Terminal Arbors of Axons That Have Formed Abnormal Connections

Abstract. *The terminal arbors of individual retinogeniculate axons that have been induced to grow into an inappropriate geniculate layer have been revealed for light and electron microscopic study by being filled with horseradish peroxidase. After a unilateral ocular enucleation in kittens, single axons from the surviving eye show terminal arbors not only within their own geniculate layers but also in the denervated layers. The new, abnormal arbors arise from the terminal segments of arbors that lie within the nondenervated layer and make patterns of synaptic contacts that appear normal.*

Abnormal axonal growth, or "sprouting," has been demonstrated in several parts of the nervous system by a variety of techniques (*1, 2*). Generally, the abnormal growth occurs when one axonal system is damaged and another moves into the sites vacated as a result of the injury. The invasion of new territory has been demonstrated by fiber degeneration methods (*3–5*), by the anterograde transport of tritiated amino acids (*6, 7*) or by histochemical means (*8*); the formation of new synaptic connections has been shown by electron microscopic (*9*) and electrophysiological (*10*) techniques.

The methods used in the past have shown that an axonal population can grow into a newly denervated region. However, in the central nervous system these methods have not displayed the structure of the newly grown terminal arbors, nor have they shown how an axon distributes its terminal arbors between the original terminal field and the newly invaded territory. We now describe a method for revealing the terminal arborizations of individual axons that have sprouted.

When axons and their terminals are filled with horseradish peroxidase (HRP) (*11*), they resemble Golgi-impregnated processes; consequently the morphology of individual sprouted axons can be examined. It thus becomes possible to evaluate (i) the extent to which any one axon gives off terminals within its normal zone of innervation and (ii) the extent to which it has invaded new territory. Further, since HRP is electron-opaque, it is possible to examine the axons electron microscopically and to compare synaptic contacts made in the normal zone with those made in the newly invaded territory.

The retinogeniculate pathway of the cat provides a particularly useful site for studying abnormally induced axonal growth. The normal dorsal lateral geniculate nucleus is laminated, and each lamina receives input from only one eye. The laminar arrangement of retinogeniculate axons has been demonstrated by several different methods (*12, 13*) including the HRP method used here (*14*). This last method shows that the terminal arbors of individual axons do not cross laminar borders and generally do not enter the interlaminar zones. After unilateral eye enucleation, the axons from the surviving eye can invade the denervated geniculate laminae (*4, 5, 7*). Since this is altogether foreign territory for these axons, the abnormal axonal growth can be clearly identified on the basis of the laminar localization of the terminals. Further, the electron microscopic appearance of normal retinogeniculate axon terminals has been described in the cat (*15, 16*); hence, the fine structure of the abnormally grown axons and their synaptic relationships can be readily compared with the normal.

Optimal sprouting of retinogeniculate axons occurs if one eye is removed during the first week of a kitten's life (*4, 5, 7*). Eyes of eight kittens were enucleated between 5 and 8 days after birth, and the animals were reared for 3 to 12 months. A small quantity (0.1 to 0.2 μl) of concentrated HRP (50 percent solution of Sigma Type VI) was then injected into

