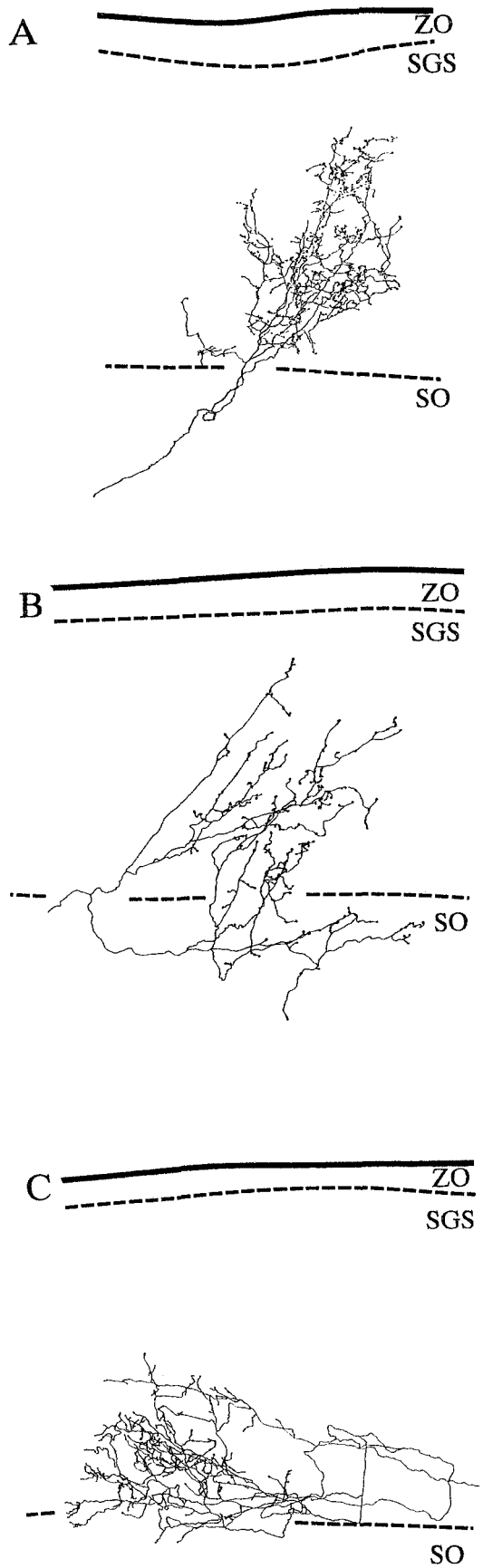


Fig. 13. Terminal arbors in the superior colliculus of seven Y axons from the contralateral eye, represented in the coronal plane. Receptive field coordinates are as follows: **A**, (+50°, −10°); **B**, (0°, −24°); **C**, (+45°, −5.0°); **D**, (+10°, −20°); **E**, (+15.5°, −5.0°); **F**, (+3.5°, −3.0°); and **G**, (−12°, −3.0). Scale bar in G also applies to A–F.



also able to extend this analysis to the entire retinal arbor. This is illustrated in Figure 18. We found that, when entire geniculate and midbrain arbors are considered, X axons showed no significant correlation between receptive field eccentricity and number of boutons ($r = +0.10$; $P > 0.1$), while Y axons did ($r = -0.77$; $P < 0.001$). Furthermore, the difference between correlations for the X and Y axons is significant ($P < 0.001$).

As noted, a negative correlation exists for Y axons between eccentricity and number of boutons for the lateral geniculate nucleus ($r = -0.85$; $P < 0.001$) but not superior colliculus ($r = -0.20$; $P > 0.1$), and this difference in relationship between terminal arbors from the same axons is statistically significant ($P < 0.001$). However, Figure 19 shows that a limited relationship does exist for these arbors in the superior colliculus. Although there was no obvious relationship between either overall eccentricity or azimuth and arbor size ($P > 0.1$ on both comparisons, whether all Y cells are considered or only the subset representing the contralateral hemifield), there was a significant negative correlation between elevation and arbor size ($r = -0.60$ and $P < 0.02$ for all Y cells; $r = -0.65$ and $P < 0.02$ for the subset representing the contralateral hemifield).

Figure 18 also shows the relationship between retinal eccentricity and the soma sizes of the α and β classes of ganglion cells; the soma size data are redrawn from the data of Leventhal (1982). These are the presumptive parent cells for the axons of the present study, α cells giving rise to X axons, and β cells giving rise to Y axons. Note that, for both classes, soma size increases monotonically with eccentricity. The number of X axons in our sample is too small for detailed statistical comparisons, but, for the Y axons, it is clear that the negative correlation between number of boutons and eccentricity contrasts with the increasing size of β cells along this dimension ($P < 0.001$).

