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Morphology of Retinogeniculate X and Y Axon Arbors in Monocularly Enucleated Cats

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ABSTRACT

We examined the terminal arbors of single, physiologically identified retinogeniculate X and Y axons from the remaining retinas of adult cats raised from birth with monocular enucleation. These were compared with arbors of X and Y axons in normally reared cats. We used intra-axonal injections of horseradish peroxidase to label each axon after recording its response properties. While the axons in monocularly enucleated cats exhibited normal response properties, both X and Y axons in these cats had abnormally large terminal arbors. Each of the hypertrophied X arbors appeared to be completely confined to the single geniculate lamina A or A1 appropriate to its eye of origin (i.e., lamina A for the contralateral retina and lamina A1 for the ipsilateral retina). In contrast, in addition to their normal terminations, most of the Y arbors seemed to extend well into laminae normally innervated only by the retina that was removed. Thus most or all of the translaminar sprouting previously reported for monocularly enucleated cats appears to reflect extensions of Y axon arbors. These data, in addition to earlier, analogous data from young kittens and cats reared with monocular lid suture, suggest the following sequelae during postnatal development: the retinogeniculate X arbors mature first and develop exuberant arbors that are later competitively pruned as the Y axons expand their innervation of the lateral geniculate nucleus; monocular lid suture prevents the Y axons from succeeding in this competition, so they fail to establish normal arbors and cannot reduce the exuberant X arbors; monocular enucleation offers a less resistant path in the denervated laminae for the rapidly growing Y arbors from the remaining eye, and the expansion of these arbors there reduces the competitive pressure on the exuberant X arbors. Thus, in monocularly enucleated cats, sprouting is limited to Y axons, either because only they possess the capacity to sprout or because they are in the midst of a period of relatively rapid growth at the time of the neonatal enucleation. The X axon arbors are also abnormally large within their appropriate laminae. This occurs presumably because they are able to maintain their immature exuberance, although we cannot rule out the possibility that they are pruned and later regrow to the final size seen in our experiments.

Key words: X-cells, Y-cells, visual system development, sprouting

In the cat, the pathway from the retina through geniculate laminae A and A1 to visual cortex is actually composed of two parallel and functionally distinct neuronal streams known as the X and Y pathways (for reviews, see Stone et al., '79; Lennie, '80; Sherman and Spear, '82; Sherman,

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'85a). Retinogeniculate axons of one (X or Y) class almost always innervate geniculate neurons of the same class, and the latter provide the parallel X and Y inputs to cortex. Not only do these pathways seem to subserve different functional roles for vision (e.g., Ikeda and Wright, '72; Stone et al., '79; Lennie, '80; Sherman, '79, '85a), but they also develop differently. In general, the X pathway matures earlier than does the Y pathway (Daniels et al., '78; Mangel et al., '83; Sur et al., '84), and development of these pathways is affected differently by various forms of early visual deprivation (reviewed in Movshon and Van Sluyters, '81; Sherman and Spear, '82).

Morphological studies based on intra-axonal labeling with horseradish peroxidase (HRP) of physiologically identified axons have revealed several such developmental differences for the terminal arbors of X and Y retinogeniculate axons. For instance, during normal development, the X axons innervate laminae A and A1 with exuberant arbors before the Y axons begin to develop adultlike arbors; as the Y arbors develop, the exuberant X arbors are pruned back to their adult form. This apparently occurs because of competition between X and Y arbors during their development (Sur et al., '84). Furthermore, early monocular deprivation results in the development from the deprived eye of abnormally large X arbors and severely restricted Y arbors, as if the later-developing Y axons were rendered incapable of competitively pruning the already established X arbors (Sur et al., '82).

Within the cat's visual system, these retinogeniculate axons represent the most peripheral neuronal elements for which dramatic effects of early visual deprivation have been documented. Such effects on these axons have obvious consequences more centrally, including a failure of Y axons to displace X innervation of geniculate cells (Friedlander et al., '82). These axons thus provide a particularly interesting subject for studies of development. We wished to explore other aspect of their postnatal development, with special reference to developmental differences between X and Y axons.

In particular, early monocular enucleation, whether effected pre- or postnatally, alters subsequent development of retinogeniculate connections from the remaining eye and disrupts formation of geniculate lamination patterns, since these reflect ocular input (e.g., Chalupa and Williams, '84; Sretavan and Shatz, '85). In normal cats, lamina A is innervated by only the contralateral eye and lamina A1 by only the ipsilateral eye, but early postnatal removal of one eye induces axons from the remaining eye to expand into inappropriate laminae (Guillery, '72; Hickey, '75; Robson et al., '78; Robson, '81). By employing intra-axonal injections of HRP into physiologically identified retinogeniculate axons in such cats, we were able to show that X and Y axons react to monocular enucleation quite differently. The Y axons are responsible for all or most of the translaminar sprouting, and the X arbors, although also abnormally large, are confined to their appropriate laminae. We have published a preliminary version of these results (Garraghty et al., '84).

MATERIALS AND METHODS

Subjects

We collected intra-axonal data from six adult cats that were raised in our breeding colony following monocular enucleation performed within 24 hours of birth. The enucleations were performed aseptically under Metofane (Pitman-Moore) anesthesia, a long-lasting local anesthetic was installed into the orbit postoperatively, and the lids were sutured. These cats were 12–16 months of age at the time of the final, acute experiment.

Electrophysiology

Our electrophysiological procedures have been described in full elsewhere (Friedlander et al., '81; Sur and Sherman, '82; Sur et al., '82, '84) and will only be briefly outlined here. The cats were anesthetized, paralyzed, artificially ventilated, and fixed in a stereotaxic device. We placed stimulating electrodes across the optic chiasm for orthodromic activation of optic tract axons. The recording electrodes consisted of beveled micropipettes filled with 5–10% HRP in 0.2 M KCl and 0.05 M Tris. The stimulating and recording electrodes were inserted into the brain through hydraulically sealed craniotomies.

We dilated the cat's pupils, retracted its nictitating membrane with topically applied drugs, and fitted contact lenses to the corneas. These lenses were selected by retinoscopy to focus the eyes on a frontal tangent screen. The optic disk was projected onto the tangent screen by the method of Fernald and Chase ('71). Since we plotted neuronal receptive fields on the same screen, the position of each field could be related to the visual axis by noting its distance and direction from the optic disk (Sanderson and Sherman,'71). Visual stimuli consisted of bright and dark targets projected on the tangent screen and gratings generated on a cathode ray tube. The gratings were modulated in time or drifted across the screen. We could continuously modulate the grating's spatial and temporal frequency, contrast (up to 0.6), and position. Its mean illumination was fixed at 36 cd/m^2.

We studied a number of response properties of optic tract axons before filling them with HRP. These properties included response latency to optic chiasm stimulation; receptive field location, size, and center type (on or off); linearity of spatial and temporal summation to grating stimuli; sustained or transient responsiveness to standing contrasts; and responsiveness to large, rapidly moving targets. These properties (except for receptive field location and center type) were used to identify each axon as an X- or Y-cell (Enroth-Cugell and Robson, '66; Hoffmann et al., '72; Cleland et al., '74; Hochstein and Shapley, '76).

We studied each axon first during extracellular recording and then impaled it. The physiological properties of the axon were quickly rechecked during intracellular recording to verify that it was the same axon recorded extracellularly. We then used depolarizing pulses to iontophorese HRP into the axon.

Histology

General methods. Several hours after the final HRP injection, we deeply anesthetized the cat and perfused it with a mixture of 1% paraformaldehyde and 2% glutaraldehyde. The brain was removed and sectioned coronally or parasagittally on a freezing microtome at a thickness of 100 μm. Sections were treated with 3-3′ diaminobenzidine and the reaction was intensified with cobaltous chloride (Adams, '77). Many sections were also later counterstained with cresyl violet to determine laminar borders. We injected ^{3}H-proline into the remaining eyes of two of the enucleated cats 1 week prior to their recording sessions; they were anesthetized with barbiturate for these injections. In these cases, autoradiography of sections through the lateral ge-

niculate nuclei helped to establish laminar borders.

We note, as has been previously reported (e.g., Hickey, '75), that laminar borders are difficult to discern in cats monocularly enucleated during the first postnatal day. A further complication is that lamina A contralateral to the enucleated eye seems far more degenerated that does lamina A1 ipsilateral to the enucleated eye. Consequently, while clear interlaminar zones are visible in some sections through the nucleus ipsilateral to the removed eye, they are less clear in the contralateral nucleus. Finally, the appearance of interlaminar zones even in the ipsilateral nucleus is quite irregular. Generally, there is a central-to-peripheral gradient such that laminar borders are more obvious centrally in the nucleus.

Labeled axons were reconstructed through serial sections at a magnification of 670 by using a drawing tube attached to a microscope with a 50× oil-immersion objective. We identified each axon with its response properties by matching the locations of its receptive field and terminal arbor according to Sanderson's ('71) maps of the lateral geniculate nucleus. In the one case where this was not possible, axons were identified from reconstructions of electrode tracks (Table 1).

Quantitative analysis of axons. We established two quantitative measures for each recovered axon. First, we counted the number of terminal boutons for each arbor. These counts were taken from drawings of individual sections rather than from the composite reconstructions. Second, the volume of each terminal field was estimated. We did this by drawing an outline around the boutons in each section and treating these as 100-μm-thick slabs. In some cases in which wide gaps separated parts of the terminal field within a single section (e.g., the terminal arbors in laminae A and C of a single Y axon), we excluded the gaps from the volume estimates. For the sake of comparison, similar measurements were made for normal X and Y axons reported elsewhere (Sur and Sherman, '82; Esguerra et al., '85; Sur et al., '86).

No adjustments were made in these volume estimates to correct for tissue shrinkage. We instead assumed that, because we employed identical histological procedures throughout, such shrinkage should be fairly constant. Since we were interested in *relative* differences between X and Y arbors and arbors from normal and enucleated cats, any fairly constant shrinkage creates no problem. Even if the shrinkage were variable, there is no reason to suppose that it would systematically bias our results by consistently and differentially affecting X and Y arbors or those from normal and enucleated cats.

Statistics

Unless otherwise noted, the Mann-Whitney *U*-test was used for all statistical comparisons.

RESULTS

We recorded extracellularly from 85 retinal ganglion cell axons in the six monocularly enucleated cats. These included 31 axons that were classified as X-cells and 54 that were classified as Y-cells. Of these, 23 axons (eight X and 15 Y) were sufficiently well labeled with HRP to permit quantitative morphological analysis. Lightly labeled axons are not considered further. Included for comparison are eight X and 11 Y axons that were injected in normal cats and reported elsewhere (Sur and Sherman, '82; Esguerra et al., '85; Sur et al., '86).