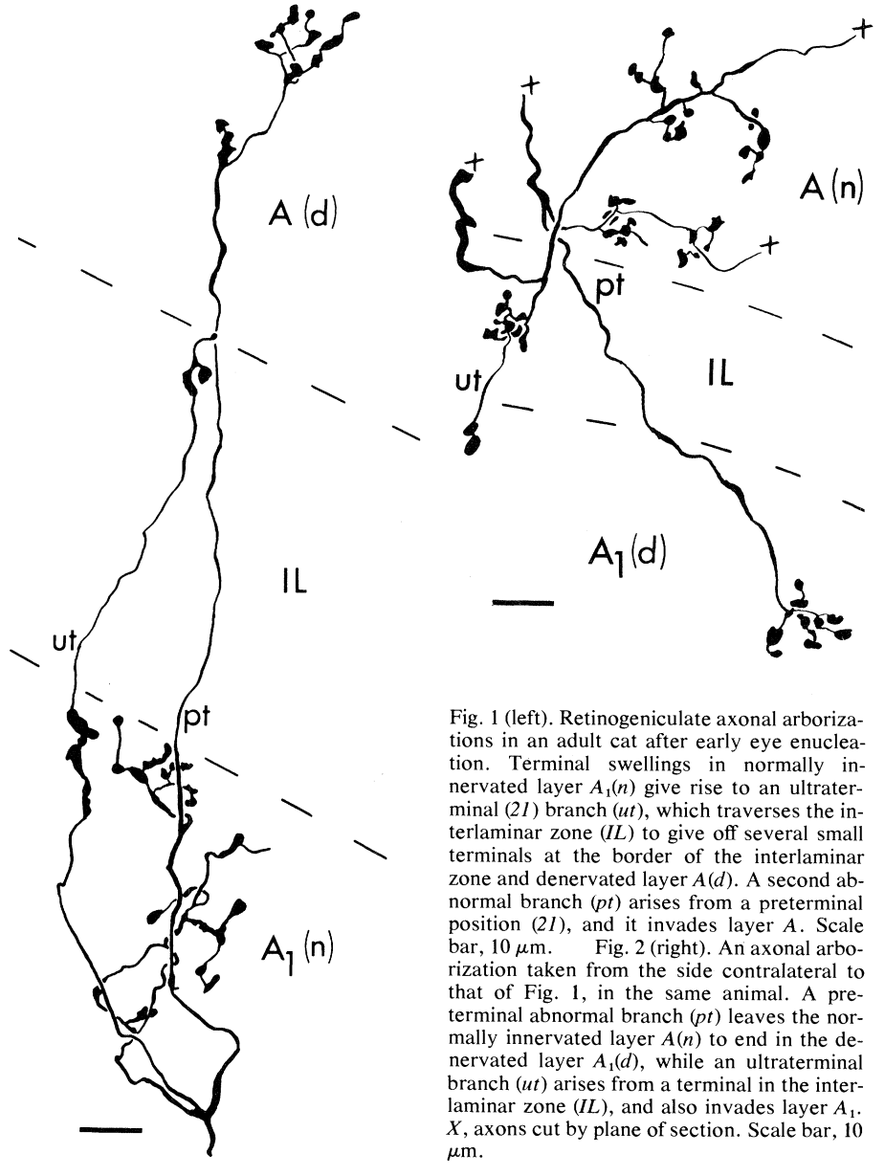

Fig. 1 (left). Retinogeniculate axonal arborizations in an adult cat after early eye enucleation. Terminal swellings in normally innervated layer $A_1(n)$ give rise to an ultraterminal (*21*) branch (*ut*), which traverses the interlaminar zone (*IL*) to give off several small terminals at the border of the interlaminar zone and denervated layer $A(d)$. A second abnormal branch (*pt*) arises from a preterminal position (*21*), and it invades layer *A*. Scale bar, 10 μm. Fig. 2 (right). An axonal arborization taken from the side contralateral to that of Fig. 1, in the same animal. A preterminal abnormal branch (*pt*) leaves the normally innervated layer $A(n)$ to end in the denervated layer $A_1(d)$, while an ultraterminal branch (*ut*) arises from a terminal in the interlaminar zone (*IL*), and also invades layer A_1. *X*, axons cut by plane of section. Scale bar, 10 μm.

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the optic tract though a stereotaxically placed Hamilton syringe (*14*). The syringe needle was used as a recording electrode and the placement of the tip in the optic tract was confirmed by recording evoked potentials through the needle while flashing light into the eye.

After 24 hours, the animals were perfused with a glutaraldehyde-paraformaldehyde fixative. The brains were stored in the fixative overnight, and 100-μm coronal sections were cut through the thalamus with a vibratome. Sections for light microscopic study were incubated in diaminobenzidine after being treated in 0.5 percent cobalt chloride (*17*). These sections were counterstained with cresyl violet. Sections for electron microscopic study were incubated in diaminobenzidine, stained for 30 minutes in 1 percent osmium tetroxide, dehydrated in ethanol, and embedded in Araldite between plastic slides. The flattened embedded sections were studied by light microscopy and regions containing labeled axons were serially sectioned at 5 μm. Areas containing labeled axons were then located, and axons sending branches across interlaminar zones into the denervated laminae were identified, drawn, and photographed. The 5-μm sections were then remounted onto Araldite blocks and serially thin-sectioned (*18, 19*).

In four of the cats, newly grown terminal arborizations of retinogeniculate axons that had invaded the denervated laminae were successfully filled with HRP (Figs. 1 and 2). The distribution of these arborizations corresponds to that described previously by other methods (*4, 5, 7*). It is striking that these arbors arise as branches of terminal arborizations formed within the normally innervated laminae. For example, Fig. 1 shows an axon from the dorsal lateral geniculate nucleus contralateral to the enucleated eye. In this experiment, lamina *A* was deprived of its normal retinal input while the ipsilateral input to lamina A_1 remained intact. Some branches of this axon are confined to lamina A_1. Furthermore, these branches form clusters of terminal swellings resembling those formed by retinogeniculate axons in lamina *A* or A_1 of normal cats (*14, 16, 20*). This same axon also gives rise to other branches that leave lamina A_1 and form similar clusters of terminal swellings both in the interlaminar zone and in the denervated lamina *A*. In this axon, the abnormally grown branches arise either from a terminal (*ut* in Fig. 1) or from a length of axon just proximal to a cluster of terminals (*pt* in Fig. 1). These would be classified as ultraterminal and preterminal sprouts, respectively, according to Barker and Ip (*21*).