DISCUSSION

Our use of biocytin as an intraaxonal label for single retinofugal X and Y axons produces much more complete labeling than possible with the previously used label (HRP). We thus confirmed and extended earlier observations. We found that every one of the six X axons branched to innervate the lateral geniculate nucleus and pretectum, but none innervated the superior colliculus. Every Y axon also innervated the lateral geniculate nucleus and midbrain, but there was some variation in midbrain terminations: most (11 of 14) innervated both pretectum and superior colliculus, one innervated only pretectum, and two innervated only superior colliculus. None of these axons innervated other known retinofugal targets, including the suprachiasmatic nucleus, the ventral division of the lateral geniculate nucleus, and the accessory optic nucleus.

Nature of retinofugal X and Y terminations

There is a voluminous literature on retinofugal projections from identified X and Y ganglion cells. A few studies, like the present, employed intraaxonal labeling after physiological identification (Bowling and Michael, 1980,

Fig. 14. Terminal arbors in the superior colliculus of three Y axons from the ipsilateral eye, represented in the coronal plane. Receptive field coordinates are as follows: **A**, (+17°, −7.5°); **B**, (+6.0°, −7.0°); and **C**, (+40°, 0°). Scale bar in C also applies to A,B.

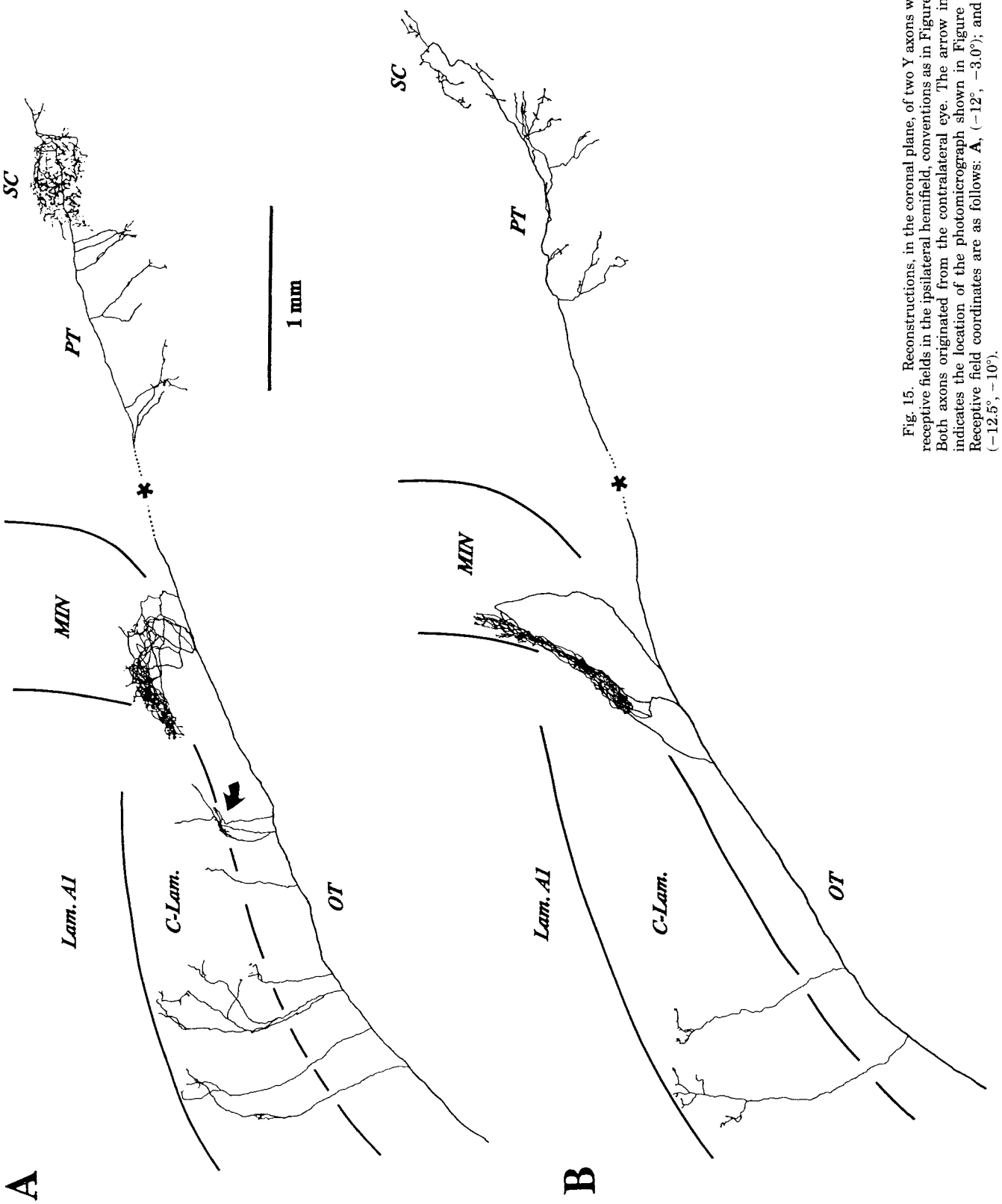


Fig. 15. Reconstructions, in the coronal plane, of two Y axons with receptive fields in the ipsilateral hemifield, conventions as in Figure 1. Both axons originated from the contralateral eye. The arrow in A indicates the location of the photomicrograph shown in Figure 16. Receptive field coordinates are as follows: **A**, (−12°, −3.0°); and **B**, (−12.5°, −10°).