Physiology of retinogeniculate X and Y axons

We found no detectable differences between the response properties of axons in monocularly enucleated cats and those of their counterparts in normal cats. On all of the tests used to identify cells as X or Y (see Materials and Methods), axons in the enucleated cats behaved normally. Also, our quantitative measures of receptive field size and response latency to optic chiasm stimulation revealed no functional abnormalities in these axons. Table 1 summarizes many of the response properties observed in the axons recorded from monocularly enucleated cats.

Receptive field center sizes varied with eccentricity. In the enucleated cats, the X axons had an average receptive field center diameter of 0.9° with an average eccentricity of 25.3°, and the Y axons had an average center diameter of 2.8° with an average eccentricity of 32.1°. For these cats, response latencies to optic chiasm stimulation varied between 0.7 and 1.1 msec for the X axons (with a mean of 0.84 msec) and between 0.4 and 0.7 msec for the Y axons (with a mean of 0.49 msec). These values for receptive field sizes and latencies to optic chiasm stimulation match previously published values for retinal axons in normal cats (Hoffmann et al., '72; Kratz et al., '79; Sur and Sherman, '82, '84).

As far as we could determine, our subpopulation of axons labeled with HRP for morphological analysis is physiologically representative of our larger population of extracellularly recorded axons. Thus, labeled X axons had an average receptive field diameter of 1.0° with a mean eccentricity of 29.0° and a mean optic chiasm latency of 0.83 msec. Labeled Y axons had receptive field sizes that averaged 2.4° with a mean eccentricity of 30.5° and a mean optic chiasm latency of 0.47 msec.

Terminal arbor morphology of retinogeniculate X and Y axons

Previous reports have documented many of the morphological differences between the terminal arbors of retinogeniculate X and Y axons in normal cats (Sur and Sherman, '82; Bowling and Michael, '84). Compared to Y axon arbors, those of X axons are smaller and contain fewer terminal boutons (see also below). X axons innervate lamina A, if derived from the contralateral retina, or lamina A1, if derived from the ipsilateral retina. In contrast, while every Y axon likewise innervates lamina A or lamina A1, those from the contralateral eye also innervate lamina C. Finally, terminal boutons of X axons tend to be relatively regular in size and spherical in shape, and they tend to occur in clumps appended to axonal branches by short stalks; those of Y axons are more variable in size and shape, and they tend to occur more diffusely *en passant* with less prominent clustering.

Since the normal morphology of retinogeniculate X and Y axon arbors has been previously described (Sur and Sherman, '82; Bowling and Michael, '84; Sur et al., '86), most of the documentation below is limited to axons from the monocularly enucleated cats. Also, our detailed analysis of the axon arbors was limited to laminae A, A1, and C because the laminar patterns within the medial interlaminar nucleus could not be readily discerned. Finally, it is important to emphasize that, in normal adult cats, all X and Y arbors are entirely restricted to geniculate laminae appropriate for their eye of origin. Also, translaminar extensions of retinogeniculate arbors into "inappropriate" laminae, while apparently present prenatally, are almost completely eliminated by birth (Shatz, '83; Shatz and Kirkwood, '84; Stretavan and Shatz, '84).

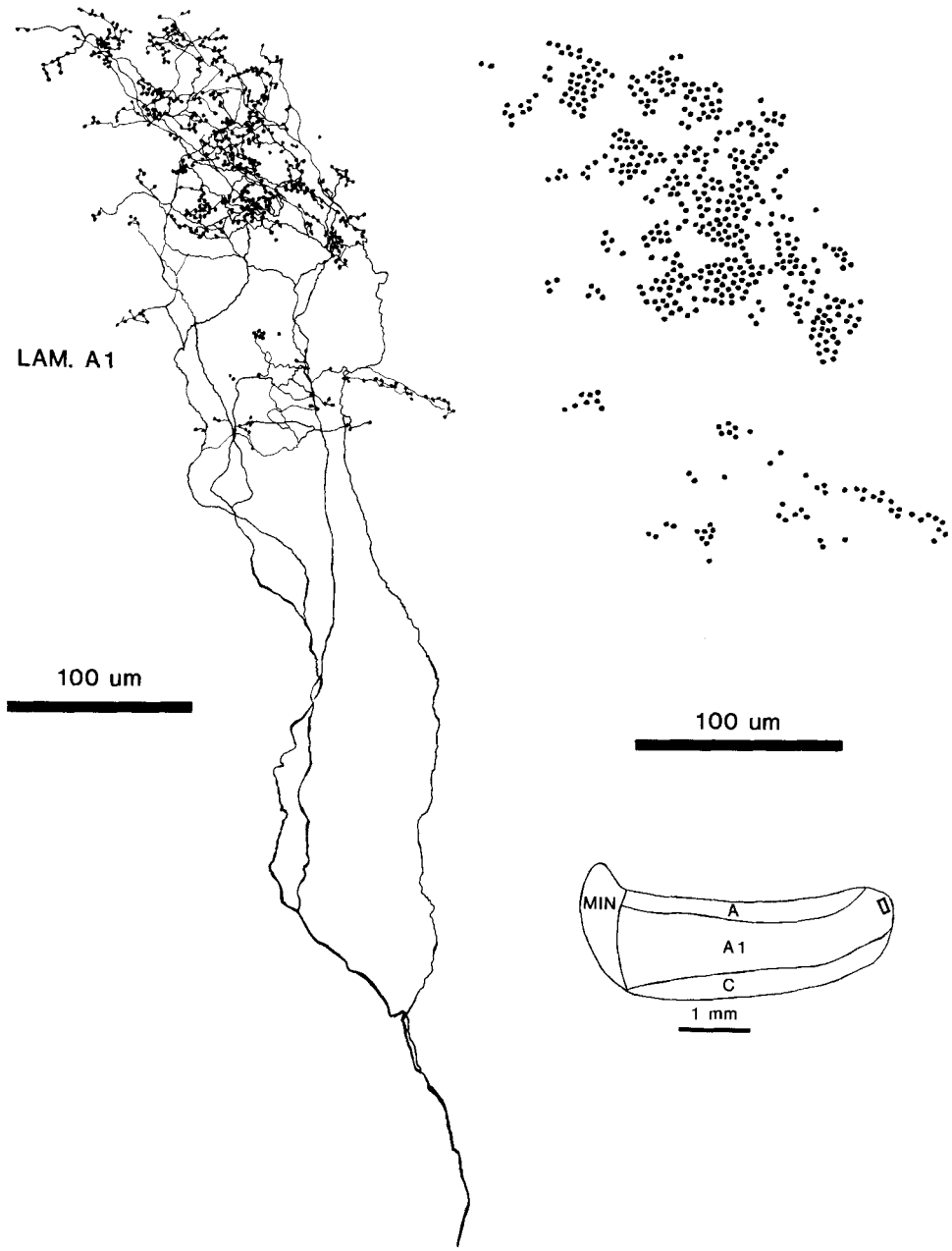


Fig. 1. Example of an HRP-labeled retinogeniculate X axon in a monocularly enucleated cat. The remaining eye is the right eye, and the axon innervates the right lateral geniculate nucleus with an arbor entirely limited to lamina A1 (LAM. A1). In the small drawing of the lateral geniculate nucleus in the lower right, the rectangle shown near the lateral border of lamina A1 depicts the region of the terminal arbor. The arbor is completely reconstructed in the drawing on the left, and the pattern on the right illustrates the location of each terminal bouton with a dot. Note that no monocular segment is illustrated for lamina A. Whether this reflects a gross distortion of lamination due to monocular enucleation or our failure to depict laminar borders accurately in the lateral portions of the lateral geniculate nucleus is not presently clear (see text for details). Abbreviations: A, Lamina A; A1, Lamina A1; C, C Laminae; MIN, Medial Interlaminar Nucleus.

Qualitative observations

X axons. Figure 1 illustrates a labeled retinogeniculate X axon from a monocularly enucleated cat. In this example, the right hemisphere is illustrated, and the remaining eye was the right eye. The axon arbor was thus limited to lamina A1, as would be expected in normal cats. Also, its terminal boutons display the size, shape, and clumping typical of normal X axons (Figs. 1, 2A). Figure 3 presents photomicrographs of another, qualitatively normal X axon that innervates lamina A from the remaining eye, which was contralateral to the terminal arbor.

Figures 4 and 5 summarize the terminal patterns for the remaining labeled retinogeniculate X axons from monocularly enucleated cats. Figure 4 depicts axons that project from the ipsilateral retina to lamina A1, and Figure 5 illustrates axons that project from the contralateral retina to lamina A. Qualitatively, each of these axons appears normal morphologically, with a relatively narrow, cylindrical arbor that occupies much of the dorsoventral extent of

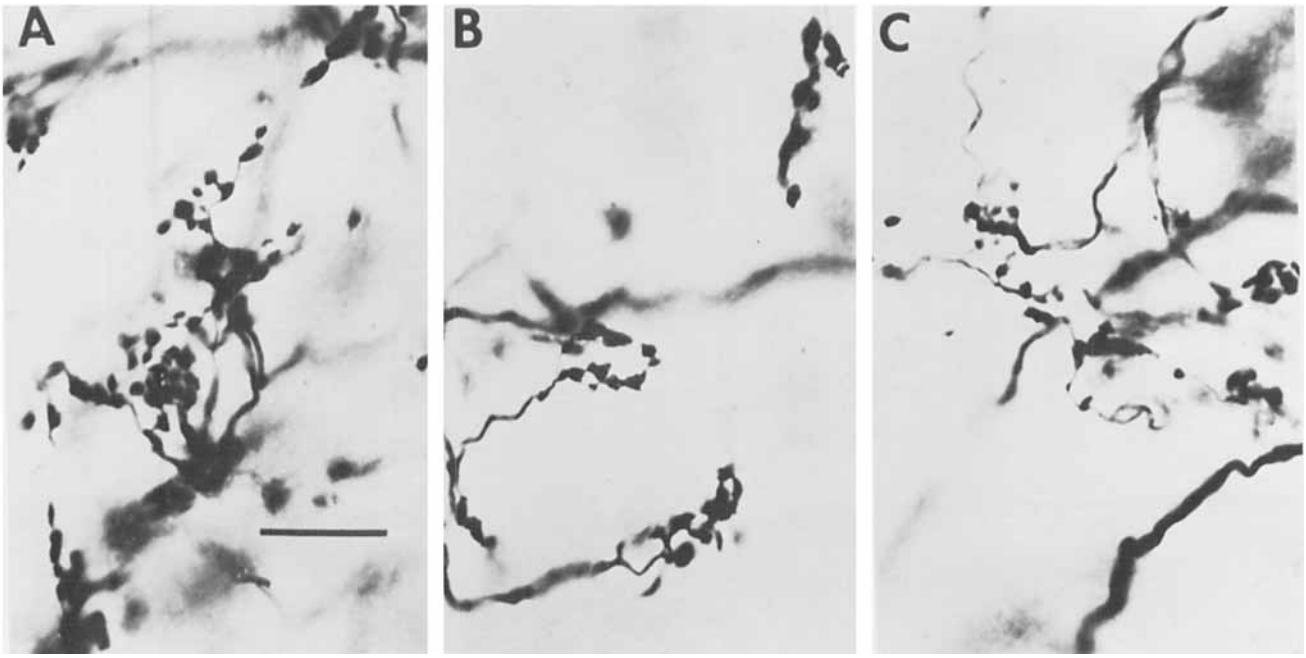


Fig. 2. Photomicrographs of portions of terminal arbors from retinogeniculate axons in monocularly enucleated cats. These examples are all located in lamina A contralateral to the remaining eye. A. X axon arbor. Note the clumped, fairly spherical terminal boutons. The scale is 20 μm and applies as well to B and C. B, C. Two Y axon arbors. Note the relatively variable sizes and shapes of the terminal boutons, which tend to occur *en passant*.

