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Regulation of Retinal Ganglion Cell Axon Arbor Size by Target Availability: Mechanisms of Compression and Expansion of the Retinotectal Projection

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ABSTRACT

The ability of pre- and postsynaptic populations to achieve the proper convergence ratios during development is especially critical in topographically mapped systems such as the retinotectal system. The ratio of retinal ganglion cells to their target cells in the optic tectum can be altered experimentally either by early partial tectal ablation, which results in an orderly compression of near-normal numbers of retinal projections into a smaller tectal area, or by early monocular enucleation, which results in the expansion of a reduced number of axons in a near-normal tectal volume. Our previous studies showed that changes in cell death and synaptic density consequent to these manipulations can account for only a minor component of this compensation for the population mismatch.

In this study, we examine other mechanisms of population matching in the hamster retinotectal system. We used an in vitro horseradish peroxidase labeling method to trace individual retinal ganglion cell axons in superior colliculi partially ablated on the day of birth, as well as in colliculi contralateral to a monocular enucleation. We found that individual axon arbors within the partially lesioned tectum occupy a smaller area, with fewer branches and fewer terminal boutons, but preserve a normal bouton density. In contrast, ipsilaterally projecting axon arbors in monocularly enucleated animals occupy a greater area than in the normal condition, with a much larger arbor length and greater number of boutons and branches compared with normal ipsilaterally projecting cells. Alteration of axonal arborization of retinal ganglion cells is the main factor responsible for matching the retinal and tectal cell populations within the tectum. This process conserves normal electrophysiological function over a wide range of convergence ratios and may occur through strict selectivity of tectal cells for their normal number of inputs.

Key words: retinotectal, synaptic specificity, population matching, hamster, axon arbor

Orderly mapping of one neuronal population onto another is a general feature of the assembly of sensory systems. Many studies have investigated the mechanisms responsible for producing organized topographic maps that preserve nearest-neighbor relationships (reviewed in Udin and Fawcett, 1988). Topographic mapping, however, is only one feature of population matching. Maintenance of appropriate convergence ratios is critical for normal function, such as preservation of spatial acuity, while allowing integration of information. Particular interconnecting populations of neurons in the nervous system have characteristic convergence ratios that range from thousands of granule cells to one cerebellar Purkinje cell (Wetts and Herrup, 1983), to the one-to-one mapping of cones to bipolar cells in the primate fovea (reviewed in Rowe, 1991). The variability allowed for individuals and species in convergence ratios is large, and the mechanisms that preserve function in the face of this variability are poorly understood (Williams and Herrup, 1988; Finlay and Pallas, 1989). Knowledge of the developmental mechanisms that produce an appropriate convergence ratio between pre- and postsynaptic populations is as essential for understanding the establishment of orderly interconnections between two populations of distant neurons as are the principles of topographic mapping.

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The problem of mapping has been well studied in the vertebrate retinotectal projection, and this system also allows investigation of the control of projection convergence and divergence. Compression of the retinal projection into a smaller than normal tectal volume was first demonstrated in the regenerating retinotectal projection of goldfish and frogs (Gaze and Sharma, 1970; Sharma, 1971, 1972; Udin, 1977). Orderly compression of the visual field map also occurs in the hamster, where detailed study of postsynaptic response properties can be made (Jhaveri and Schneider, 1974; Finlay et al., 1979b). Interestingly, normal receptive-field properties and receptive-field sizes are maintained under this whole-field compression (Pallas and Finlay, 1989).

Several mechanisms could be responsible for the compression of excess retinal axons into a reduced tectal volume. Developmental cell death does not account for it: neuronal death in ganglion cells shows only a minor increase (8%) after tectum lesions as large as 50%, and cell death rates in the tectum remain normal (Wikler et al., 1986). There are several remaining possibilities. First, terminal sites in the tectum (the dendritic arbor of tectal cells) could enlarge. Second, the density of synaptic sites on dendritic trees could increase. Third, retinotectal axon arbors could be reduced or be partially redirected.

In the partially ablated hamster tectum, adult cell size and density are normal, making it unlikely that dendritic arbor has increased (Wikler et al., 1986; Xiong and Finlay, 1993). Studies on synaptic density in both goldfish and hamster tecta suggest that postsynaptic terminal sites remain constant in the partially ablated tectum (Murray, et al., 1982; Hayes and Meyer, 1988a,b; Xiong and Finlay, 1992, in press). Pallas and Finlay (1991) showed that the number of ganglion cells projecting to a defined tectal area is supernormal in covering a larger area of visual space, but that the density of labeled ganglion cells in the retina is reduced. This suggests that at least two mechanisms operate in this case of compression: a higher percentage of ganglion cells have sole projections to nontectal targets than normal and other ganglion cells have reduced their arbor in the tectum. An abstract by Sachs and Schneider (1981) also suggests that retinal ganglion cell arbors in the tectum are reduced after partial tectal ablation.

Work with the partial tectal lesion case thus suggests strongly that the locus of regulation of afferent/target convergence is largely confined to the axon in cases of target depletion. In the case of greater available target, do axons enlarge their arbors? Monocular enucleation in hamster is a good model for this question. Neonatal removal of one eye in hamster results in the loss of the normal massive contralateral projection to the superior colliculus and an increased ipsilateral projection from the remaining eye, which is due to both sprouting of the ipsilateral projection in the temporal retina and also to a stabilized, exuberant projection from the contralateral nasal retina (Finlay et al., 1979a; Rhoades, 1980; Sengelaub et al., 1983; Thompson, 1979). Even with the increase in volume of the ipsilateral projection, retinal synaptic density is reduced in the tectum (Xiong and Finlay, 1993). It is unknown what conformation single retinotectal axons take in this larger terminal space.

Our goal in this study was to confirm or deny that axon arborization is the principal locus of plasticity in alteration of convergence ratios between interconnecting populations, and to explore the relationship between altered axonal structure and the other electrophysiological and anatomical reorganizations that occur consequent to these manipulations. We thus examined the range of alterations induced in retinotectal axon arborizations in the superior colliculus of normal hamsters, hamsters with early partial collicular lesions, and hamsters with monocular enucleations by using an in vitro horseradish peroxidase (HRP) bulk-filling procedure. Preliminary reports of this study have been made (Xiong et al., 1991; 1992).

METHODS

Thirty-six golden hamsters (*Mesocricetus auratus*) were used in this study. All animals were bred in our laboratory colony, were maintained on a 12-hour light/12-hour dark cycle, and were fed ad libitum on rat chow and water.

Neonatal surgery

Neonatal surgeries were done within 24 hours of birth, before the majority of retinal axons have formed synaptic connections in the superior colliculus (SC; Frost et al., 1979; Woo et al., 1985). Hamster pups were taken from the mother and anesthetized by induction of mild hypothermia. One group of hamster pups (the partial tectal ablation, or PT, group) received unilateral lesions of the caudal half of the right SC. The skin on the head was cut and retracted to reveal the SCs through the translucent skull. A heated needle was applied to the skull above the caudal portion of the right SC. This procedure effectively ablated the caudal part of the SC without the need to open the cranium. The skin was then sutured. The other group of hamster pups (neonatal-ENUC) received monocular enucleation. A small slit under the prospective eyelid was made and then the left eye was withdrawn with fine forceps. Hamster pups were then warmed and returned to the mother. Two control groups of hamsters, a normal groups of hamsters (normal group) received no surgery, and a second group (normal-IPSI) received monocular enucleation at adulthood.

Adult surgery and HRP injection

When the subjects were at least 3 months old (when the brain has reached its mature size), they were anesthetized by intraperitoneal injection of pentobarbitol sodium solution (15 mg/100 g). Once anesthetized, the hamsters were perfused through the heart with cold, oxygenated Ringer solution for 5 minutes. Following the perfusion, the brains were removed as rapidly as possible and the SCs were dissected out. With the aid of the dissecting microscope, a bead of HRP dried onto the tip of a micropipette was placed just under the surface of the optic tract immediately adjacent to the rostral margin of the SC, following the procedure of Hahm et al. (1991). Care was taken not to inject too deeply to avoid the unwanted staining of nonretinal axons, or backfilling of deep collicular neurons. Immediately after injection, the tissue was placed in warm, oxygenated Ringer solution and incubated for 3 to 4 hours. Oxygenation of the solution was continuously monitored by a phenol indicator. The SC tissues were then placed in a 1% paraformaldehyde and 1.25% glutaraldehyde solution for 2 to 2.5 hours followed by 30% buffered sucrose solution until the tissue sank.