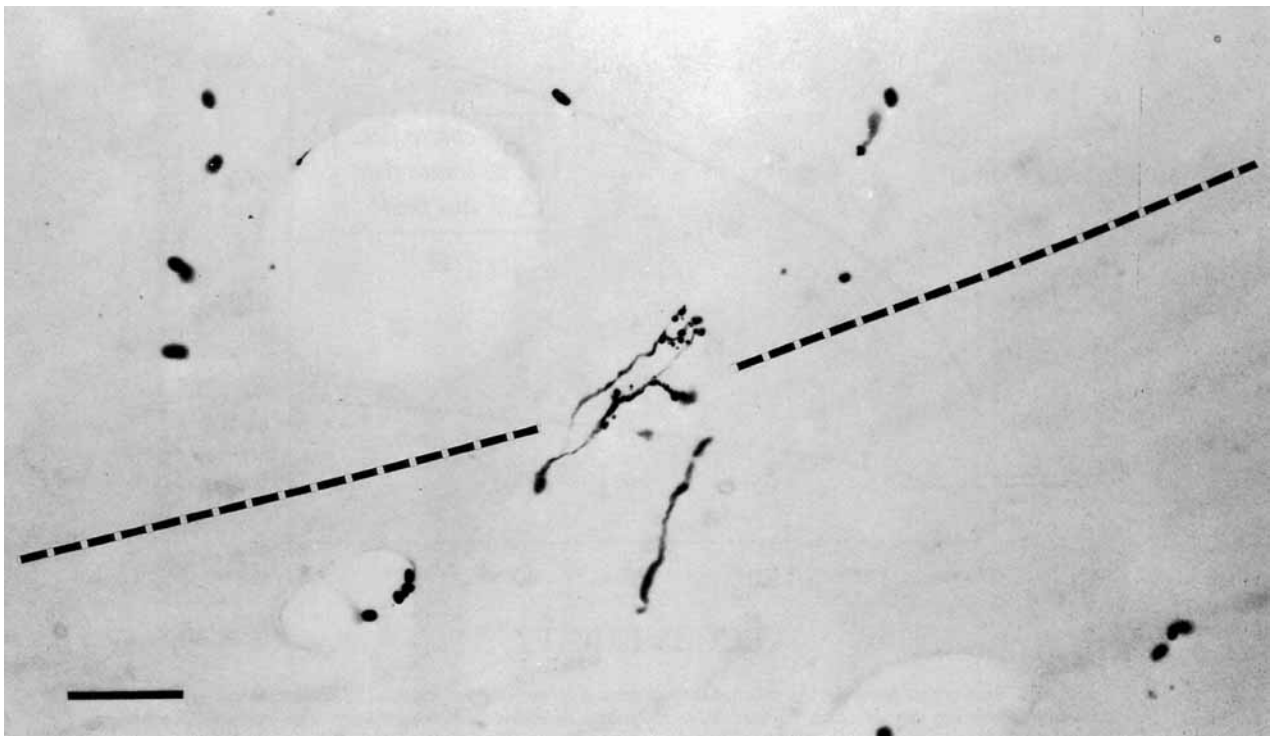


Fig. 16. Photomicrograph of terminal arbor of axon shown in Figure 15A. The dashed line represents the border between the C-laminae and the optic tract. Scale bar = 50 μm.