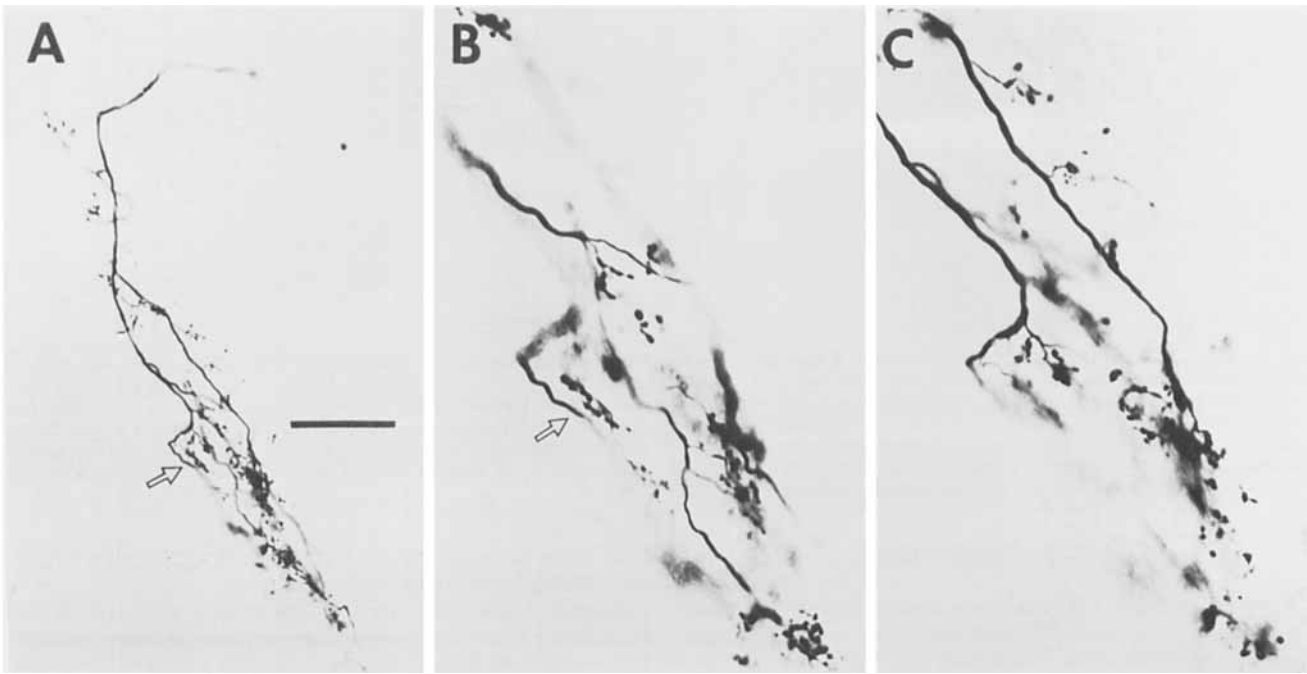


Fig. 3. Photomicrographs of a retinogeniculate X axon innervating lamina A from the remaining contralateral retina of a monocularly enucleated cat. A. Lower-power view. The axon effectively spans the vertical extent of lamina A, the laminar borders of which are just beyond the frame of the photograph. The scale represents 100 μm for A and 40 μm for B and C. B, C. Higher-power views of the same field at different focal planes. The arrows in A and B point to the same section of the arbor.

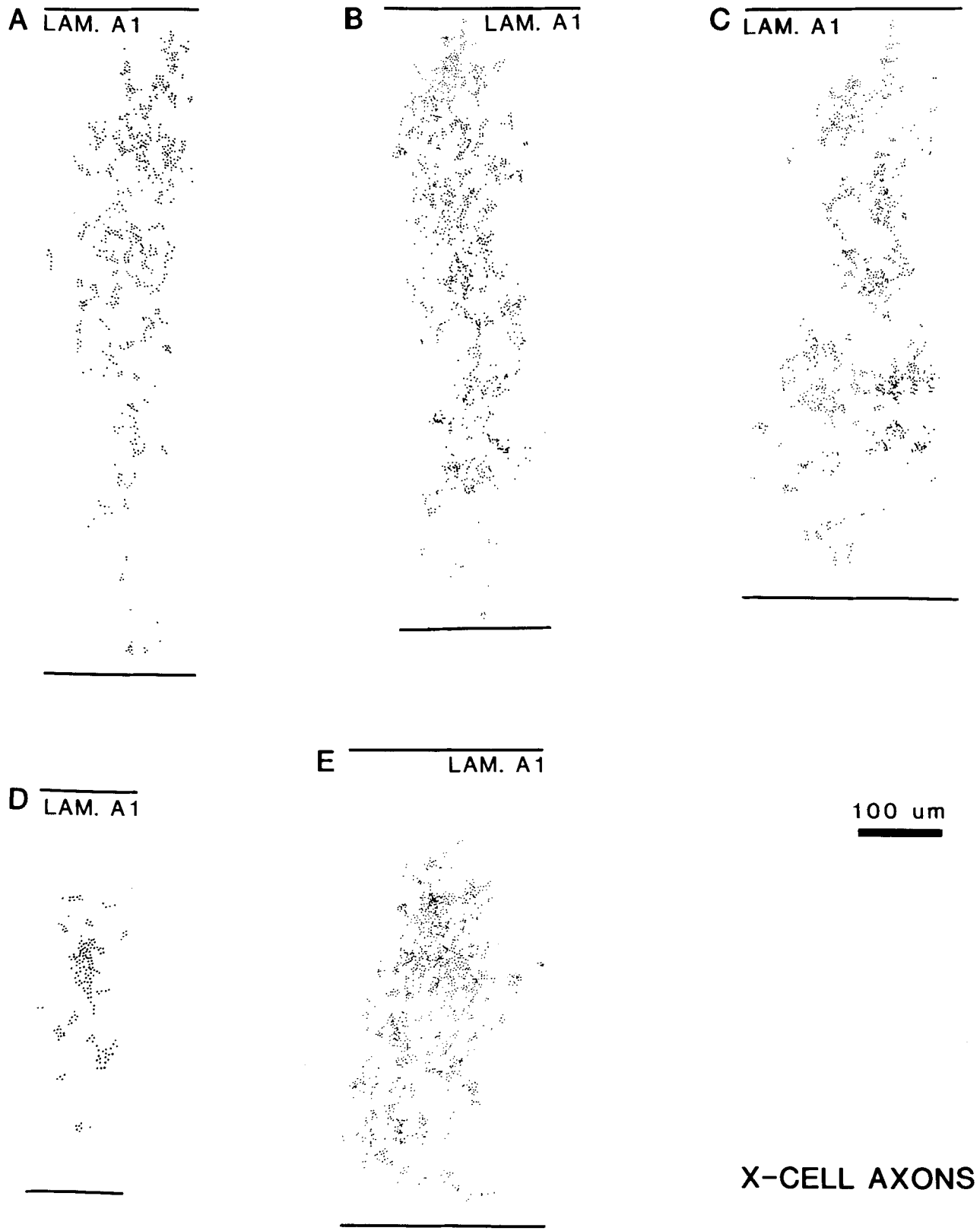


Fig. 4. Summary of terminal boutons from retinogeniculate X axons in monocularly enucleated cats. These represent ipsilaterally projecting axons that innervate lamina A1. The laminar borders are depicted by solid, horizontal lines. Each dot represents a single bouton in the same fashion as the drawing on the right in Figure 1. Note that all of these terminal arbors are completely confined to lamina A1.

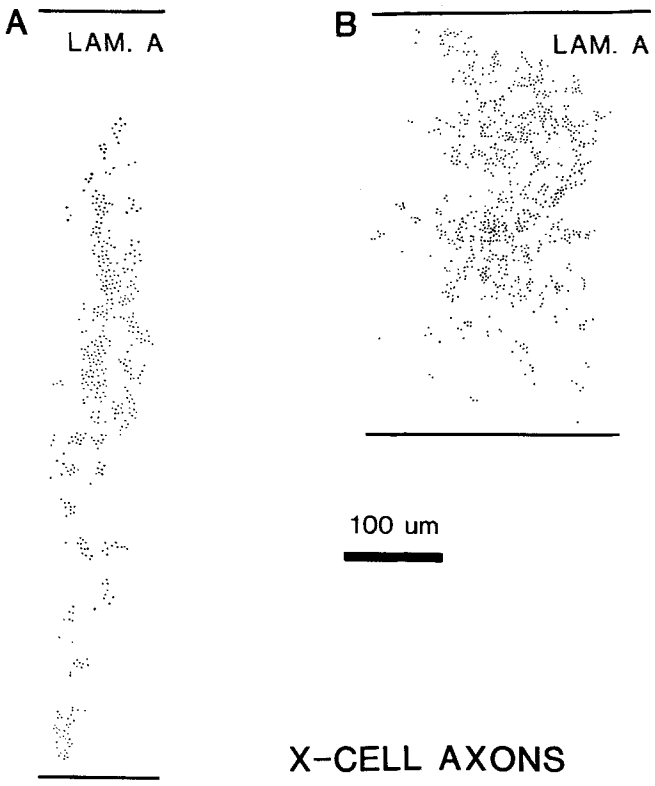


Fig. 5. Summary of terminal boutons from retinogeniculate X axons in monocularly enucleated cats. This is similar to Figure 4, except that these are contralaterally projecting axons that innervate lamina A.

its target lamina. None of the X axons recovered from monocularly enucleated cats displayed any terminal boutons in an adjacent, denervated lamina.