Histology

SCs were embedded in albumin gelatin, hardened in glutaraldehyde, and sectioned on a freezing microtome. Two different sectioning orientations were used. Some of the SCs were cut along the "axon entry plane," which we define as the plane that extends dorsoventrally perpendicu-

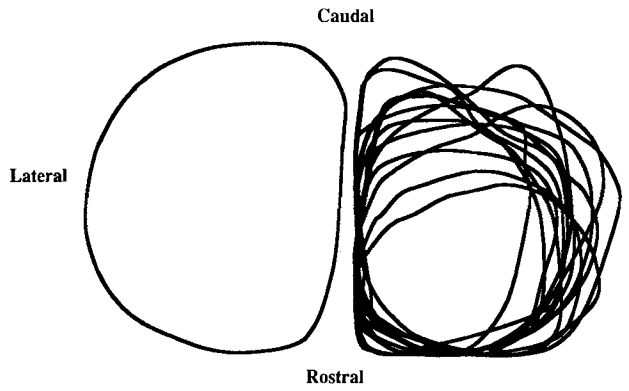

Fig. 1. **Left:** A dorsal view of the extent of the normal superior colliculus. **Right:** Overlaid dorsal view reconstructions of the 13 partial tectal lesion animals used in this study, with the remaining surface areas ranging from 35 to 70% of normal.

lar to the optic tract entering into the tectum, approximately midway between the coronal and midsagittal planes. This plane best demonstrates the depth of axon arborization in the SC along the direction of axon entry. The remainder were cut in the "retinotopic plane," which is tangent to the surface of the SC and shows best the two dimensions (approximately mediolateral and rostrocaudal) in which compression or expansion of arbor is presumed to occur. Although our measurement techniques collapse information into two dimensions for each individual axon, the use of these two sectioning planes allows us to quantify any alterations in three-dimensional distribution of the axons.

The sections were cut in 100-μm slices and collected in a 0.1 M phosphate buffered solution (pH 7.4). They were soaked in a 3,3′-diaminobenzidine (DAB) solution (100 mg DAB/200 ml 0.1 M phosphate buffer plus 5 ml of 1% cobalt chloride and 4 ml of 1% nickel ammonium sulfate) for 20 minutes and processed in the DAB solution with 3% hydrogen peroxide solution (0.64 ml of 3% H_2O_2 solution/50 ml DAB solution) for another 15 minutes (Adams, 1977, 1981). The sections were then rinsed in the phosphate buffered solution, mounted, and dried. The slides were dehydrated in graded alcohol series, cleared in xylene, and coverslipped with Permount.

Reconstruction and data analysis

Each SC was reconstructed from its serial sections to fix the position of the labeled axons, and, in the case of the PT animals, to verify the extent of the neonatal lesion (Fig. 1). Well-filled retinal ganglion cell axon arbors were drawn using a 100× oil-immersion objective with a camera-lucida drawing tube. Axons found in either the left or right SC of the normal group were drawn; however, only axons in the SC found ipsilateral to the remaining eye of the normal-IPSI group were drawn. As to experimental groups, only axons found in the left SC of the neonatal-ENUC group (ipsilateral to the remaining eye) and only axons found in the lesioned right SC of the PT group were drawn. Those axons that could be isolated and that either ended in a cluster of boutons or had several densely stained branches were chosen for drawing. To avoid drawing axon collaterals of tectal cells backfilled from the HRP implant, all axons were traced to the optic tract where parent axon diameters were measured. Any small varicosity was counted as a bouton.

Several quantitative measurements were made for each axon arbor by using a computer-assisted digitizing pad. The total length of arborization from the first bouton or branch within the superficial gray layer of the SC was measured, as well as average branch length. The number of boutons and branches were counted. The boutons per length of axon were calculated by taking the ratio of the bouton number and total arbor length. Arbor areas were estimated from the minimum polygon that enclosed the reconstructed arbors. Analysis of variances were performed by the SYSTAT program.

RESULTS

Methodological concerns in axon labeling: Quality of transport, identity, spatial distribution, and size distribution of labeled fibers

The principal considerations in choosing axons for drawing were quality of fill and relative isolation from other labeled axons. A photomicrograph of one such axon arbor is shown in Figure 2. Whether an individual axon is well isolated depends mainly on where it is located with respect to other filled axons, and there is no obvious reason to suspect this procedure should produce a biased sample of the population. In total, 72 axons were drawn: 26 from the normal group, 8 from the normal-IPSI, 27 from the PT group, and 19 from the neonatal-ENUC group. In the normal group, 15 axons came from tissue cut along the retinotopic plane and 11 axons came from tissue cut along the axon entry plane. In the PT group, 15 axons were in the retinotopic plane and 12 in the axon entry plane; and in the neonatal-ENUC group, 12 axons were in the retinotopic plane and 7 in the axon entry plane. In the normal-IPSI group, all of 8 axons were in the retinotopic plane.

Axon arbors of normal, neonatal-ENUC, and PT animals were distributed widely across the dorsal surface of the SC from rostral to caudal and from medial to lateral, as illustrated in Figure 3. Axon arbors of normal-IPSI animals were distributed in the rostral one-third of the SC. Retinotectal axon arbors are not normally found in the caudalmost region of the neonatal-ENUC tecta, because the expanded ipsilateral projection fills principally the rostral two-thirds of the colliculus. The path traveled by normal and PT axons was similar. Retinal ganglion cell axons traveled in a relatively tight band in the lower part of the superficial layers in both PT and normal hamsters, and turned toward the surface of the SC to arborize. This arrangement was different in the SCs of monocularly enucleated animals, where retinal ganglion axons traveled in a looser and wider band that was closer to the collicular surface (Fig. 4).

Measurements of axon diameter were similar in normal and PT animals. The average axon diameter in normal hamsters is 0.68 ± 0.27 μm (SD). Axons of PT animals have a similar average diameter (0.66 ± 0.28 μm) to normal arbors, which is not significantly different (Fig. 5A,C). The axons of the normal-IPSI group are significantly thicker than axons of the normal group (normal-IPSI, 1.00 ± 0.31 μm; $F = 8.128, P < 0.05$). This result suggests that normal-IPSI axons and normal axons belong to different populations of retinal ganglion axons. There is no difference in mean diameter of axons between neonatal-ENUC (0.93 ± 0.25) and normal-IPSI groups (Fig. 5B,D).

Although different axon diameter distributions may be indicative of separate cell classes (Mooney and Rhoades,

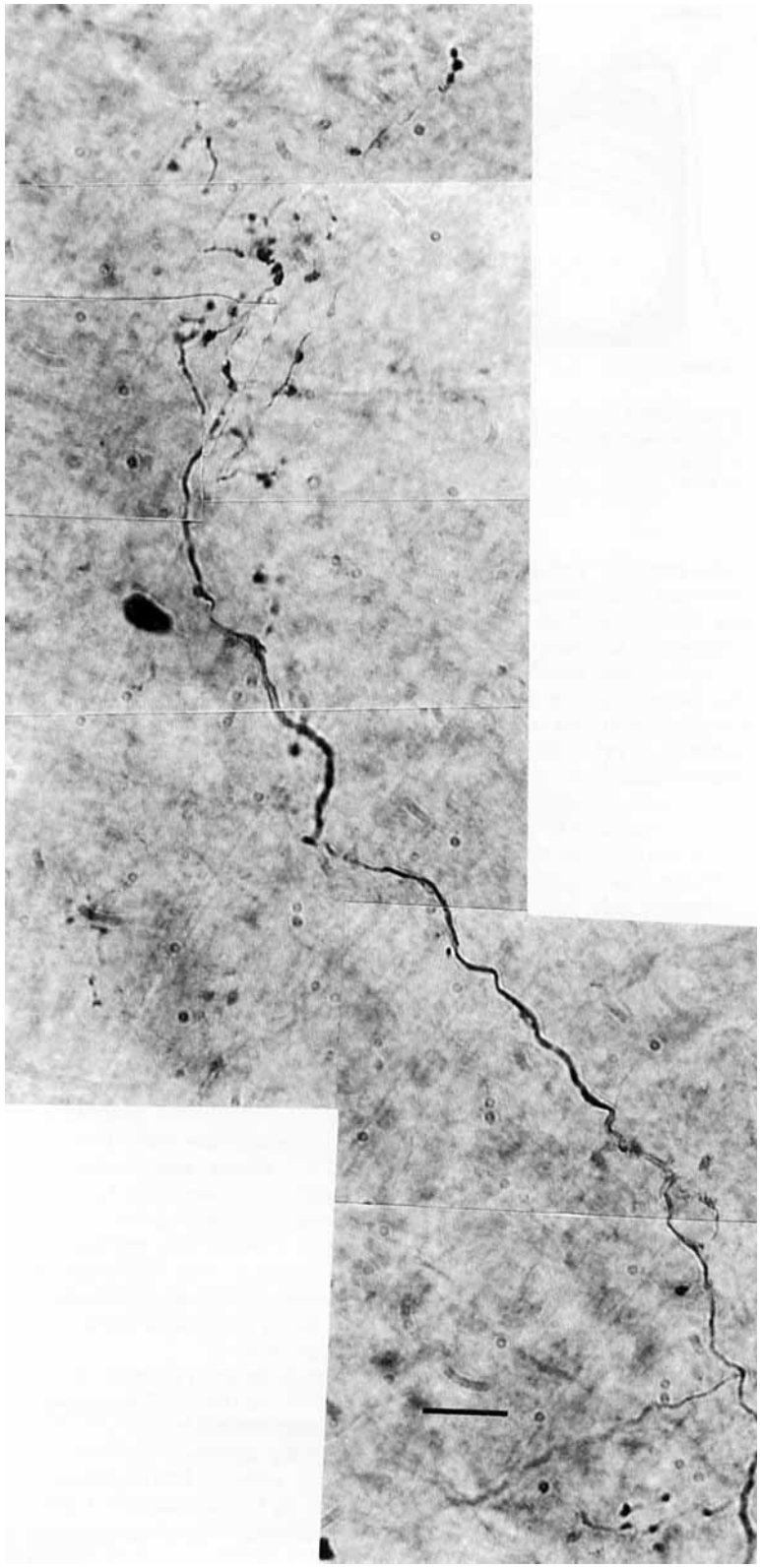

Fig. 2. Example of horseradish-peroxidase-labeled axon arbor in the superficial grey layer of the superior colliculus of a normal hamster. Scale bar = 20 μm.