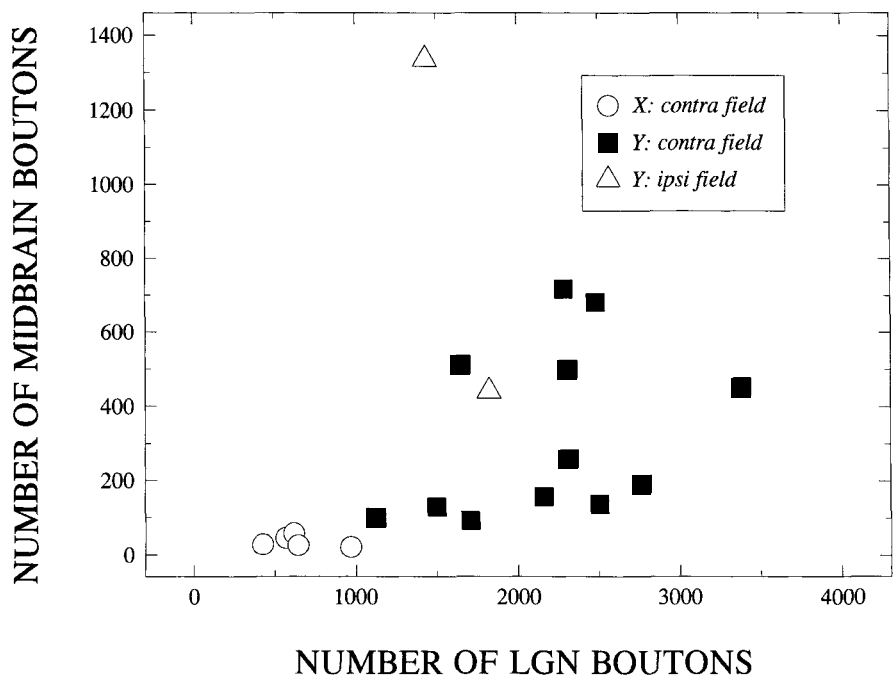


Fig. 17. Scatterplot showing number of boutons for each axon in the midbrain (pretectum plus superior colliculus) and lateral geniculate nucleus. Axons with receptive fields in the hemifield contralateral (contra field) and ipsilateral (ipsi field) are indicated.

1984; Sur and Sherman, 1982; Sur et al., 1987). Others make use of antidromic activation or retrograde labeling of retinal ganglion cells from putative termination sites (Fukuda and Stone, 1974; Kelly and Gilbert, 1975; Illing and Wässle, 1981; Leventhal, 1982; Koontz et al., 1985; Leventhal et al., 1985; Sawai et al., 1985). With antidromic

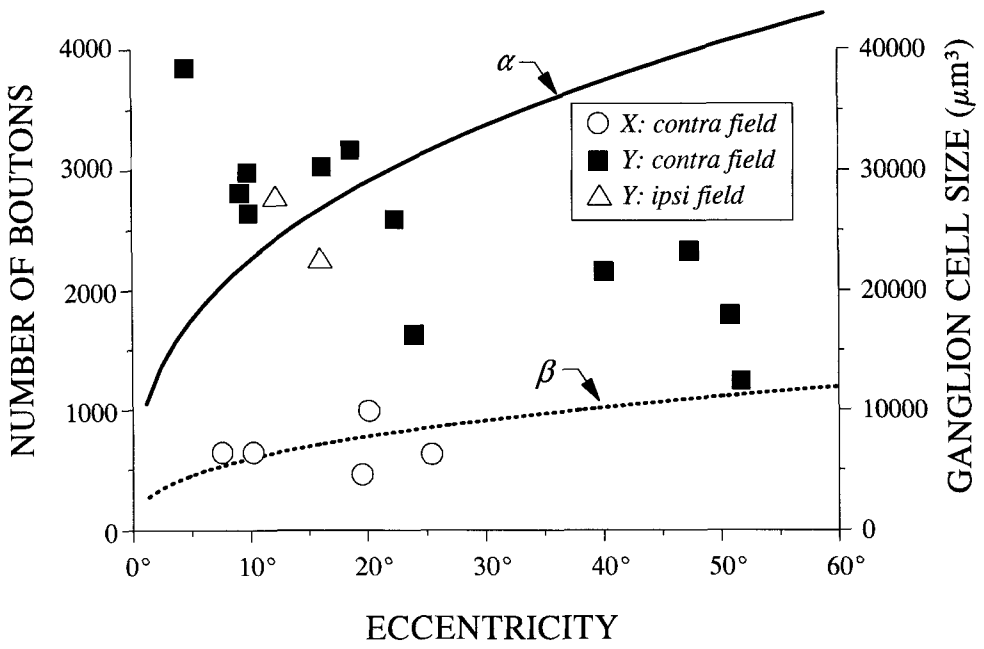


Fig. 18. Scatterplot showing the relationship of total number of boutons (i.e., in both thalamus and midbrain), ganglion cell size of α (Y) and β (X) cells, and receptive field eccentricity. The left ordinate indicates bouton number, and the right ordinate indicates cell size. The cell size data, which are represented by the solid and dashed curves, are redrawn from Leventhal (1982).

stimulation, it is straightforward to identify the recorded cell as X or Y based on standard physiological criteria. Interpretation of retrograde labeling is made possible by studies that demonstrate that X and Y cells have different somadendritic features: X cells have β morphology (small somata, dense but restricted dendritic arbors, and medium-caliber axons), and Y cells have α morphology (large somata, dense and large dendritic arbors, and thick axons).

Projections of retinal X axons. Our present results are in close agreement with prior conclusions regarding terminations of retinal X axons in the lateral geniculate nucleus. Every β or X cell innervates the dorsal division of the lateral geniculate nucleus (Cleland et al., 1971; Hoffmann et al., 1972; Fukuda and Stone, 1974; Illing and Wässle, 1981; Leventhal, 1982; Sur and Sherman, 1982; Bowling and Michael, 1984; Leventhal et al., 1985; Sur et al., 1987), and none innervates the ventral division (Spear et al., 1977; Bowling and Michael, 1984; Sur et al., 1987). Each of these axons innervates lamina A or A1, depending on the retina of origin, and a small minority also innervates the C-laminae and/or the medial interlaminar nucleus (Wilson et al., 1976; Dreher and Sefton, 1979; Leventhal, 1982; Leventhal et al., 1985; Sur et al., 1987). As was reported by Sur et al. (1987) before the present report, we found no obvious physiological difference between those X axons that innervate only lamina A or A1 and those few that also innervate the C-laminae or medial interlaminar nucleus.