Y axons. Although some arbors from retinogeniculate Y axons in the monocularly enucleated cats seemed grossly normal while others showed clear evidence of translaminar invasion of denervated laminae, all displayed terminal bouton morphology typical of normal Y axons. That is, these boutons were irregular in size and shape, and they usually were more diffusely distributed throughout the terminal arbor. Parts B and C of Figure 2 are photomicrographs of representative examples of these boutons.

Figure 6 illustrates a Y axon from the remaining eye of a monocularly enucleated cat that innervates the ipsilateral lamina A1. The terminal arbor seems qualitatively normal with no evident translaminar extension into lamina A or C. Its arbor is noticeably broader than those of the X axons (see Figs. 1, 4, 5), a difference also seen in normal cats (Sur and Sherman, '82; Bowling and Michael, '84). Figure 7 summarizes the terminal pattern of the other two ipsilaterally projecting Y axons with qualitatively normal arbors limited to lamina A1.

Figure 8 shows the three ipsilaterally projecting axons with abnormal terminal patterns that extend beyond lamina A1. These axons that had clear translaminar sprouts also tended to have more terminal boutons (mean of 1,560) than those shown in Figures 6 and 7, which did not sprout translaminarly (mean of 1,242). Their average terminal volumes, however, were nearly identical, as were their physiological properties (see Table 1). In the examples in Figure 8, it is lamina C that is inappropriately innervated by translaminar growth. While we have yet to see an example of an ipsilaterally projecting Y axon with sprouts into lamina A in a monocularly enucleated cat, such innervation clearly exists (Guillery, '72; Hickey, '75; Robson et al., '78; Robson, '81), and we have seen such sprouting after monocular enucleation combined with lid suture of the remaining eye (see the following paper, Garraghty et al., '86).

Nearly all of the Y axons projecting contralaterally exhibited what we interpreted as inappropriate innervation of lamina A1. Figures 9 and 10 illustrate two such examples. The widths and overall shapes of these axon arbors are qualitatively normal for Y axons, as is the appearance and distribution of individual terminal boutons (Fig. 10B,C). Nonetheless, there is translaminar invasion of the terminal arbor into the previously denervated lamina A1. This seems to occur as a result of a dorsal extension of the arbor in lamina C and a ventral extension of the arbor in lamina A.

Figures 11 and 12 summarize the remaining terminal patterns of the contralaterally projecting Y axons. All but one of these exhibit clear extensions of terminal arbor into lamina A1. The one exception had a receptive field in the monocular segment (Fig. 12E). Since all of the inappropriate innervation of lamina A1 seen in the other axons illustrated in Figures 11 and 12 roughly follow retinotopic lines of projection (Sanderson, '71), it may be that axons innervating the monocular segment fail to invade lamina A1 because of retinotopic constraints. Finally, it is worth noting that the axon illustrated in Figure 12A is a Y axon that failed to innervate lamina C from the contralateral retina (Sur and Sherman, '82; Sur et al., '82, '84). Such axons are only rarely seen in normal cats (cf. Fig. 7 of Bowling and Michael, '84).

In summary, 11 of the 15 Y axons in the monocularly enucleated cats exhibited extensions of terminal arbor that innervated inappropriate geniculate laminae. This included three of six ipsilaterally projecting axons and eight of nine contralaterally projecting axons, the only exception among the latter being an axon from the monocular segment. This contrasts with the situation among X axons, for which none of the eight examples clearly innervated an inappropriate lamina. This difference between X and Y axons is statistically significant ($P < .001$, chi-square test).

Quantitative observations

Our qualitative impression was that the retinogeniculate axon arbors from the monocularly enucleated cats were larger and had more terminal boutons than did their counterparts from normal cats. To verify this impression, we measured the volume of each labeled arbor within laminae A, A1, and C and counted the number of boutons contained therein. Figure 13 summarizes these data taken for the eight X and 15 Y axons from the monocularly enucleated cats. Shown for comparison are data for eight X and 11 Y axons from normal cats.

For both X and Y axons, the mean volume of the terminal arbor was significantly greater in monocularly enucleated than in normal cats ($P < .01$ for both axon classes). The average volume for X axons was 0.00614 mm^3 in monocularly enucleated cats versus only 0.00239 mm^3 in normal cats. For Y axons, the average volume was 0.0194 mm^3 in monocularly enucleated cats versus only 0.0116 mm^3 in normal cats. Similarly, for both X and Y axons, the number of boutons was greater in monocularly enucleated cats than in normal cats, although the effects of monocular enuclea-

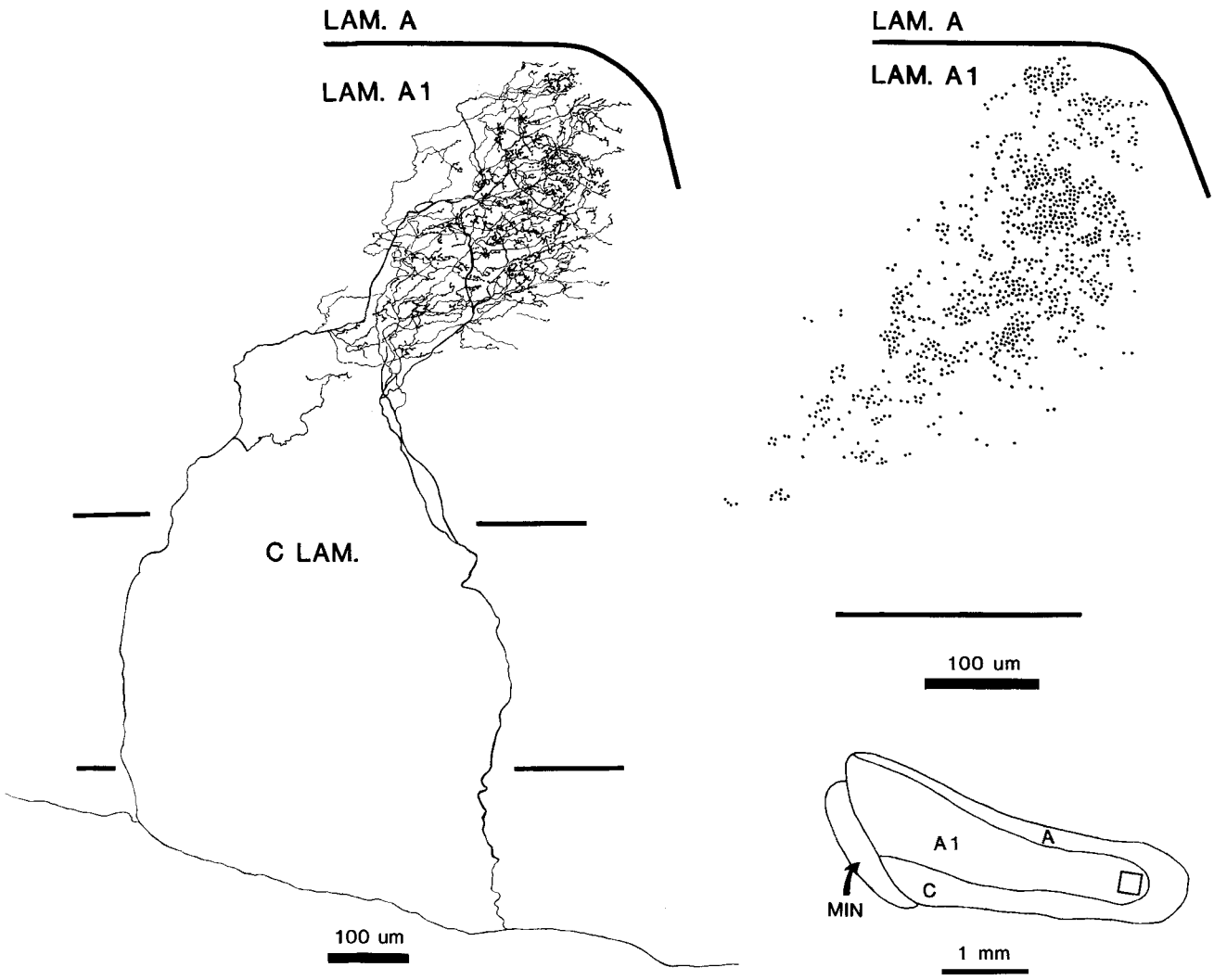


Fig. 6. Example of an HRP-labeled retinogeniculate Y axon in a monocularly enucleated cat; conventions as in Figure 1. The remaining eye is the right eye, and the axon innervates the right lateral geniculate nucleus with an arbor entirely limited to lamina A1.

tion were less dramatic on bouton numbers than on the volume of terminal arbors. The average number of boutons for X axons was 803 in monocularly enucleated cats versus 571 in normal cats ($P < .05$). For Y axons, the average numbers were 1,258 in monocularly enucleated cats versus 1,004 in normal cats, a difference that is less statistically significant ($P < .10$). Therefore, both X and Y axon arbors are larger in monocularly enucleated cats than in normal cats, although only the Y axons exhibit expansion into previously denervated laminae. Finally, since the abnormal increase in the volume of terminal arbors was greater for these retinogeniculate axons than was the abnormal increase in bouton numbers, it follows that the density of boutons was lower in monocularly enucleated cats than in normal cats. For X axons, the average bouton densities were 142,000/mm^3 in monocularly enucleated cats and 256,000/mm^3 in normal cats, a statistically significant difference ($P < .001$). For Y axons, the averages were 73,000/mm^3 in monocularly enucleated cats and 98,000/mm^3 in normal cats ($P < .05$).

DISCUSSION

Pathway tracing studies have shown that retinogeniculate axons from the remaining eye of a monocularly enucleated cat can abnormally innervate the previously denervated geniculate laminae (Guillery, '72; Hickey, '75; Robson et al., '78; Robson, '81). We extended these observations at the single axon level in monocularly enucleated cats with the technique of intra-axonal HRP labeling of physiologically identified retinogeniculate axons. This approach has revealed dramatic differences in the reaction of X and Y axons to neonatal monocular enucleation. Both axon classes develop abnormally large terminal arbors in the lateral geniculate nucleus, but only the Y axons appear to extend into the previously denervated laminae. This difference between retinogeniculate X and Y axons is consistent with other developmental differences previously noted for these axons and helps to clarify the underlying mechanisms controlling their development.