For this and other such sprouted axons, the terminal swellings in the interlaminar zone and in abnormally innervated lamina *A* are similar to those in lamina A_1. Equally important is that all of the terminals closely resemble in size, shape, and arrangement those seen in laminae *A* and A_1 of normal cats. That is, the terminal swellings range in size from 1 to 7 μm; they can have smooth contours or scalloped, crenulated surfaces; they may arise singly or form clusters with other swellings (*14*).

A second example of axonal sprouting is from the lateral geniculate nucleus on the same side as the enucleated eye (Fig. 2). Lamina A_1 is denervated while lamina *A* receives its normal retinal inputs. The terminal configurations of these axons resemble those of Fig. 1. Single axons have terminals within lamina *A* and also send branches into the denervated lamina A_1. The sprouted branches arise from portions of the retinogeniculate axons within lamina *A* and then double back into lamina A_1. That is, even though the retinogeniculate axons must traverse lamina A_1 on their way to lamina *A*, they have not been seen to give off collateral branches to lamina A_1 as they pass through it. Instead, all of the branches that can be traced arise from portions of axons within lamina *A* or the adjacent interlaminar zone. It appears as though the abnormal growth is not induced from the axonal stem [nodal sprouting of Barker and Ip (*21*)], but arises from the terminal arbor or from a terminal swelling as in Fig. 1.

In order to study the fine structural appearance of these sprouted axons and to determine the extent to which their synaptic relationships resemble those of normal retinogeniculate axons, we have studied several of the HRP-labeled

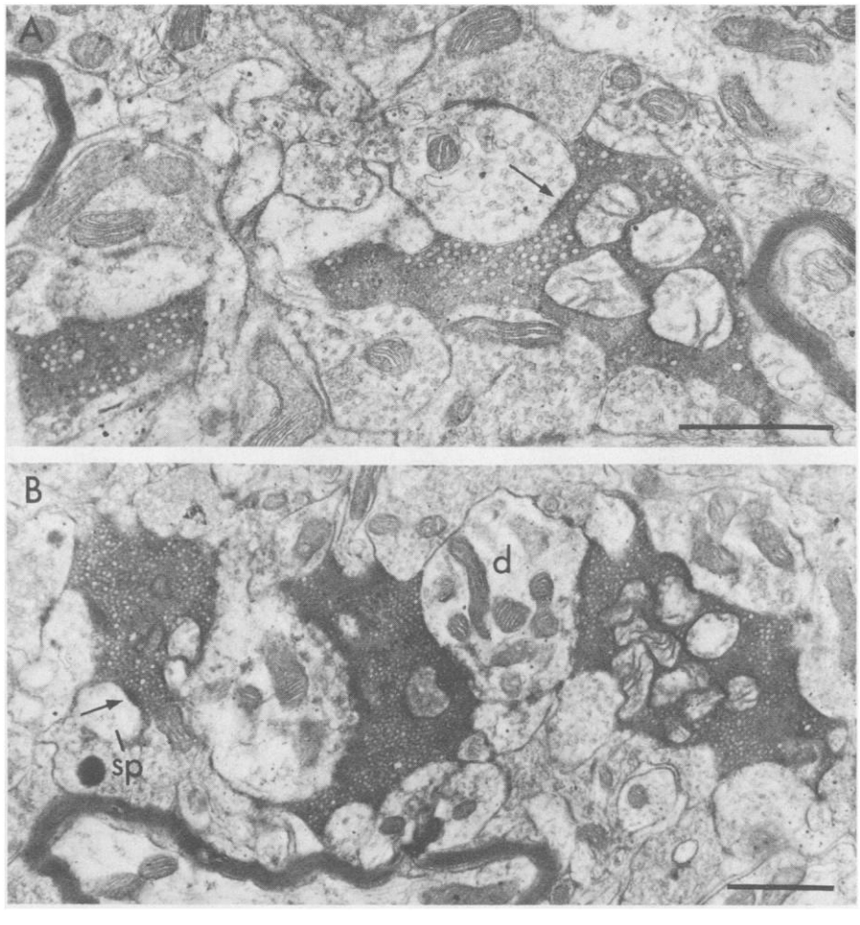

Fig. 3. (A) Labeled retinogeniculate axonal terminal in the denervated layer *A*. This terminal is a part of an arbor that arose from an axon in the normally innervated layer A_1. The profiles surrounding this terminal form a typical glomerular configuration, but in this section only one of them receives a synaptic contact from the labeled terminal (arrow). Scale bar, 1 μm. (B) Section through a cluster of labeled retinogeniculate terminals, also from denervated layer *A*. In this more complex synaptic zone, a single dendritic profile (*d*) is contacted by two labeled terminals. Note synapse (arrow) onto spine (*sp*). Scale bar, 1 μm.