Our observations on the midbrain projection patterns of retinal X axons seems to be in disagreement with prior literature on this subject. Studies based on retrograde transport of horseradish peroxidase and on antidromic stimulation of retinal ganglion cells indicate that only a small proportion of retinofugal X axons innervate the pretectum (Cleland and Levick, 1974; Fukuda and Stone, 1974; Koontz et al., 1985; Leventhal et al., 1985), and some claim that they innervate the superior colliculus (Wässle and Illing, 1980; Koontz et al., 1985; Sawai et al., 1985; but see Hoffmann, 1973; Kelly and Gilbert, 1975; Leventhal et al., 1985). Unfortunately, quantitative estimates of the fraction of X cells that innervates the midbrain vary widely with the technique used to study this. For instance, retrograde transport of HRP indicates that no β cells (Ballas et al., 1981) or exceedingly few (< 1%) of these cells (Koontz et al., 1985; Leventhal et al., 1985) innervate the midbrain, whereas up to 50% of recorded retinal X cells can be antidromically activated from the midbrain (Fukuda and Stone, 1974; Sawai et al., 1985). Furthermore, Sur and colleagues (Sur and Sherman, 1982; Sur et al., 1987) report that essentially every one of their sample of intracellularly labeled retinogeniculate X axons branches in the optic tract, with a thicker branch innervating the dorsal lateral geniculate nucleus and a thinner one entering the brachium of the superior colliculus; the latter branches could not be traced to terminal arbors. In noting the apparent discrepancy between retrograde transport and intracellular labeling, Koontz et al. (1985) suggest that perhaps "only a minority of beta cells branch and continue to the brachium, but their fibers are thicker than those that do not, which makes them easier to impale and inject." Perhaps this could also be applied to the antidromic stimulation experiments (Fukuda and Stone, 1974; Sawai et al., 1985): if the midbrain-projecting X cells have thicker axons, they might also have larger somata, which would render them more likely to be sampled electrophysiologically. An entirely different explanation offered by Sur et al. (1987) is that most or all X axons indeed branch to innervate the midbrain but that the remarkably thinner branch entering the brachium of the superior colliculus cannot support effective retrograde trans-

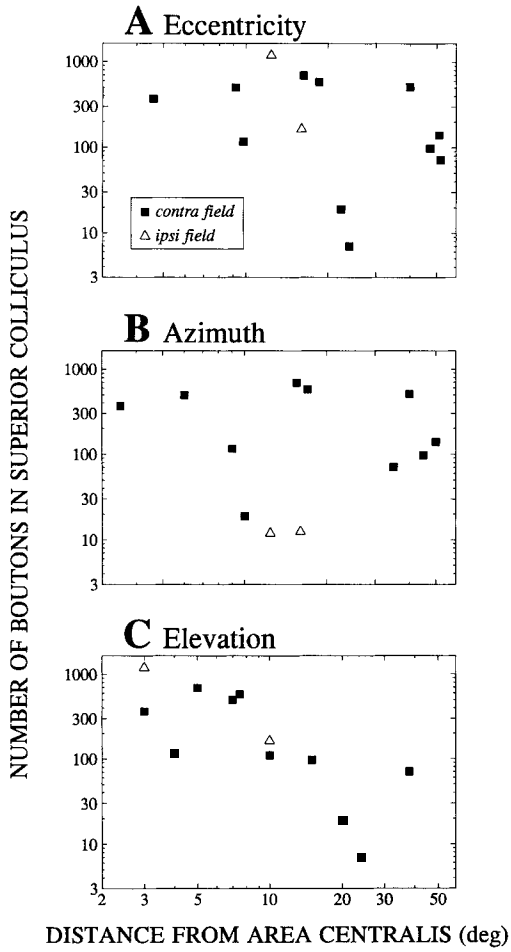


Fig. 19. Scatterplots showing relationship between receptive field coordinates and number of boutons for Y axon arbors located in superior colliculus. **A:** Plot of bouton number vs. overall receptive field eccentricity. **B:** Plot of bouton number vs. receptive field azimuth. **C:** Plot of bouton number vs. receptive field elevation.

port of horseradish peroxidase, and a thin branch may also lead to frequent failure of antidromic conduction at the branch point.