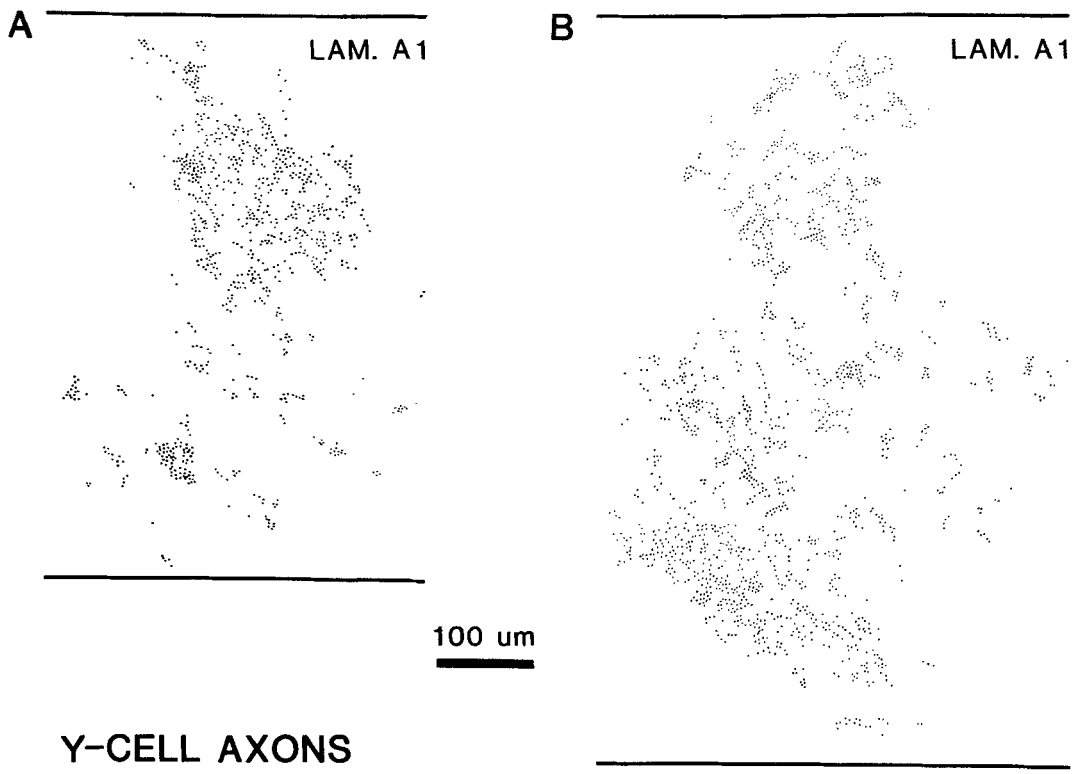


Fig. 7. Summary of terminal boutons from ipsilaterally projecting retinogeniculate Y axons in monocularly enucleated cats; conventions as in Figure 4. Like the example illustrated in Figure 6, these axon arbors are entirely confined to lamina A1, which is normally the appropriate terminus for these axons.

Retinogeniculate innervation patterns in monocularly enucleated cats

A major conclusion from our data is that the translaminar expansion of retinogeniculate axon arbors seen in monocularly enucleated cats is not a generalized response of all retinogeniculate axons in these cats. Rather, none of the X axons of our sample exhibited such expansion, while most of the Y axons did. It thus seems plausible that all or most of the translaminar expansion seen in monocularly enucleated cats (Guillery, '72; Hickey, '75; Robson et al., '78; Robson, '81) reflects the abnormal development of Y axons.

This conclusion must be qualified by the uncertainty of our assignation of laminar borders in the monocularly enucleated cats of this study. It is possible that we overemphasized the extent of normally innervated laminae and thereby missed limited sprouting of some retinogeniculate X axons. While this would not alter one of our major conclusions—namely, that the Y axons exhibit considerably greater ability to form translaminar sprouts than do X axons—we feel that evidence from a parallel set of experiments renders it most unlikely that we failed to detect such sprouts of X axons. In cats raised with monocular enucleation at birth paired with lid suture of the remaining eye, retinogeniculate X arbors are completely confined to their appropriate laminae, and this conclusion is less ambiguous because the laminar borders are much more clearly defined in these cats (see the following paper, Garraghty et al., '86). Yet X axon arbors in these monocularly enucleated and lid sutured cats are also abnormally large, with numbers of boutons comparable to those in cats with monocular enucleation alone. Given the differential development of X and Y retinogeniculate axon arbors, possibly involving competition between the two classes of afferents (see below), monocular enucleation coupled with lid suture should favor the expansion of X arbors even more than monocular enucleation alone. The fact that X arbors in the monocularly enucleated and lid-sutured cats still remain confined to their appropriate lamina suggests strongly that they are similarly confined in monocularly enucleated cats (see also Discussion in the following paper, Garraghty et al., '86).

Axonal sprouting versus lack of retraction. An interesting issue regarding this expansion of retinogeniculate Y axon arbors is whether or not it reflects actual sprouting of terminal processes. It may instead reflect the stabilization of an immature pattern of retinogeniculate innervation in which many axon arbors exuberantly invade most or all laminae. Shatz and her colleagues (Shatz, '83; Shatz and Kirkwood, '84; Sretavan and Shatz, '84) have demonstrated, however, that while retinogeniculate axons from the two eyes of prenatal kittens have overlapping arbors in laminae A and A1, little or no overlap is present by birth. Since the enucleations in the present study were performed very soon after birth, the abnormal growth of retinogeniculate Y axon arbors evident in the previously denervated laminae, particularly given its magnitude, reflects true sprouting and not a failure to retract immaturely exuberant arbors.

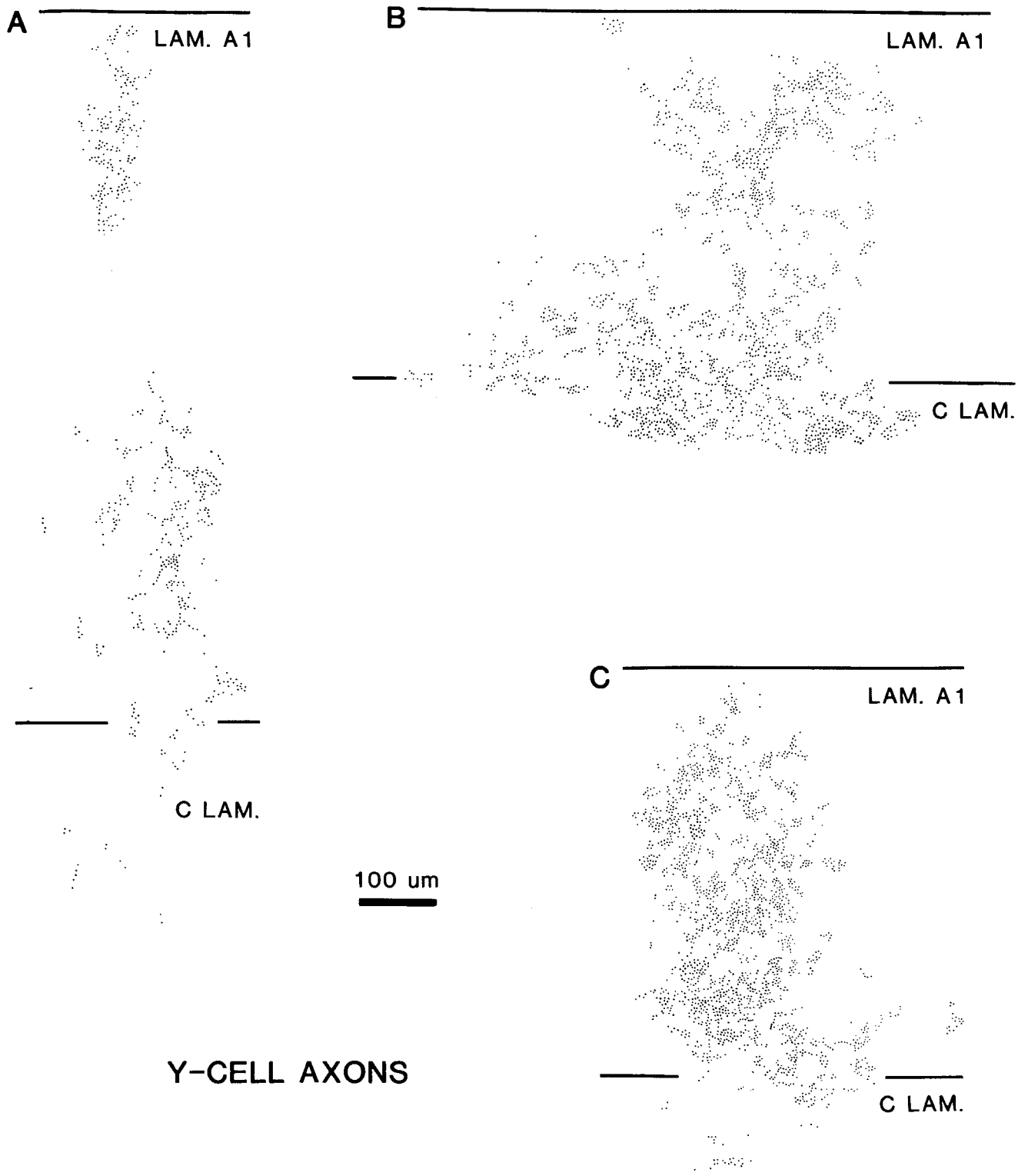


Fig. 8. Summary of terminal boutons from ipsilaterally projecting retinogeniculate Y axons in monocularly enucleated cats; conventions as in Figure 4. These axons differ from those depicted in Figure 7, because their arbors clearly extend into lamina C, which is normally an inappropriate terminus for these axons.

TABLE 1. Summary of the Physiological Properties of the Retinogeniculate Axons From Monocularly Enucleated Cats That are Analyzed in Detail in the Present Study, and the Figure(s) in Which They are Illustrated

Axon class	Center type	Center size	Optic chiasm latency (msec)	Eccentricity	Figure
X	On	2.5°	0.8	68°	1
X[1]	On		0.75		3, 4A
X	On	0.8°	0.75	47°	2A, 4B
X	On	0.8°	0.8	15°	5A
X[2]	On	1.7°		11°	5B
X	On	0.4°	0.9	12°	5C
X	On	0.8°	0.9	41°	5D
X	On	0.8°	0.9	9°	5E
Y	Off	3.0°	0.5	57°	6
Y	On	2.3°	0.5	25°	7A
Y	On	2.0°	0.4	35°	7B
Y	On	2.3°	0.4	27°	8A
Y[1]	On		0.5		8B
Y[1]	On		0.4		8C
Y	Off	2.5°	0.5	32°	9
Y	Off	4.2°	0.4	29°	10, 12B
Y	Off	3.3°	0.5	18°	2B, 11A
Y	Off	1.5°	0.5	5°	11B
Y	On	1.9°	0.5	13°	2C, 11C
Y	On	1.6°	0.5	49°	12A
Y[1]	On		0.5		12C
Y[1]	On		0.5		12D
Y	On	2.0°	0.5	46°	12E

[1]These results were from a single cat in which ^{3}H-proline was injected into the remaining eye. The optics were sufficiently blurred in this cat due to the injection that retinal axons would not respond to small spots of light, and we could define their receptive field locations only roughly. Statistical comparisons involving the morphology of X and Y axons in normal and monocularly enucleated cats were identical whether these axons were included or not. The descriptive values given in the text include these data.
[2]This unit could not be driven electrically by optic chiasm stimulation.