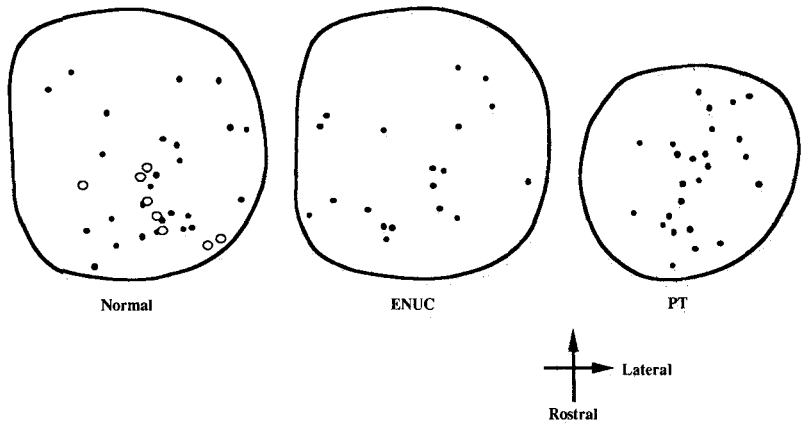

Fig. 3. Spatial distribution of axon arbor centers represented with respect to the dorsal surface of a right superior colliculus for normal, normal-IPSI, neonatal-ENUC, and PT groups. The locations of PT arbors are placed on a "average" partial tectum of the mean rostrocaudal length by fixing their rostrocaudal and mediolateral positions proportionately to the average tectum borders. Dots represent locations of normal arbors; circles represent locations of normal-IPSI arbors.

1990), we have not tried to identify these classes here. Because both neonatal partial tectal ablation and neonatal enucleation can change the pattern of early cell death, produce different retinal ganglion cell size distributions in the retina, and change central branching patterns, size of arbor, and location of arborization (Finlay et al., 1979a,b; Crain and Hall, 1980; Sengelaub and Finlay, 1981; Sengelaub et al., 1983; Wikler et al., 1986; Pallas and Finlay, 1991), it is suspect to attempt to classify axons in these groups a priori on the normal dimensions of size and location. In the Discussion, we will address to what degree changes in axon and arbor size reflect altered populations of neurons that contribute axons versus direct increases or decreases in axon or arbor size.

Morphology of normal arbors

Representative arbors from six axons of normal hamsters taken from the retinotopic plane, tangential to the collicular surface, are illustrated in Figure 6. The average cross-sectional tangential area covered by the axon arbors on the collicular surface (retinotopic plane) was 16,300 ± 1,300 μm^2 and the average cross-sectional area covered by each arbor perpendicular to the collicular surface (axon entry plane) was 36,900 ± 2,600 μm^2; samples of axons from the axon entry plane are shown in Fig. 4; samples of axons in the retinotopic plane are shown in Fig. 6; for summary graph, see Fig. 11. Unlike the cross-sectional area, measures of bouton number and branch number were independent of the plane of section (as would be expected), with an average of 43 ± 17 branches and 167 ± 69 boutons per axon. The average total length of the arbors measured 1,196 ± 444 μm.

Monocular enucleation in the adult hamsters allow us to capture the structure of normal ipsilateral projections from the remaining retina to the SC. In Figure 7, we show four normal ipsilateral retinal axon arbors in the retinotopic plane. In general, axon arbors were larger, with a mean area of 36,000 ± 2,900 μm^2, $F = 5.322$, $P < 0.05$; Fig. 11. The total length of arbors of normal-IPSI hamsters (mean = 1,825 ± 719 μm) was significantly longer than those of normal hamsters ($F = 6.791$, $P < 0.05$). The average bouton number (149 ± 65) and branch number (42 ± 18) were similar to axon arbors from the normal group. Therefore, the bouton density is more sparse in the normal-IPSI axon arbors than the normal arbors as measured by bouton per length ($F = 9.816$, $P < 0.05$, Fig. 13).

Morphology of PT arbors

Every aspect of axon arbor size and complexity was reduced in the PT cases. Examples of seven representative drawings of PT arbors in the retinotopic plane are shown in Figure 8 and are reproduced at the same scale as the normal arbors in Figure 6 (see also the sample of an arbor in the axon entry plane in Fig. 4). Axon arbors cover less area of the SC in both the axonal entry plane and retinotopic plane (Fig. 11). Axon arbors were smaller, less than half of the normal areal extent: the mean terminal fields were 9,400 ± 810 μm^2, whereas normal arbors covered 26,600 ± 830 μm^2; $F = 20.209$, $P < 0.05$; Fig. 11. The arbor length was reduced ($F = 25.449$; $P < 0.05$; Fig. 12A), and the arbors had significantly fewer boutons ($F = 12.758$; $P < 0.05$; Fig. 12B), which gives an average of 106 ± 50 boutons. There was also a significant reduction in total branching: the average number of branches for PT arbors was 25 ± 11; $F = 17.366$; $P < 0.05$; Fig. 12C.

The intrinsic structure of individual axon branches was not markedly altered. The mean length of individual branches did not differ from normal. Bouton density was normal along the length of the reduced arbors, as assessed by dividing total boutons by total arbor length (PT mean = 0.136 ± 0.72 boutons/μm; normal mean = 0.139 ± 0.043; Fig. 13). Visual comparison of the pattern of clustering of boutons in normal and PT groups revealed no interesting differences and was not pursued.

We were also interested to see if, in addition to the overall effect of partial tectal ablation, there was a correlation between lesion size and any metric of arbor size: specifically, did the animals with the largest lesions have the smallest arbors? The lesion sizes of the SC ranged from 30 to 70% in reconstructed surface area (Fig. 1). Within this range of lesion sizes, however, we found only a modest relationship of lesion extent and any of our axonal arbor measurements, none of which reached statistical significance, although all were positive (area, $r^2 = 0.188$; branches, $r^2 = 0.216$; bouton number, $r^2 = 0.193$). For example, shown in Figure 9 is a scatter plot of bouton number versus