Our present data support the suggestion of Sur et al. (1987) by adding to the population of labeled retinofugal X axons, each of which branches to innervate the midbrain. Also, whereas previous studies seem quite divided on the point of whether any retinal X axons innervate the pretectum and superior colliculus (Fukuda and Stone, 1974; Wässle and Illing, 1980; Leventhal et al., 1985; Sawai et al., 1985), our data quite consistently support the conclusion that these midbrain branches of retinal X axons innervate the pretectum but not the superior colliculus. Unfortunately, there are two possible and incompatible interpretations for all of these results. First, it may be that all retinal X axons form a branch that innervates the pretectum, but both antidromic stimulation and retrograde transport of tracer lead to many false-negative observations due to the fine caliber of these branches. This seems plausible, especially insofar as the electrophysiological properties of the intraaxonally labeled retinal X axons, including conduction velocity which reflects axon caliber, are indistinguishable from the known properties of retinal X cells gleaned from the literature. Another reason to favor this interpretation is given below, after we consider the pattern of midbrain terminations from retinal Y axons. Second, perhaps < 1% of retinal X axons actually branch to innervate the pretectum, but these are of the largest caliber among this axon class and are sampled with considerable bias during electrophysiological recording. Nonetheless, the question of the fraction, identity, and termination patterns of retinal β/X cells that innervate midbrain regions bear further study.

Projections of retinal Y axons representing the contralateral hemifield. Like β/X cells, every α/Y cell representing the contralateral hemifield innervates the dorsal lateral geniculate nucleus; each Y cell innervates lamina A or A1, depending on the retina of origin, and most also branch to innervate the medial interlaminar nucleus and/or the C-laminae (Fukuda and Stone, 1974; Bowling and Michael, 1980, 1984; Illing and Wässle, 1981; Leventhal, 1982; Sur and Sherman, 1982; Leventhal et al., 1985; Sur et al., 1987). These patterns imply that the different divisions of the lateral geniculate nucleus (i.e., the A-laminae, the C-laminae, and the medial interlaminar nucleus) are generally innervated by the same individual retinogeniculate Y axons, and, thus, any differences seen among these divisions cannot be explained simply on the basis of different populations of retinal Y afferents.

Most or all of these Y axons, in addition to innervating the dorsal lateral geniculate nucleus, branch to innervate the superior colliculus and/or the pretectal complex (Hoffmann, 1973; Fukuda and Stone, 1974; Kelly and Gilbert, 1975; Schoppmann and Hoffmann, 1979; Bowling and Michael, 1980, 1984; Wässle and Illing, 1980; Illing and Wässle, 1981; Sur and Sherman, 1982; Leventhal et al., 1985; Sur et al., 1987). However, the question of the proportion of Y cells that branch to innervate both pretectum and the superior colliculus was unresolved from the literature. Leventhal et al. (1985) find that 80% of their α cell population can be retrogradely labeled from the superior colliculus, while only 28% were labeled from the pretectum. They further argued that some of the α cells labeled from pretectum "may have been labeled as a result of injury to fibers of passage to the SC [superior colliculus]." However, Sawai et al. (1985) reported that only 58% of their retinal Y cells could be antidromically activated from the superior colliculus, while 93% were activated from the pretectum. The great difficulty with interpretation of these experiments depending on antidromic activation or retrograde transport is controlling spread of current or label between two such closely spaced structures as the superior colliculus and the pretectum. Our dependence on orthograde transport of label and our ability to visualize terminal boutons obviates these limitations. Our data indicate that neither previous view is quite correct and, instead, that virtually all retinal Y axons branch to innervate both pretectum and the superior colliculus. Thus, nearly all of the Y cell information from the retina is transmitted centrally by the same axons to the lateral geniculate nucleus, the pretectal complex, and/or the superior colliculus.

It is interesting, in the context of possible electrode sampling biases, that our observations of retinal Y projection patterns do not match those reported by Leventhal et al. (1985) based on retrograde transport. Although it is plausible that the thinner X axons may well be divided into a relatively thick subgroup that is well sampled and a

relatively thin one that is missed by electrodes (see above), the mere fact that X axons are routinely sampled makes it unlikely that the much thicker Y axons are subject to significant sampling biases. However, we find a much higher percentage of these axons innervating the pretectum than did Leventhal et al. (1985), 83% vs. 28%, and our percentage innervating the superior colliculus is also somewhat higher (91% vs. 80%). The most likely explanation for this discrepancy is the failure of the HRP label in many retinal Y axons to transport retrogradely across fine branch points in the midbrain. If this is so, then it seems equally likely that such a failure of retrograde transport in X axons would result in many retinal β cells failing to transport label from the pretectum despite the presence of a terminal arbor there.

Projections of retinal Y axons representing the ipsilateral hemifield. A projection to the lateral geniculate nucleus and superior colliculus from retinal ganglion cells in the contralateral temporal retina, which thus represents the ipsilateral (or "wrong") hemifield, has been reported in the past (Sanderson and Sherman, 1971; Harting and Guillery, 1976; Guillery et al., 1980; Rowe and Dreher, 1982; Lee et al., 1984). However, our two examples of Y axons represent the only observations of terminal patterns for such cells. Our small sample precludes any confident conclusions to be formed about this curious pathway. Nonetheless, there are two aspects of these cells that seem worthy of comment. First, like the other Y axons, these cells branch to innervate the lateral geniculate nucleus, pretectum, and superior colliculus. Second, within the lateral geniculate nucleus, the terminal arbors are confined to the medial interlaminar nucleus and/or C-laminae in a remarkably diffuse arbor, and neither penetrates the A-laminae. Unfortunately, it is difficult to imagine any functional significance for this projection pattern.