Since the X axons in the monocularly enucleated cats also exhibit abnormally large arbors, albeit within their appropriate laminae, it is possible that they also sprout. However, it seems more likely that these arbors simply fail to contract from their immature exuberant state (see also below). The alternate explanation, that the X arbors undergo a more-or-less normal phase of retraction followed by a secondary phase of expansion due to the enucleation, seems unnecessarily complex to us. We must emphasize, however, that we cannot rule out this latter possibility with our presently available evidence.

Ipsilateral versus contralateral sprouting in Y axons. In our material, sprouting seemed more pronounced and common among the contralaterally projecting retinogeniculate Y axons than among those projecting ipsilaterally. For two reasons, however, we are reluctant to draw strong conclusions from these observations. First, due to the above-mentioned distortions in laminar patterns following monocular enucleation, it was easier to determine the borders between laminae in the lateral geniculate nucleus contralateral to the remaining eye than in the ipsilateral nucleus. Consequently, we conservatively assigned laminar borders ipsilateral to the remaining eye in such a way that we may well have biased our results against detecting sprouting there. Nonetheless, the ipsilaterally projecting Y axons were hypertrophied from normal and some clearly invaded lamina C. Second, observations from pathway tracing studies (Guillery, '72; Hickey, '75; Robson et al., '78; Robson, '81) clearly point to invasion of lamina A from axons of the remaining ipsilateral retina. However, our enucleations were performed within 1 day of birth, whereas these other studies included later (up to 7–10 days postnatal) enucleations. These later enucleations result in a less ambiguous pattern of geniculate lamination (cf. Hickey, '75), and this may make the sprouting easier to detect.

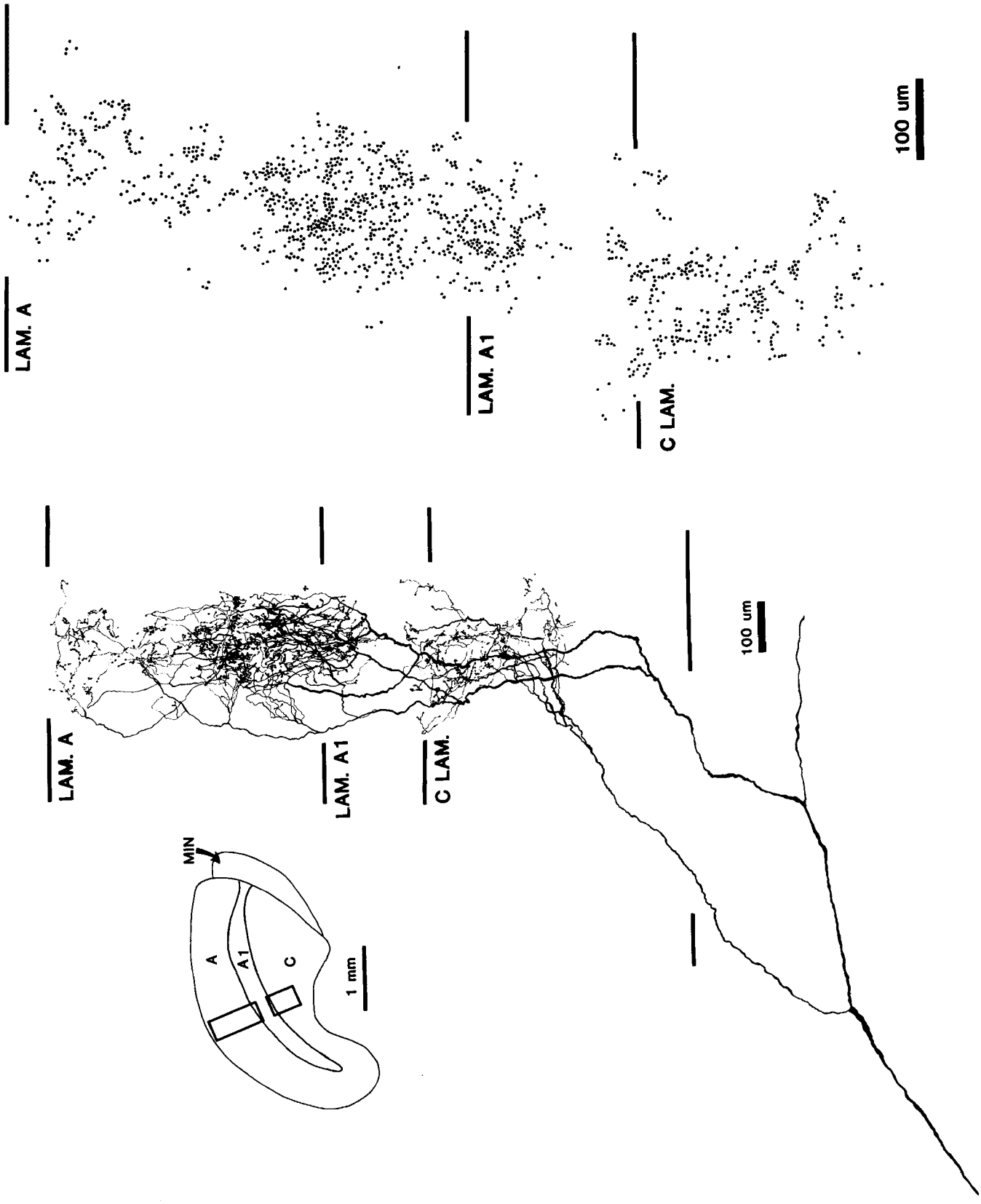
Retinotopic nature of sprouting. All of our examples of translaminar sprouting faithfully followed lines of projection within the retinotopic map of the lateral geniculate nucleus (Sanderson, '71). Prior studies of monocularly enucleated cats have stressed an additional form of sprouting that does not seem to obey retinotopy—that is, invasion of the monocular segment from axons of the remaining ipsilateral eye (Hickey, '75; Robson, '81). Such sprouts could arise from three sources. First, axons terminating in the most lateral regions of lamina A1 could sprout into the adjacent denervated monocular segment of lamina A, and Robson ('81) has shown such growth using bulk-filling methods. We have also seen such growth in monocularly enucleated and visually deprived cats (Garraghty et al., '86). Second, it is possible that some of these monocular segment sprouts do not derive from X or Y axons. They might, for instance, derive from W axons. Although W axons do not normally innervate lamina A or A1, they might do so in monocularly enucleated cats. W axons may be particularly drawn to the monocular segment ipsilateral to the remaining eye if no X or Y axons sprout to occupy this region. Finally, monocular segment growth could result from the stabilization of a small, normally occurring ipsilateral retinal projection to the monocular segment, which is rather common in normal cats (Polley and Guillery, '80).

Differential development of retinogeniculate X and Y axons

As noted above, considerable evidence already exists that retinogeniculate X and Y axons develop at different rates and possibly by different mechanisms (reviewed in Sherman, '86b). In normal kittens at 3–4 weeks of age, retinogeniculate X axons have larger arbors with more boutons than are found for X axons in adults, while Y arbors in these kittens are much smaller, with fewer boutons than seen in adults (Sur et al., '84). Retinogeniculate X axons thus seem to develop earlier than do the Y axons. In many developing pathways, the first axons to innervate a terminal zone form exuberant arbors that become pruned as other axons develop innervation to this zone (e.g., Purves and Lichtman, '80; Cowan et al., '84). Perhaps the earlier-developing X axons form exuberant arbors in the lateral geniculate nucleus at 3 weeks of age, and these are later cut back through some sort of competitive process as retinogeniculate Y axons begin to develop their innervation.

The results of rearing with monocular lid suture are consistent with the interpretation that some sort of competitive interaction occurs between developing retinogeniculate X and Y axons (Sur et al., '82; and the following paper, Garraghty et al., '86). Lid suture seems to interfere with the ability of Y axons to prune the exuberant, earlier-

Fig. 9. Example of an HRP-labeled retinogeniculate Y axon in a monocularly enucleated cat; conventions as in Figure 1. The remaining eye is the right eye, and the axon innervates the left lateral geniculate nucleus. Although most of the terminal arbor is located in laminae A and C, some of it extends into lamina A1, which normally receives retinal input only from the ipsilateral eye.