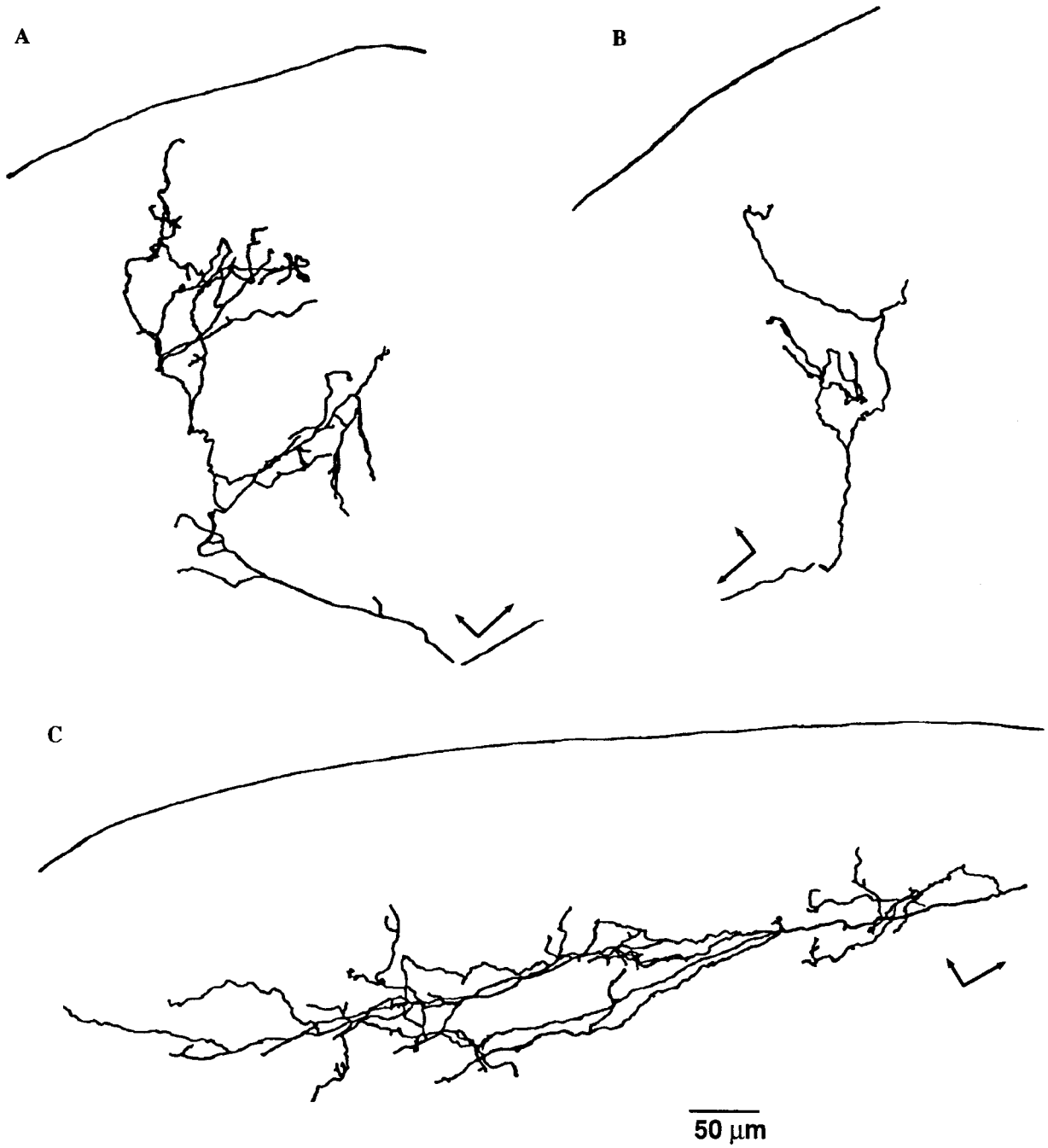

Fig. 4. Examples of normal, neonatal-ENUC, and PT arbors in the axon entry plane. For each axon, the long arm of the orientation arrow points rostrally, and the short arm dorsally. The location of the dorsal surface of the superior colliculus is represented above each axon. Axons of normal (**A**) and PT (**B**) groups typically enter the colliculus in a tight bundle in the stratum opticum and turn sharply dorsally to arborize; ENUC axons (**C**) travel in a less compact bundle and arborize along their length.

residual tectal area for the PT animals, which includes the range of normal bouton numbers. A more extensive parametric study with more animals at each lesion size probably would be necessary to fully explore this relationship.

Morphology of arbors in monocular enucleates

Two sample axon arbors from the ENUC group, both drawn in the retinotopic plane, are shown in Figure 10. The two different planes of section captured different aspects of the altered distribution of the axon arbor (see also Fig. 4 and summary graph in Fig. 11). Axons in enucleates spread extensively over the collicular surface but restrict the depth of their arborization with respect to the collicular surface, which has been observed in prior anatomical and physiological studies (Finlay et al., 1979b; Rhoades, 1980; Thompson, 1979). Thus, the mean area occupied by the ENUC arbors is significantly larger than normal for the retinotopic plane by covering a mean area of 106,000 ± 9,700 μm^2 compared

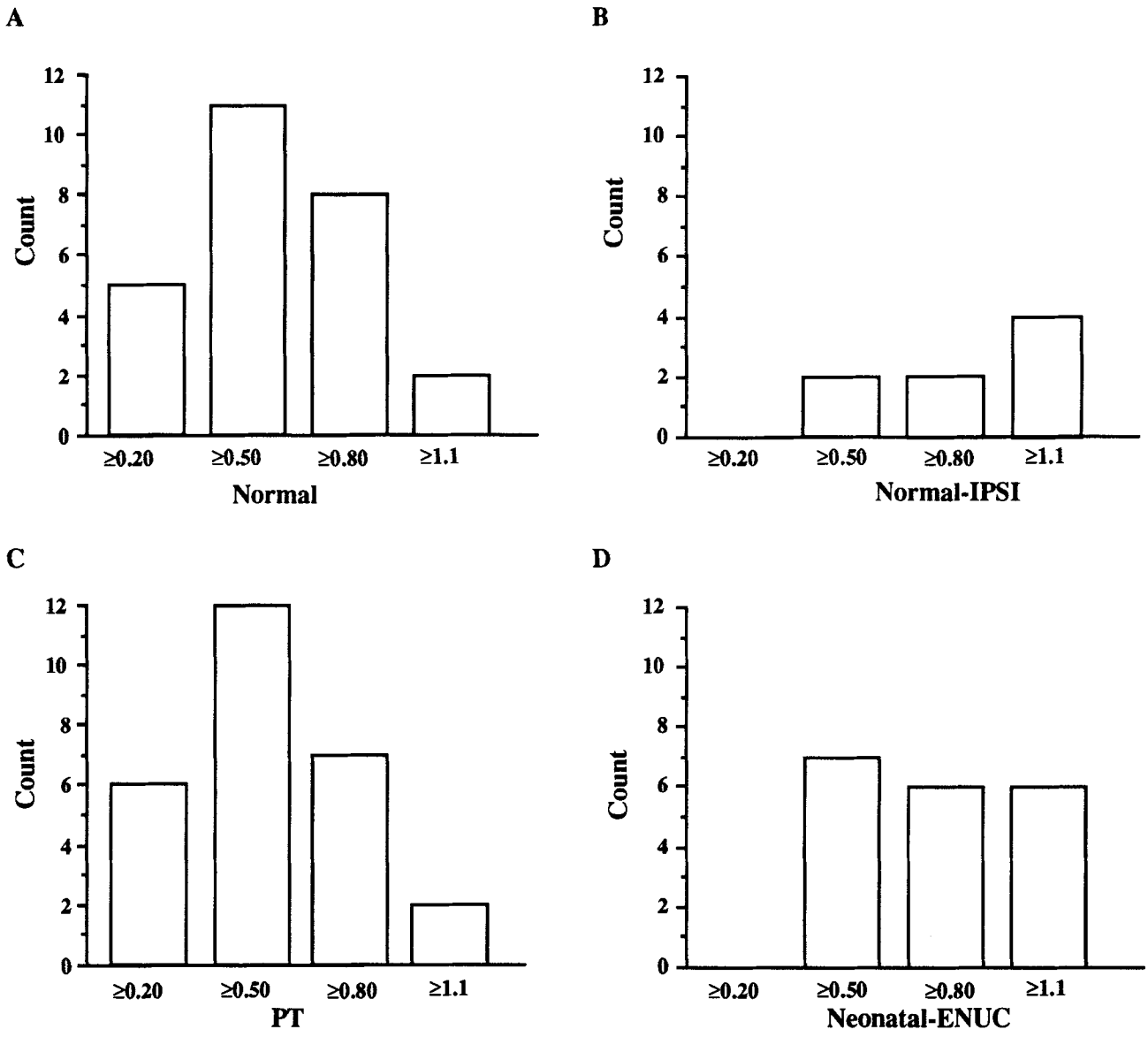

Fig. 5. Distribution of retinotectal axon diameters of axon arbors from normal (**A**), normal-IPSI (**B**), PT (**C**), and neonatal-ENUC (**D**) hamsters. Normal-IPSI and neonatal-ENUC axons have an average diameter similar to the normal group; the PT and normal groups are similar.

with the normal-IPSI arbor size of 36,000 ± 2,900 μm^2; F = 5.513; $P < 0.05$. In the axon entry plane, perpendicular to the collicular surface, they extended farther rostrocaudally but were restricted in their dorsoventral extent. The area of arbors in this plane is 45,000 ± 1,600 μm^2. This preference for occupation of the dorsal aspect of the SC in both depleted or "compressed" projections has been noted in several other contexts (Finlay et al., 1979a,b; Finlay, 1979; Rhoades, 1980; Thompson, 1979). Because we found no difference in area comparing normal and neonatal-IPSI axons for the retinotopic plane, we did not explore the difference of neonatal-IPSI and normal-IPSI in the axon entry plane.

The remaining measures of dendritic size and complexity were elevated consistent with increase in arbor size in neonatal enucleates. The mean arbor length and bouton and branch numbers from the ENUC arbors were higher than that of the normal arbors (Fig. 12A–C). Neonatal-ENUC arbors measured 2,580 ± 1,090 μm, much longer than normal-IPSI arbor length (mean = 1,800 ± 720, F = 4.368, $P < 0.05$). Neonatal-ENUC axons gave rise to an average of 217 ± 122 boutons and 64 ± 34 branches,