Differences between retinal X and Y axons. A number of differences have been previously described between the distribution of retinal X and Y arbors in the lateral geniculate nucleus (Bowling and Michael, 1980, 1984; Sur and Sherman, 1982; Sur et al., 1987), and these differences have generally been confirmed here. These differences include a greater number of terminal boutons for Y axons; a greater tendency for Y axons to innervate the C-laminae and medial interlaminar nucleus; terminal boutons that occur mostly en passant in Y axons and within clusters in X axons; and a tendency for Y arbors to decrease with eccentricity of their receptive fields, a feature not seen in X arbors.

Because of our inclusion of midbrain projections of these retinal axons, we can extend somewhat this list of differences, primarily in terms of extent of the arbors. Most Y axons innervate both the pretectum and the superior colliculus, but X axons innervate only the former. As a result of this, X axons project fewer terminal boutons beyond the lateral geniculate nucleus to the midbrain than do Y axons, whether expressed in terms of relative percentage of the total arbor or in terms of actual number of boutons.

Other targets of retinal X and Y axons. There is no prior published evidence for retinofugal X or Y inputs to the suprachiasmatic nucleus, the ventral lateral geniculate nucleus, or the accessory optic nuclei. Our present observations are consistent with this, although uncertain retrograde transport of intracellularly injected biocytin limits our conclusion concerning the suprachiasmatic nucleus.

Organization of terminal arbors in the midbrain

Pretectum. As was noted in Results, our material does not permit us to define precisely which nuclei of the pretectum receive retinal X and Y inputs, but most terminal boutons seem to be localized within the nucleus of the optic tract. There are no descriptions in the literature of an unambiguous retinotopic map in the pretectum. Some studies report partial or limited maps (Graybiel and Berson, 1980; Koontz et al., 1985; Kubota et al., 1987). In most prior experiments, maps could be obscured by unclear nuclear boundaries, by overlap in orderly maps for different classes (e.g., from W, X, and Y axons) that offset slightly, or by other factors. In several cases, we have reconstructed several axon arbors in the pretectum from a single experiment (see Figs. 8–11), and, although the observations are few in number, they offer an unobscured view of any maps that might be present. We find evidence for only a very crude map limited to elevation, and this supports the earlier suggestions (Graybiel and Berson, 1980; Koontz et al., 1985; Kubota et al., 1987).

Another sort of organization suggested for retinal axons terminating in the pretectum is a curious pattern of clustering. For instance, Koontz et al. (1985) described an unexpected clustering seen in orthograde labeling of this pathway, and Nabors and Mize (1991) recently showed that these clusters of retinal terminations are localized amongst clusters of postsynaptic cells that stain positively for the calcium binding protein calbindin-D. It is interesting, in this context, that many of our reconstructed axons, both X and Y, appeared to terminate in the pretectum in several distinct clusters. It is not yet clear what the significance of this clustering is, but our data do indicate that they represent significant clustering among single axons and do not represent territories innervated uniquely by different retinal axon populations.

Superior colliculus. A number of anatomical and electrophysiological studies have documented the rich retinal innervation of the superior colliculus, with the superficial layers being the primary target of this input (reviewed by Huerta and Harting, 1984; Berson, 1988; Stein and Meredith, 1991). Most or all of the retinocollicular input derives from W and Y cells, and the possibility of a small X input (Sawai, et al., 1985) has been discussed above. Our data suggest that the retinal Y cell contribution to this innervation terminates primarily in the lower stratum griseum superficiale, with limited encroachment into the upper stratum griseum superficiale and the upper stratum opticum. These results are consistent with estimates derived from current source density analysis (Freeman and Singer, 1983). Hoffmann (1973), relying on orthodromic activation data, suggested that the retinal Y input further extends throughout the stratum opticum and into the dorsal stratum griseum intermediale (see also, Berson, 1988). Our data do not support this idea but instead suggest that any cells receiving direct retinal Y input and located in these deeper layers of the superior colliculus must receive this input on dorsally projecting dendrites (cf. Berson and McIlwain, 1982).

Several prior studies have shown that the retinal input to the superior colliculus declines markedly at the tectal representation of the area centralis (Graybiel, 1975; Mize, 1983; Huerta and Harting, 1984). Interestingly, we found no significant reduction in the number of boutons of Y

axons with receptive fields at low eccentricities. Instead, the strongest correlation that we found for bouton number in the superior colliculus suggests the opposite relationship, because these numbers decrease with elevation from the area centralis. We did not recover axons with receptive field eccentricities within the central 4°, and, had we done so, perhaps we would have observed a reduction in bouton numbers for Y axons with such central receptive fields. However, the decline in retinal input reported previously (Graybiel, 1975; Mize 1983) appears to extend across a larger representation of the visual field, large enough to include our sample of Y axons. In the context of these previous studies, our data thus suggest that the decline in retinal input reflects a decrease in the retinal W cell innervation and not that of Y axons.