LAM. A
LAM. A1
C LAM.
100 um
LAM. A
LAM. A1
C LAM.
100 um
MIN
A
A1
C
1 mm

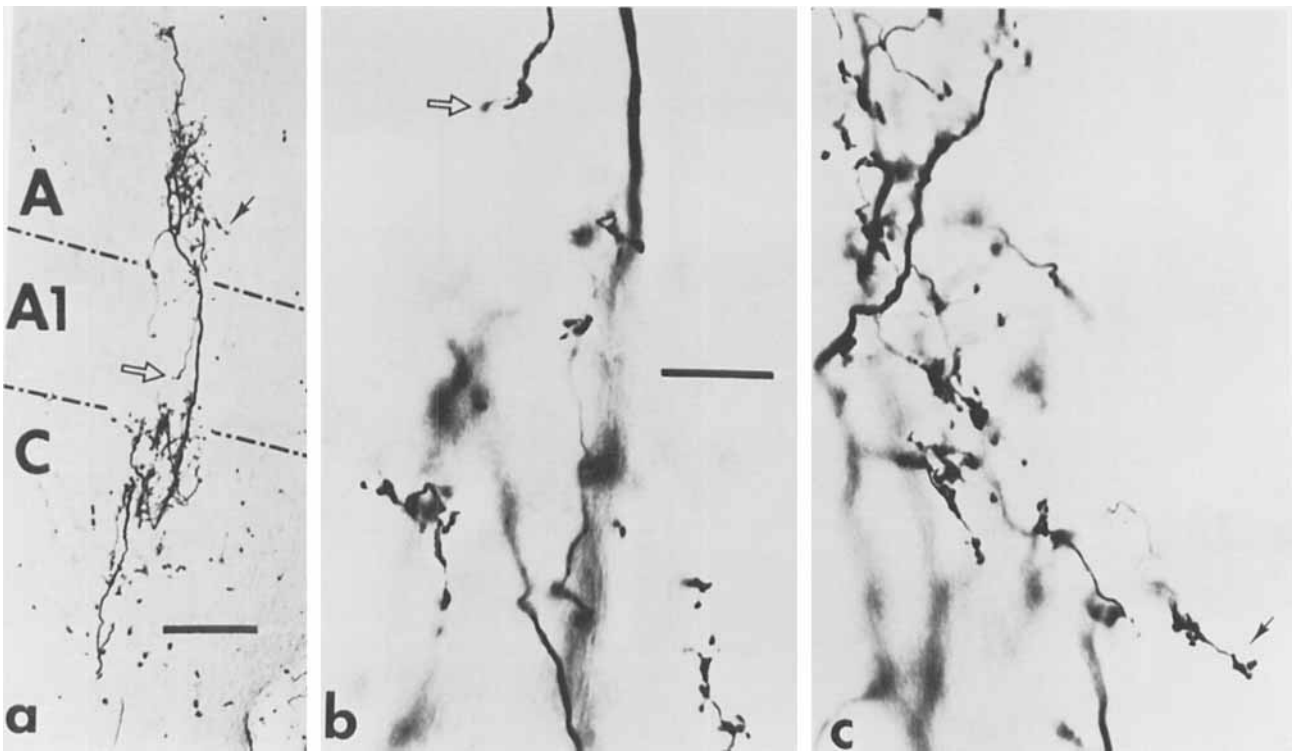


Fig. 10. Photomicrographs of another contralaterally projecting Y axon from a monocularly enucleated cat. A. Lower-power view. The borders between laminae A (A), A1 (A1), and C (C) are shown by dashed lines. The scale bar is 200 μm. B. Higher-power view of terminal boutons in the previously denervated lamina A1. The scale is 40 μm and applies to C as well. C. Higher-power view of portion of terminal arbor in lamina A. The open arrows in A and B point to the same region of the arbor, as do the solid arrows in A and C.

developed X axon arbors. X axons from the deprived retinas thus maintain abnormally large arbors in lamina A or lamina A1, and Y axons from these eyes fail to develop normal arbors there. However, the development of Y axon arbors from the deprived retinas is patently retarded only in regions where X arbors are already present. Thus deprived Y axons from the contralateral retina often fail to innervate lamina A with a normal arbor, but they form normal arbors in lamina C, where X arbors never develop to any significant degree (cf. Sur and Sherman, '82; Sur et al., '84; Friedlander et al. '85; Bowling and Michael, '84). Further evidence of such competitive interactions is provided in the following paper (Garraghty et al., '86). We can explain many, but not all, of the observations of the present experiments in the context of such competitive interactions between developing retinogeniculate X and Y axons.

Given these developmental differences between retinogeniculate X and Y axons and evidence for competition between these axon classes during development, there are three likely explanations for the observation that only the Y axons exhibit translaminar sprouts as a result of neonatal monocular enucleation. First, the X axons may be at such great competitive disadvantage with respect to the Y axons in terms of forming translaminar sprouts into the denervated laminae that they are barred from these laminae by the Y axons that monopolize terminal space there. In a parallel set of experiments presented in the following paper (Garraghty et al., '86), competitive advantage conferred to the X axons by lid suturing the remaining eye failed to elicit translaminar sprouts in these axons. This makes it less likely that the X axons fail to sprout into the denervated laminae simply because of a competitive disadvantage. The second plausible explanation derives from the different developmental periods for these retinogeniculate X and Y axons. Since X axons mature earlier than do Y axons (Sur et al., '82; reviewed in Sherman, '85b), it may be that by birth only the latter axons look forward to dramatic growth and have the capacity to form translaminar sprouts. The X axons may have already passed through a sort of "critical period" by birth and no longer possess the capacity to form translaminar sprouts. If so then a much earlier prenatal enucleation should elicit such sprouting from X axons. However, preliminary results suggest that this is not the case (Sretavan, et al., '85). The final, and in our opinion most likely, explanation is that the retinogeniculate X and Y axon classes may develop via qualitatively different mechanisms such that only Y axons are endowed with the potential to form translaminar sprouts. These possibilities are more completely discussed in the following paper (Garraghty et al., '86).

In any case, only the Y axons seem to have the capacity to form terminal arbors in the denervated laminae. They

Fig. 11. Summary of terminal boutons from contralaterally projecting retinogeniculate Y axons in monocularly enucleated cats; conventions as in Figure 4. Note that, in addition to their innervation of laminae A and C, all three terminal arbors extend into lamina A1, which is abnormal for axons from the contralateral retina.