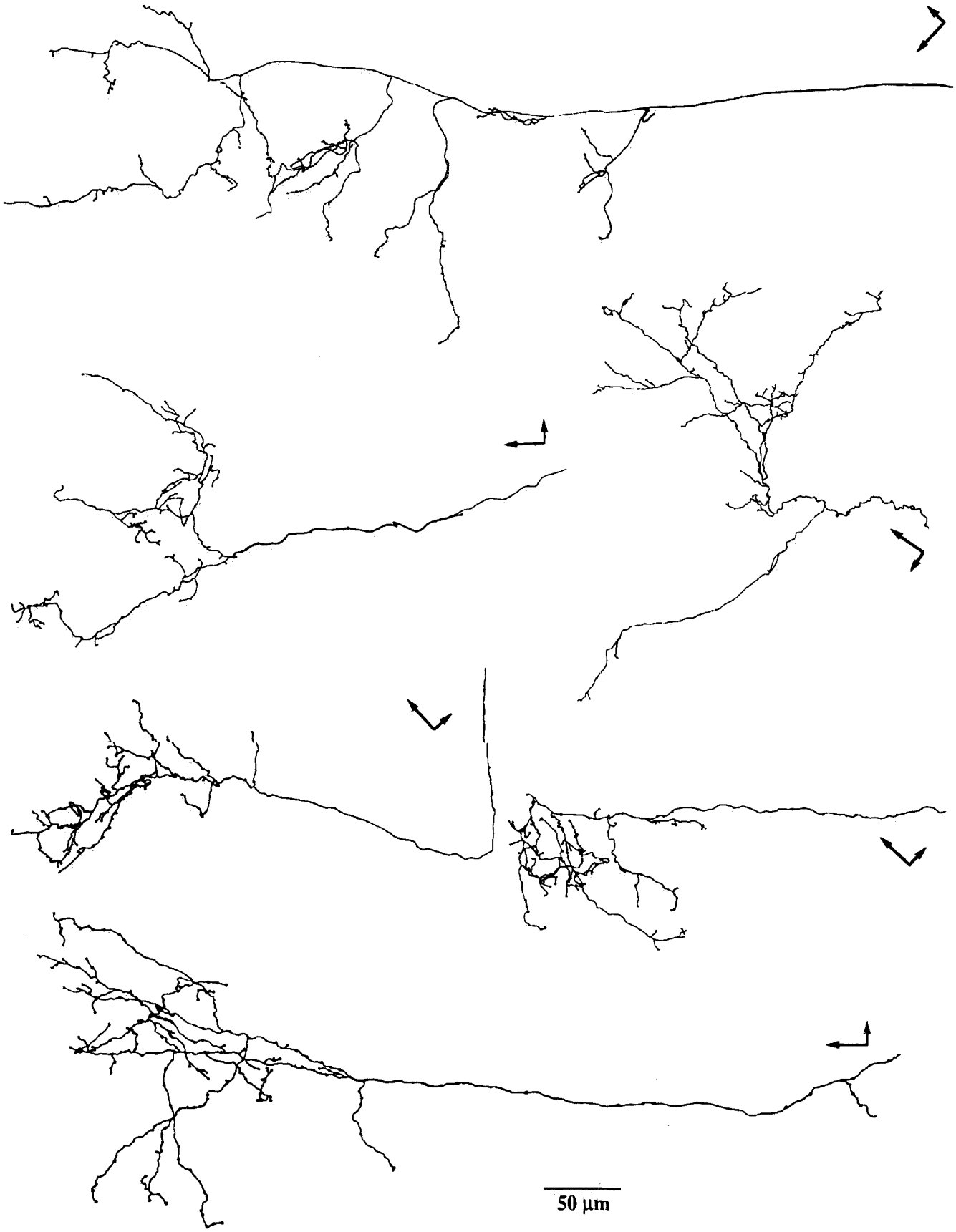

Fig. 6. Examples of axon arbors in the retinotopic plane of the normal hamster superior colliculus. For each axon, the long arm of the orientation arrow points caudally, and the short arm laterally.

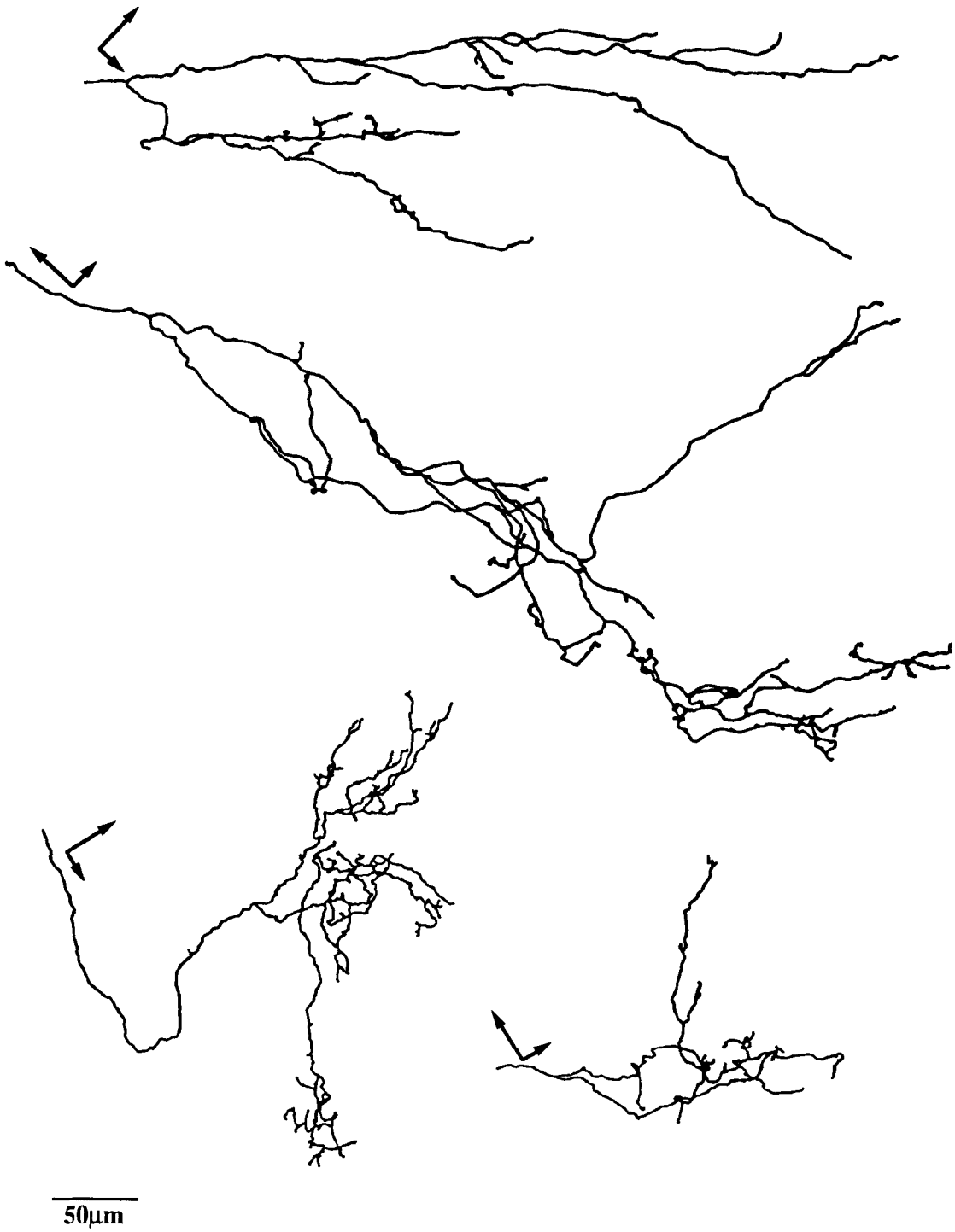

Fig. 7. Examples of axon arbors in the retinotopic plane of the superior colliculus of normal-IPSI hamsters. For each axon, the long arm of the orientation arrow points caudally, and the short arm laterally.

compared with normal-IPSI, which have 149 ± 65 boutons and 42 ± 18 branches (for the bouton number comparison, $F = 3.553$, one-sided $P < 0.05$; for the branch number comparison, $F = 4.836$, $P < 0.05$). The bouton-per-arbor length of neonatal-ENUC arbors is not different from that of normal-IPSI.

DISCUSSION

Methodological considerations

Quality of arbor filling. The arbor extent of normal retinocollicular axons in the hamster has been described previously with HRP (Sachs and Schneider, 1984; Mooney