Determinants of axon arbor size

The "pruning" hypothesis. As was noted in the opening paragraphs, our data permit a comparison of arbors directed to different targets from the same parent axons, which in our examples are thalamic and midbrain targets from retinal axons. One popular hypothesis for the formation of arbors is the so-called pruning hypothesis, which, through analogy to gardening, suggests that arbors made smaller in one sector will become larger in another (Schneider, 1973). This idea predicts that a larger arbor formed in one target by an axon would result in a smaller arbor being formed in another target or targets, and vice versa. Figure 17 shows that this does not seem to be the case for retinal X and Y axons; a larger arbor in the lateral geniculate nucleus does not correlate with a smaller one in midbrain; indeed, if there is any correlation at all, it is in the opposite direction. We thus conclude from our data that normal development of retinofugal arbors for X and Y axons is not controlled by a mechanism analogous to pruning.

Relationship between soma and arbors size. It has frequently been suggested that the size of a soma is correlated with the extent of axonal arbor it must support. For instance, the smaller size of geniculate somata in deprived laminae of the lateral geniculate nucleus of cats and monkeys raised with visual deprivation has been related to the presumed smaller arbors these cells support in cortex (see, e.g., LeVay et al., 1980). Sur et al. (1987) noted that their observation of smaller arbors of Y axons in the lateral geniculate nucleus with greater eccentricity of their receptive fields ran counter to this suggestion, because α cells, which are the parent somata of the Y axons, become larger with eccentricity (see Fig. 18). However, because Sur et al. (1987) could not analyze the entire arbor because their HRP label did not reveal the midbrain arbors of the Y axons, they could not be absolutely certain of their conclusion. We have been able to reveal the entire arbors of these axons, and we can confirm the suggestion of Sur et al. (1987). As is summarized in Figure 18, the total amount of terminal boutons supported by Y axons decreases with eccentricity as the average size of their parent α cell somata increases.

Significance of thalamic and midbrain targets

Each of our sample of labeled X and Y axons branched to innervate both thalamus and midbrain. Although we cannot yet completely rule out the possibility that our sample is biased and unrepresentative, we have discussed above why we believe our sample to be indeed typical of retinal X and Y projections. This means that virtually all information carried out of the retina by these parallel neuronal streams is simultaneously and in parallel distributed to the lateral geniculate nucleus and to the midbrain. The pathway to the lateral geniculate nucleus continues to visual cortex, and that to the midbrain is more difficult to follow. One functional significance of these midbrain structures is that they seem to be involved in various reflex behaviors (oculomotor, etc.) that may not involve visual cortex. However, both the pretectum and the superior colliculus innervate the lateral geniculate nucleus, as well as other visual thalamic regions such as parts of the pulvinar and lateral posterior nucleus (Huerta and Harting, 1984; Graybiel and Berson, 1980), and they thus also contribute to pathways reaching visual cortex. Therefore, there are at least two main routes of information to visual cortex, the retino-geniculo-cortical route and the retino-midbrain-thalamo-cortical route. Both routes share the same retinal output information, as represented by the X and Y pathways.

There is an important proviso to be made regarding this conclusion. Recently, Schmidt and Hoffmann (1992) were able to identify retinal Y inputs to pretectal cells innervating the lateral geniculate nucleus but failed to identify any with X inputs. However, they used orthodromic stimulation to identify the retinofugal inputs to these pretectal cells, and there are several conditions under which retinal X inputs would be hard to reveal. For instance, if both X and Y axons converge onto the same pretectal cells, only the faster conducting Y axons would be readily revealed by their techniques; also, if the faster conducting Y axons, when massively and synchronously activated by electrical shocks, initiate inhibitory activity in the pretectum, this activity might prevent expression of the later arriving X volley. Thus, although the data of Schmidt and Hoffmann (1992) clearly indicate the presence of retinal Y input to this pretectal innervation of the lateral geniculate nucleus, they do not rule out the presence of a retinal X contribution.

Regardless of whether the route through the pretectum emanates from both X and Y retinal axons or from only the latter, this route clearly exists. The more direct retino-geniculo-cortical route seems to be purely a means of providing relatively raw sensory information to cortex, while the information relayed to cortex via the midbrain presumably carries more processed information. For instance, the pretectum and superior colliculus together seem to be involved in various reflex pathways for eye movements, among other functions (Hoffmann and Distler, 1986; Simpson et al., 1988; Stein and Meredith, 1993), and the pathway to cortex through these structures may provide a signal to cortex that combines the raw retinal signal with eye movement or positional information.

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