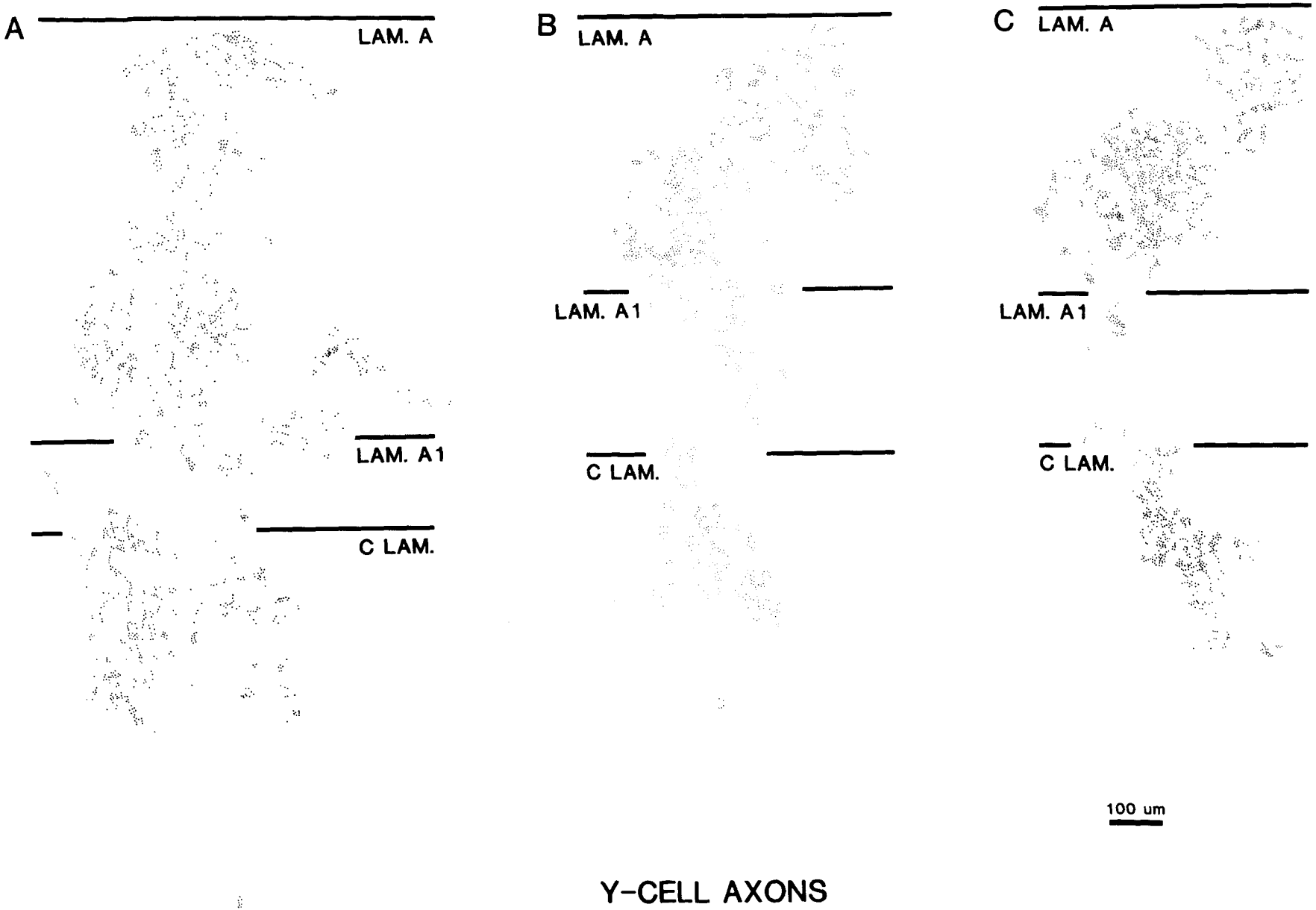

A
LAM. A
LAM. A1
C LAM.
B
LAM. A
LAM. A1
C LAM.
C
LAM. A
LAM. A1
C LAM.
100 um
Y-CELL AXONS

Figure 11

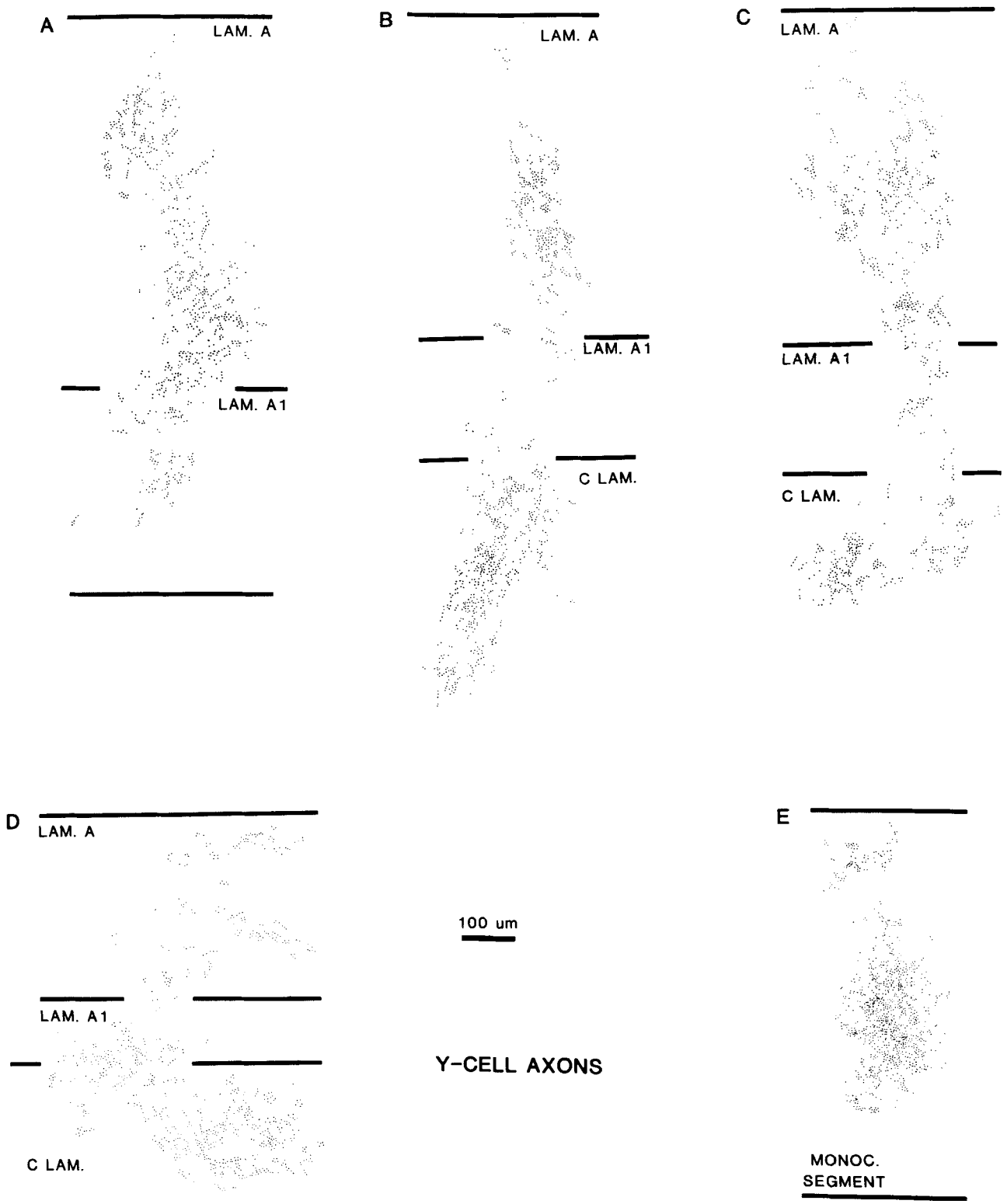


Fig. 12. Summary of terminal boutons from additional contralaterally projecting retinogeniculate Y axons in monocularly enucleated cats not illustrated in Figures 9 and 11; conventions as in Figure 4. A. Axon with innervation of lamina A and an extension of its terminal arbor into lamina A1, but without innervation of lamina C. B–D. Axons with terminal patterns much like those illustrated in Figure 11. E. Axon with innervation of the monocular segment of lamina A and/or lamina C with no detectable innervation of lamina A1.

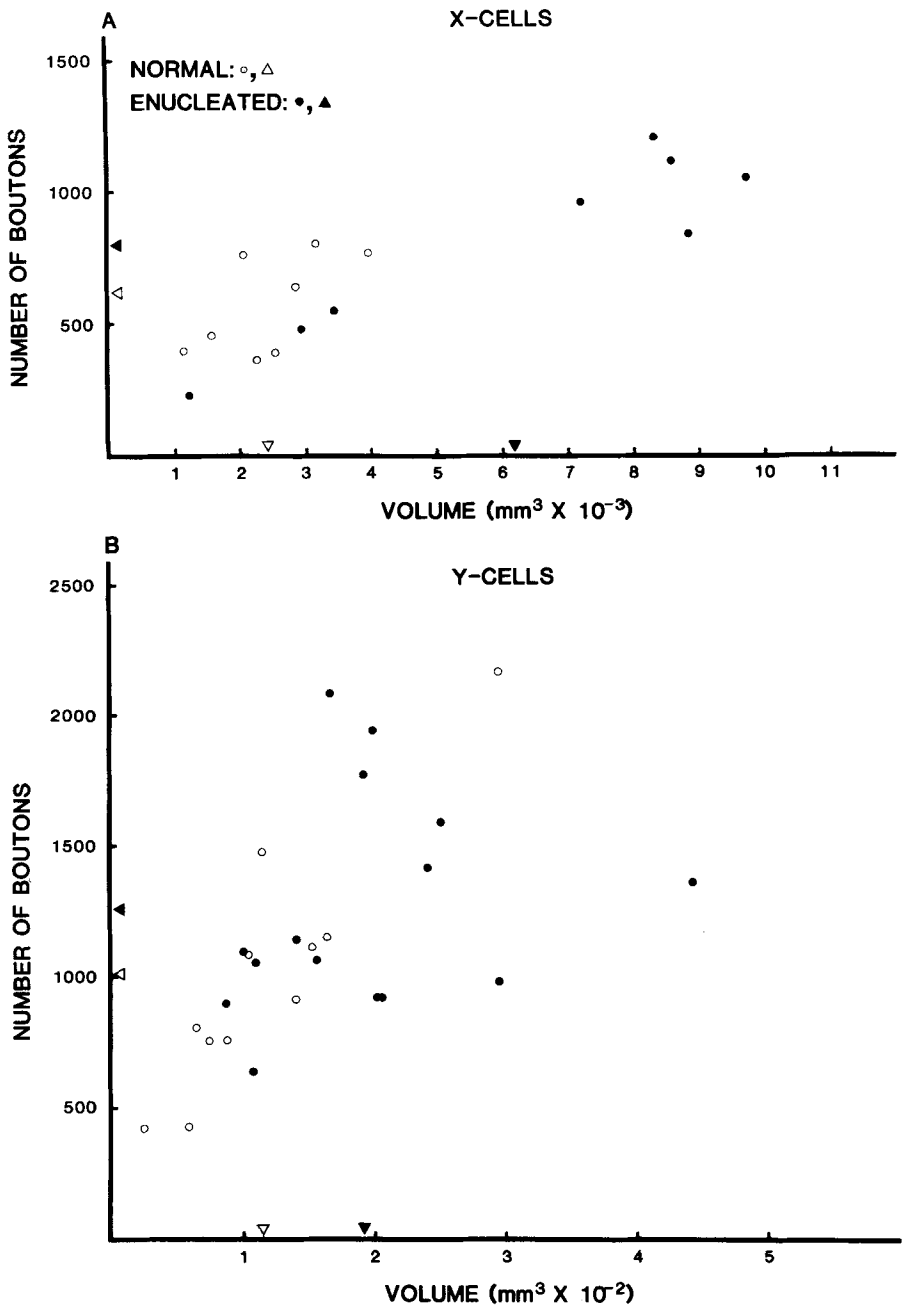


Fig. 13. Plots of the volume of terminal arbor for each retinogeniculate axon versus the number of terminal boutons each contains. These measurements were limited to laminae A, A1, and C, and they consequently do not include the medial interlaminar nucleus. These plots represent the axons from the monocularly enucleated cats and axons from normal cats reported elsewhere (Sur and Sherman, '82; Esguerra et al., '85), and they separately depict these relationships for X axons in A and Y axons in B. Each circle represents a single axon. The triangles on each axis reflect the average values for each axonal population. Note the different scale magnitudes on the abscissae in A and B.

may do so because the denervated laminae offer an avenue for growth of Y axon arbors that is less resistant than one involving competition with the already present X arbors, and this reduced competitive pressure permits the Y arbors to hypertrophy, mostly via expansion into the previously denervated laminae. This process also reduces the competitive pressure on the X arbors, which allows them to maintain their abnormally large sizes and numbers of terminal boutons. The use of such competitive interactions between precociously exuberant X axon arbors and later-developing Y axon arbors to explain our results in monocularly enucleated cats seems parsimonious, because a single competitive

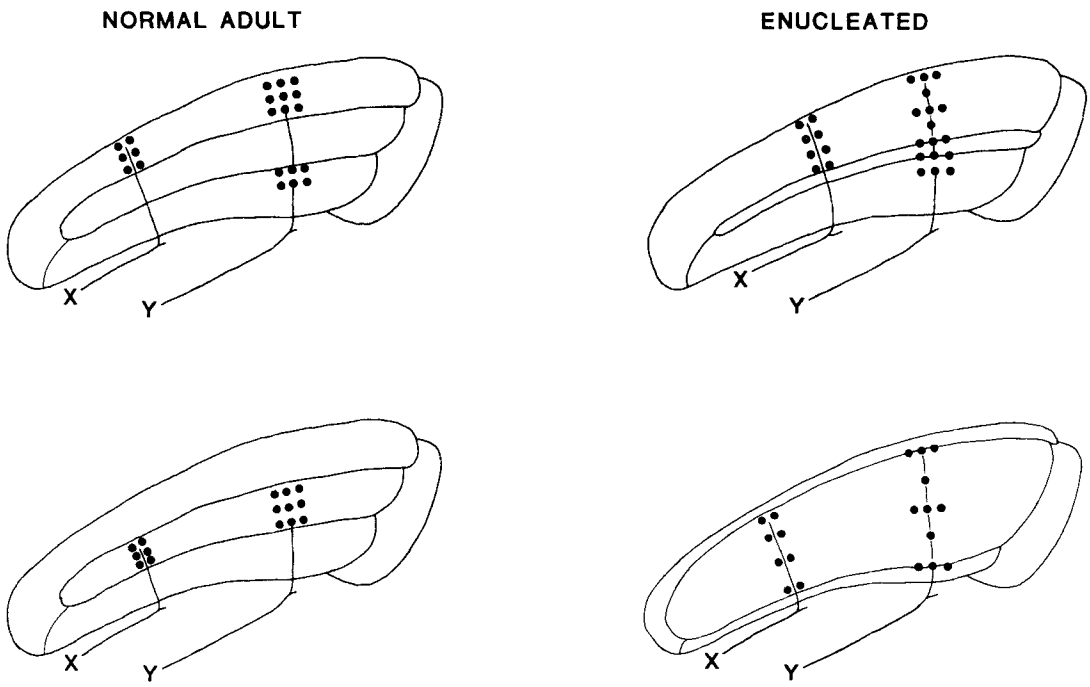


Fig. 14. Schematic summary diagram of results reported in this paper. The dots represent terminal boutons, and only arbors in laminae A, A1, and C are depicted. In the normal adult cat, retinogeniculate X and Y axons terminate exclusively within their appropriate laminae as shown. In monocularly enucleated cats, both X and Y axon arbors are abnormally large. For X axons, this hypertrophy occurs entirely within lamina A or lamina A1, depending on which is appropriate for the axon's eye of origin. All of the translaminar sprouting into inappropriate laminae is confined to arbors of the Y axons. However, this sprouting may be more pronounced for contralaterally projecting Y axons than for ones projecting ipsilaterally.

mechanism can be invoked to explain much of the different developmental phenomena related to retinogeniculate X and Y axons.

Conclusions

Figure 14 summarizes our results. Monocularly enucleated cats exhibit retinogeniculate axons from the remaining eye that have abnormally large terminal arbors containing more than the normal number of boutons. This is true for both X and Y axons. However, the hypertrophy of each X axon arbor is confined to lamina A or lamina A1, and the lamina is always appropriate for the retina of origin (i.e., ipsilateral or contralateral) for the X axon. In contrast, the Y axons form sprouts into the previously denervated geniculate laminae. We thus conclude that most or all of the translaminar sprouting found in monocularly enucleated cats (Guillery, '72; Hickey; '75; Robson et al., '78; Robson, '81) reflects Y axons and not X axons. This is yet another example of a fundamental developmental difference between the X and Y pathways.

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