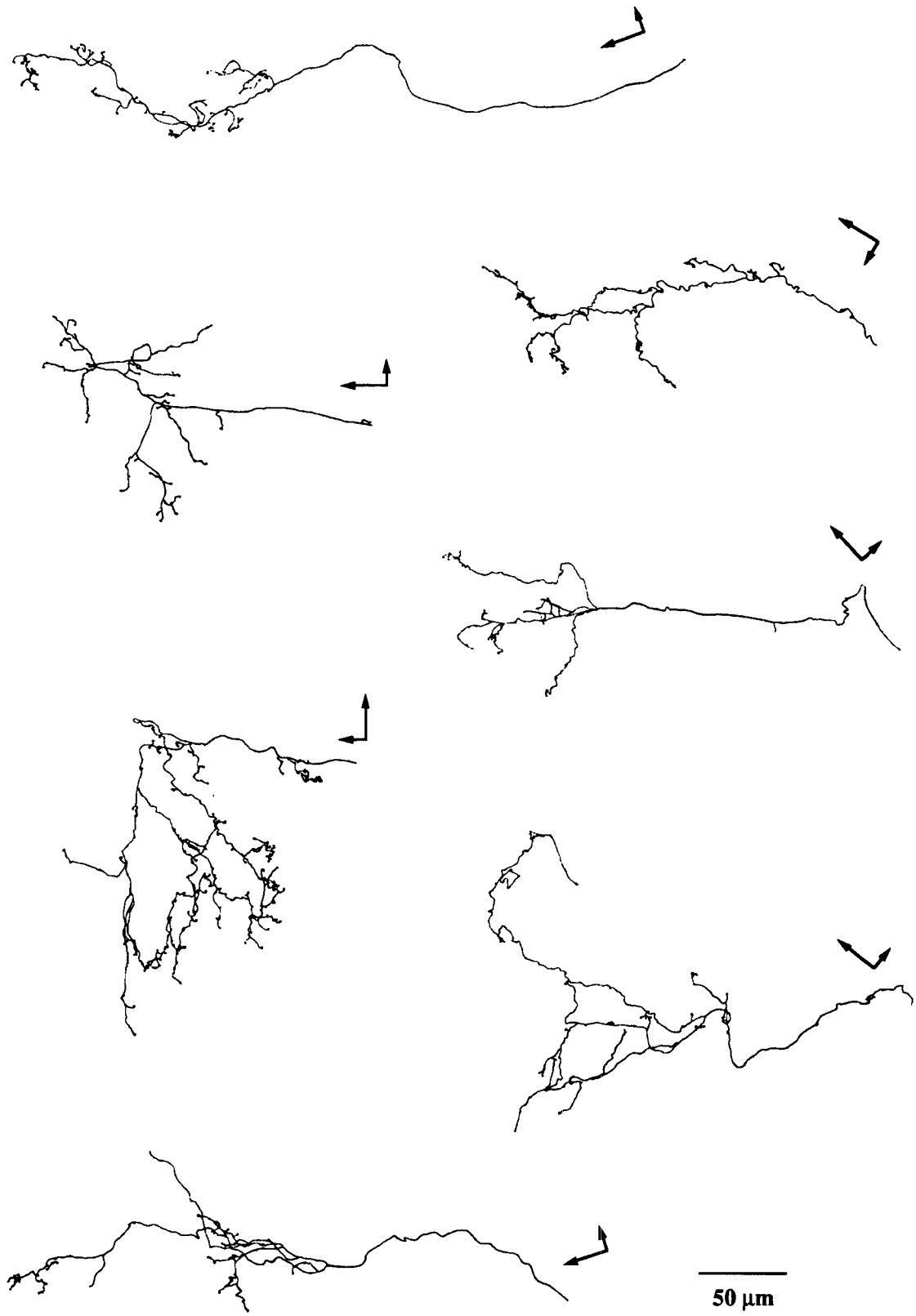

Fig. 8. Examples of axon arbors in the superior colliculus of PT animals, retinotopic plane. For each axon, the long arm of the orientation arrow points caudally, and the short arm laterally.

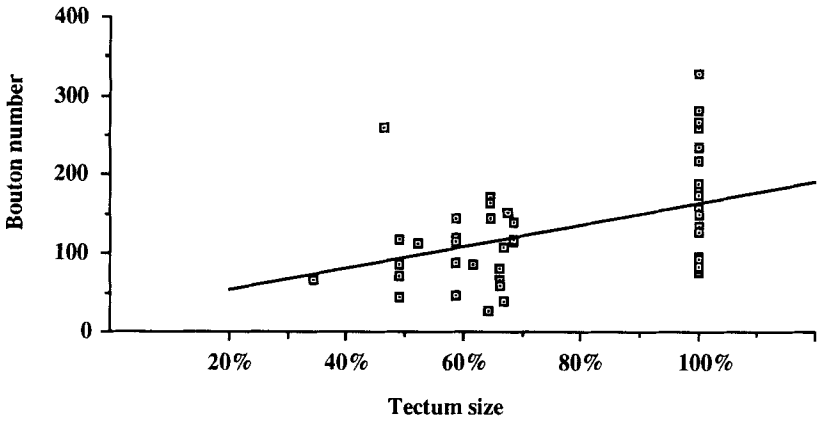

Fig. 9. Scatter plot of total bouton number for 27 axons versus the size of the remaining colliculus after partial tectal lesions, with regression line. The distribution of bouton number of normal axons is also represented.

and Rhoades, 1990), and their measurements of cross-sectional area or mediolateral extent in the coronal plane are in reasonably good agreement with the corresponding measurement in our "axon entry plane." For example, Mooney and Rhoades distinguished two classes of axon: type 1, with arborizations in the stratum opticum, axon diameter of 0.84 μm and a cross-sectional arborization area of 78,059 μm^2; and type 2, principally arborizing in the superficial grey layer, with an axon diameter of 0.58 μm and a cross-sectional area of 33,238 μm^2. Our average cross-sectional area, including both types, is 38,868 μm^2. Because our mean axon diameter, 0.68 μm, and distribution of arbors indicates a greater relative proportion of smaller arbored, type 2 axons in our sample, our recovered axon arbors fall within the range of sizes that would be expected from these prior studies. A few big axon arbors in our sample may be type 1 axons, which covered area up to 89,550 μm^2.

There are some striking differences, however, in the appearance of the fills in these three studies apart from arbor extent, which may relate to their methodological differences. Mooney and Rhoades (1990) intracellularly filled individual retinocollicular axons with HRP after electrophysiological characterization and recovered axon arbors after an in vivo transport period of up to 8 hours, whereas Sachs and Schneider (1984) essentially used the same in vitro HRP brachium-placement method employed here, with 2–4-hour incubations. The Mooney and Rhoades axons, although of comparable areal extent, show much more dense branching and a greater number of boutons, about 390 per axon versus the 170 we report (Sachs and Schneider [1984] did not count boutons, but the axon arbors and bouton densities they drew resemble ours). The difference between the intracellular fill procedure and extracellular placement is apparent both in the photomicrographs and in the reconstructions, so it is unlikely that the criteria for naming boutons and drawing axons is the source of difference in these studies.

Both procedures have benefits and pitfalls. Intracellular injection of HRP for a longer period of transport can potentially fill an identified single axon completely, and the assumption that only a single axon has been filled can be used to recover widely separated processes, even if intervening connections cannot be traced. However, there are at least three possible pitfalls: (1) it is possible that more than one axon might transport HRP with this method; (2) because of the extreme difficulty of intracellularly filling SC axons (only 20 axons were filled in 350 animals in the Mooney and Rhoades [1990] study), it seems unlikely that the sample of axons is random, particularly with respect to axon size; and (3) the longer survival time might lead to the first stages of degeneration in a transected axon.

The extracellular bulk-fill technique produces a number of filled axons with relative ease, unidentified except by the location of the HRP placement and their terminal zone, and probably has less bias toward large size in selection of axons. Because many axons are filled, each axon branch must be laboriously registered and followed to its termination; more potential for error might exist for misregistration of axons, but more precautions are taken to insure that only one axon is counted. The shorter survival time raises the possibility of less complete filling but reduces the probability of artifacts of axonal degeneration. A direct comparison of bulk fills (Hahm et al., 1991) to intracellularly filled axons (Roe et al., 1990) in studies of X axons in the LGN by the same laboratory group showed no consistent differences in the quality of filling. At this point, it seems that only comparison of additional techniques will resolve the question of which technique appropriately captures the details of axon configuration. At any rate, we feel confident that our results accurately reflect the ordinal relationship of arbor sizes of the partial tectal, normal, and neonatal enucleate groups central to the goal of this study.

Identity of axons filled. At the location at which we made our injections, close to the SC and on the superficial aspect of the brachium, retinal ganglion cell and posterior corticotectal axons are the only two populations with input to the colliculus that can be labeled, and of those two, the corticotectal is very much a minority population (type L2 as described by Sachs and Schneider, 1984; for pattern of fiber distribution, see Giolli et al., 1978). Furthermore, cortical afferents tend to have fewer axonal arborizations than retinal afferents and widely separated branches (Sachs and Schneider, 1984), which are very different from the axon arbors we sampled in the neonatal-ENUC axon arbors. Thus, it is unlikely that large axons observed in the neonatal-ENUC arbors represent a significant proportion of the corticotectal axons.

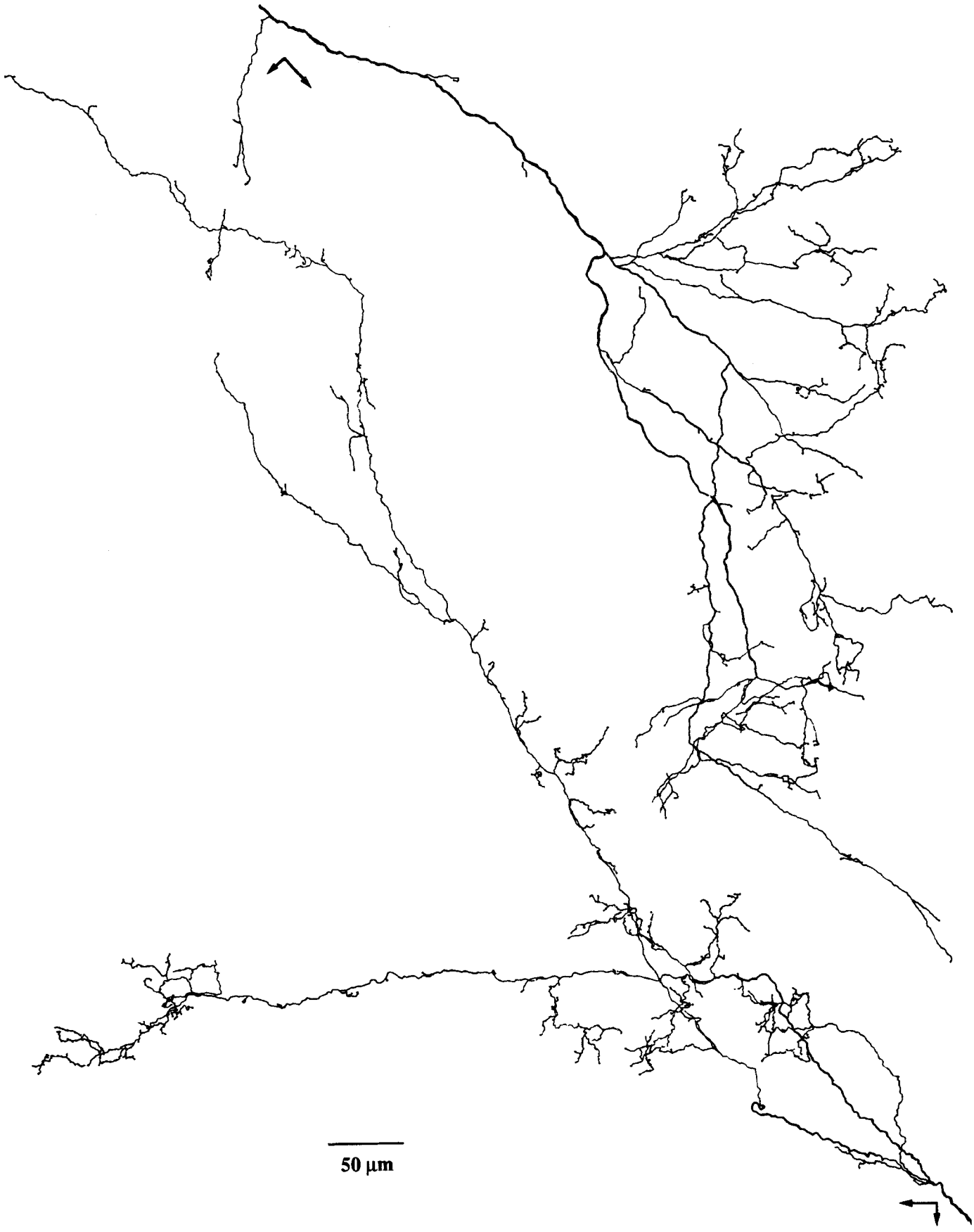

Figure 10

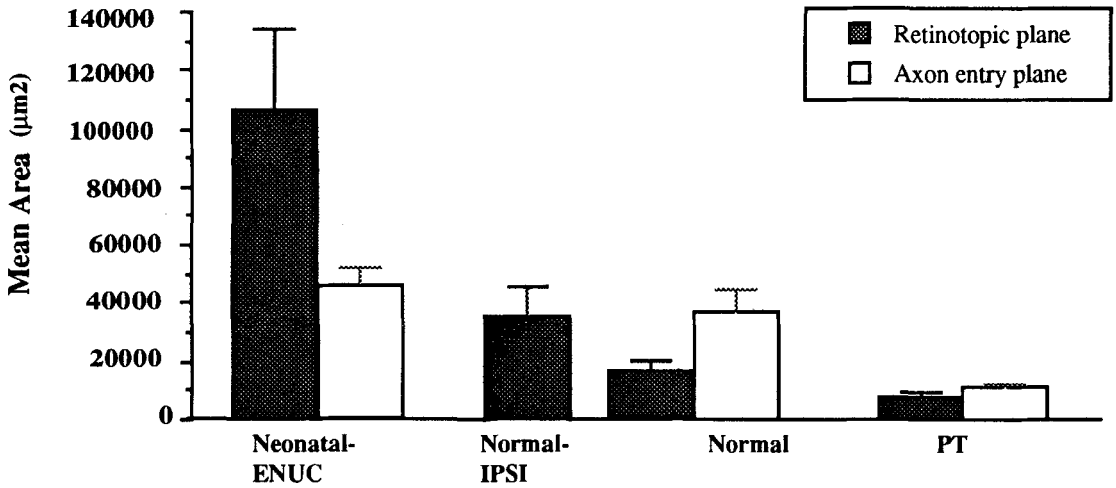

Fig. 11. Mean area of axon arbors in the superior colliculus of normal, normal-IPSI, neonatal-ENUC, and PT hamsters. Black bars show mean area of axon arbors drawn from the retinotopic plane. White bars show mean area of axon arbors drawn from the axon entry plane.

Other sources of input to the colliculus, such as the parabigeminal nucleus, ventral lateral geniculate body, and medial and frontal cortices, take a more ventral approach to the colliculus or, for the most part, occupy the ventral aspect of the optic tract (Beckstead, 1979; Huerta and Harting, 1984; Harting et al., 1991) and cannot be labeled by this method even after contralateral enucleation (Sachs and Schneider, 1984).

Change in cell populations or arbor?

Normal hamster retinocollicular axon arbors in the SC are of several types and occupy different laminar positions in the superficial layers (1,2 classification: Mooney and Rhoades, 1990; U/L classification: Sachs and Schneider, 1984). Two types of axons are seen most frequently. Type 2 (or U) axons have a mean axon diameter of 0.58 μm and arborize in the stratum griseum superficiale (SGS), and the arbors are tightly clustered with dense boutons. Type 2 (or L1) axons are thicker (mean diameter, 0.84 μm) and innervate a larger area across several laminae, including the SGS, the stratum opticum (SO), and the upper stratum griseum intermediale (SGI). Sachs and Schneider (1984) showed that L1 class axons in the rostral colliculus (potentially representing the temporal ipsilateral retina) are larger than the remaining L1 axons. We observed a similar relationship of type of arbor, depth of arborization, and axon diameter as the other studies for the subset of axons that were sectioned in the same plane.

Although potential compression and expansion will be a force on all axons, these manipulations also could alter the composition of the populations that project to the SC. In the case of the PT manipulation, there is relatively little induced cell death as a result of the manipulation (Wikler et al., 1986). Injections of HRP into the partial colliculus for lesions of approximately 50% label supernormal numbers of retinal ganglion cells of the normal size distribution; for larger lesion sizes, the cell number drops, and the cells labeled tend to be of large diameter (Pallas and Finlay, 1991). For all but the largest PT lesion, therefore, it is likely that we have labeled a reasonably typical population of retinal ganglion cells whose arbors have been induced to become smaller.

Monocular enucleation spares a considerable proportion of cells from normal cell death (Sengelaub and Finlay, 1981), and this sparing is most extensive in the largest-celled population in the temporal retina (Sengelaub et al., 1983). It is therefore likely that the population of remaining retinal ganglion cells that project to the ipsilateral tectum are enriched by at least 15–20% in neurons contributing large-caliber axons. However, it is also clear that the arbor extents of enucleate axons fall well outside the normal range, and again, whereas a change in contributing population may contribute to the changed population statistics, growth in terminal arbor must also have occurred. Unresolved, and of interest, is whether different fiber types respond in distinct ways to increased or decreased target volume.

Correlation of morphology with physiology

Monocular enucleates. The anomalous ipsilateral projection of the remaining eye forms an expanded, functional projection in the colliculus compared with the normal ipsilateral projection (Finlay et al., 1979b; Thompson, 1979; Rhoades, 1980). Receptive fields in this projection are abnormally large and habituate rapidly, and responding cells are most likely to be found in the upper half of the superficial grey layer. Retinotopy is orderly in the mediolateral (superior to inferior visual field) axis, but disorderly in the rostrocaudal (nasal to temporal) axis, with occasional spatially separated receptive fields giving rise to responses in a single location in the colliculus. We found that the appearance of the axon arbors is complementary to the electrophysiology. Axons arborize where responsive cells are found in the dorsal aspect of the superficial grey layer. The somewhat subnormal bouton density, compared with the normal axon projection, may produce sluggish and

Fig. 10. Examples of two axon arbors in the retinotopic plane of the superior colliculus of neonatal-ENUC hamsters. For each axon, the long arm of the orientation arrow points caudally, and the short arm laterally.

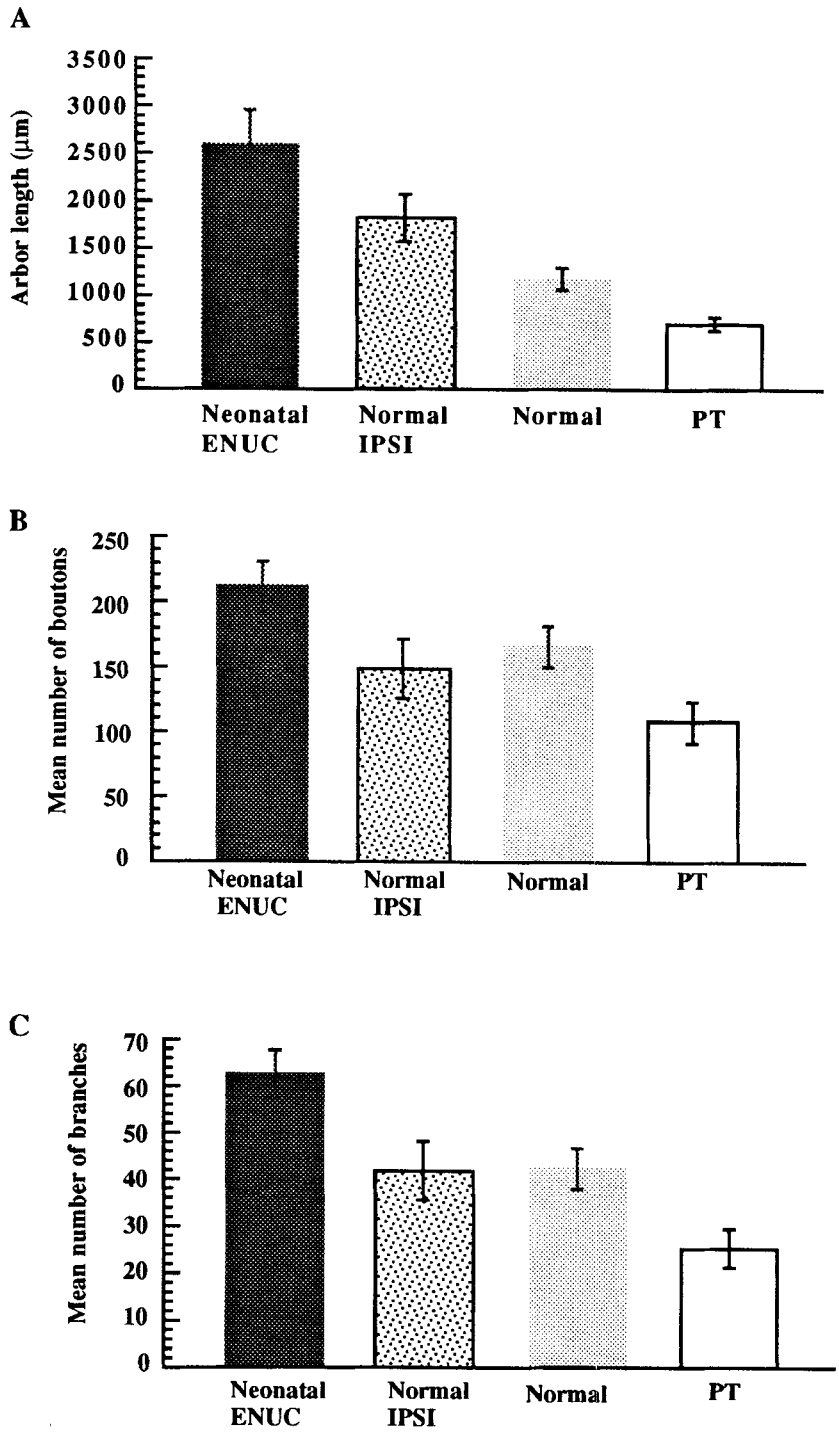

Fig. 12. (**A**) Mean arbor length (μm) and standard error for neonatal-ENUC, Normal-IPSI, normal, and PT hamsters. (**B**) Mean number of branches and standard error of axon arbors in the superior colliculus (SC) of neonatal-ENUC, normal-IPSI, normal, and PT hamsters. (**C**) Mean number of boutons and standard error of individual arbors in the SC of neonatal-ENUC, normal-IPSI, normal, and PT hamsters.

habituating responses. The wide, multiply branched arbors correlate well with multiple and abnormally large receptive fields. On consideration, however, these result are paradoxical. If there are fewer competitors for terminal area, a single ganglion cell could concentrate its arbor in any position and exclude other cells entirely. Yet the receptive fields are larger than normal and spatially disorganized. Perhaps the subnormal synaptic density of this projection (Xiong and Finlay, 1993) does not allow postsynaptic cells to be driven at a high enough rate to allow normal activity-dependent sorting to occur, or nonretinal inputs form the principal basis for whatever sorting does occur.

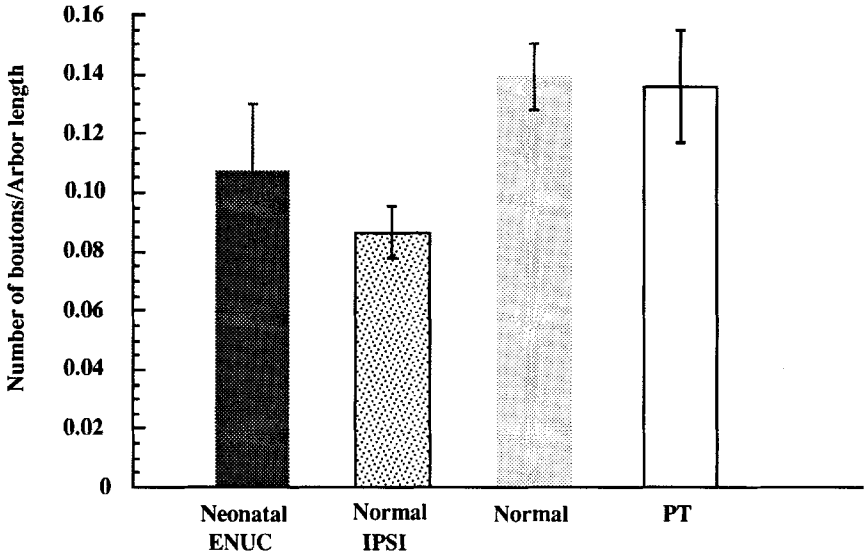

Fig. 13. Number of boutons per length of axonal arbor and standard error for neonatal-ENUC, normal-IPSI, normal, and PT hamsters.

Developmental studies, coupled with pharmacological manipulations, could resolve whether these large arbors represent stabilized exuberant projections or induced growth.

Partial tectal animals. Electrophysiological study of partial tectal ablated animals shows remarkably little change in receptive field properties when compared with normal animals. Pallas and Finlay (1989) recorded from single tectal cells in both partially lesioned and intact tecta. The distribution of receptive field size, preferred stimulus size, and preferred stimulus velocity is apparently unaffected by the early lesion, although there were some changes in the pattern of surround suppression such that inhibition fell off faster with distance in the lesioned animals. The constancy of receptive-field size is particularly surprising because the lesions create an increase in the afferent:target ratio. At the same time, multiunit receptive fields increase in size (Finlay et al., 1979a,b), which suggests that there is a stimultaneous compression of the visual field map onto the tectum and a decreased amount of overlap between the receptive fields of adjacent tectal cells. Two hypotheses were put forth that could explain these results: either individual ganglion cells reduce their arbor to share the limited space in the tectum, or not all of the ganglion cells project to the tectum. The present study and Pallas and Finlay's (1991) study show that both mechanisms play a role in keeping constant, the number of ganglion cells innervating each tectal cell and thus preserving function under conditions of decreased target space. The large disparity in the arbor size of ganglion cells from lesioned, compared with normal, tecta show that arbor reduction is a major component of the compensation mechanism.

The locus of plasticity in population mapping

We have shown that the axons of the retinal ganglion cells possess great plasticity in the area they occupy, and the total length, number of branches, and number of boutons they possess. In the case of partial tectal lesions, retinal ganglion cells can survive with less than half of their normal areal extent, whereas after enucleation arbors can (at least) be more than twice as extensive. What developmental factors control the afferent:target convergence ratio in the retinocollicular topographical mapping process?

We now have the following facts about the features of changed convergence in the case of the partial tectal lesion by using the case of a 50% lesion (lesions in excess of this amount produce a different solution to the topographic mapping problem; see Finlay, 1979; Pallas and Finlay 1991). (1) The entire visual field is represented. Multiunit receptive fields are larger than normal (Finlay et al., 1979), whereas, at the single unit level, receptive field sizes are of normal size (Pallas and Finlay, 1989). (2) Cell death in the retina is only slightly elevated over normal (Wikler et al., 1986), and a supernormal number of cells projects to each tectal location (Pallas and Finlay, 1993). (3) Cell density is normal in the partially ablated tectum, which indicates no excess neuropil (Wikler et al., 1986) and synaptic density is normal (Xiong and Finlay, 1993). (4) The volume of axon arbor is decreased, commensurate with axon length, number of branches and boutons.

Therefore, for this numerical matching problem, the locus of plasticity appears to lie in the volume of presynaptic arbor. The substructure of the arbor does not change: branch length and bouton density are normal. On the postsynaptic side, synaptic density is unaltered and the spatial convergence on collicular cells does not change, that is, each retinal ganglion cell may contact fewer tectal targets, but each tectal cell receives its normal number of inputs. Similarly, in the regenerating retinotectal system of goldfish, there is no evidence for up-regulation of either dendritic arbor or synaptic density in response to increased convergence (Hayes and Meyer, 1988b). In the control of normal neuron death in development, target regulation of afferent neuron number occurs early in development, and target manipulation can produce large increases and decreases in the neuron numbers of afferent populations. The reverse, the regulation of target neuron numbers by afferents, occurs overall later in development, and relatively small target neuron loss due to massive denervation is the principal effect reported (reviewed in Pallas and Finlay, 1989; Finlay, 1992). We thus hypothesize generally for

numerical matching problems that the afferent population and its axon arbor is the source of plasticity, whereas the target population with its stable dendritic arbor and synaptic density is the principal constraint.

Interestingly, there are a number of manipulations that regulate the size of dendritic arbor, but they are all features of activity or metabolic state that indicate a changed functional state: increased sensory stimulation (Coss and Globus, 1979; Greenough, 1984) or hormonal manipulation (Kurz et al., 1986), for example. Removal of primary afferents, such as in monocular enucleation, does cause a decrease in dendritic arbor (e.g., Kelly and Cowan, 1972; Brunso-Bechtold and Casagrande, 1981), but this may reflect the lowered activity level of the target neurons themselves rather than a direct effect of afferent volume on dendritic volume. If the volume of dendritic arbor at a particular synaptic density during development sets the size of a functional system, altered activity or changed hormonal state will have maximum effect if their actions are at this critical site.

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