sprouts with an electron microscope. Figure 3A shows a labeled retinogeniculate terminal from a previously denervated lamina *A*. Although the cytoplasm of this labeled profile is filled with the dark reaction product, the marker has not crossed the axonal plasmalemma, nor has it crossed the membranes of the synaptic vesicles or mitochondria. Thus, it is possible to see that the synaptic vesicles are round and that the mitochondria have a pale appearance due to their widely spaced cristae and pale intercristal substance. These features are characteristics of normal retinogeniculate axon terminals (*15, 16, 19*).

The labeled profile also forms synaptic contacts like those described previously for normal retinogeniculate axons (Fig. 3A). It is surrounded by an arrangement of profiles in a configuration resembling a synaptic glomerulus in a normal nucleus. Some of these profiles contain pleomorphic synaptic vesicles. In this micrograph the labeled terminal is presynaptic to one of these profiles (arrow) but is not postsynaptic to any of them. Thus, even though this terminal is from an abnormally grown axon branch, it has formed patterns of synaptic contacts that appear to be quite normal (*15, 16, 19*).

Figure 3B shows another example from denervated layer A in the same animal. Profiles labeled with HRP are involved in a more complex synaptic zone. These profiles contact dendrites, terminals with pleomorphic vesicles and spinelike processes. In addition, a single dendritic profile is contacted by two labeled profiles, both from the same axon. Although this last feature differs somewhat from the usual concept of the lateral geniculate glomerulus, we have observed similar clusters of retinogeniculate terminals in normal adult cats [(*19*); see also (*16*)].

We have demonstrated that a single axon can innervate its normal field and also an abnormally invaded field. The pattern of axonal branching indicates that the axon goes to the correct site first and then sends terminal branches to the new site. The classical picture that sprouts are induced to form from intranodal axonal segments (*21*) does not appear to apply to this interlaminar sprouting in the lateral geniculate nucleus. Instead, it would appear that terminal growth has continued beyond the stage at which one eye was removed and that some of the newly grown terminals invaded the denervated sites.

The phenomenon is probably growth that is abnormal in direction but not necessarily abnormal in amount. That is, the growth of the axons would have occurred even without the eye removal, but the denervation of some geniculate layers has allowed the axons to grow into inappropriate layers (*2, 4, 7, 21*). This interpretation assumes that, at the time of enucleation, the retinogeniculate axons are segregated into their normal layers. Axonal degeneration and autoradiographic studies (*22*) at early postnatal ages show that this is the case. Furthermore, in Golgi material from kittens as young as 8 days, O'Leary (*13*) never saw an axon that sent branches to both laminae *A* and A_1.

In a normal cat, one retinogeniculate axon does not invade two adjacent geniculate laminae (*13, 14*). The crossed and the uncrossed axons have distinct and well-specified terminal fields (*12, 22*). On this basis one might have anticipated that an axon with terminals in lamina *A* would not also develop terminals in lamina A_1. Our results show that the normal selectivity has been destroyed by the early eye removal. That is, either the specific character of the denervated layer is lost after the eye removal, so that inappropriate axons can invade it, or the formation of these specific connections depends entirely upon the properties of the retinogeniculate axons. Further studies with our method of the early development of retinogeniculate axons in normal and monocular kittens may show how the retinogeniculate axons define their terminal fields.

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Lead Exposure During Infancy Permanently Increases Lithium-Induced Polydipsia

Abstract. *Lead (200 milligrams per kilogram) was administered daily by intubation to Long-Evans rats on days 3 through 30 of life. Thirty to 180 days after cessation of lead administration, the lead-treated rats were consistently more polydipsic after lithium administration (2 millimoles per kilogram per day) than were pair-treated controls. Lithium increased the plasma renin activity equally in both the lead-treated and the control groups. These data are evidence that there may be permanent neural changes induced by postnatal exposure to lead that are manifested by pharmacological challenge with lithium.*

Acute lead intoxication (plumbism) is a clinical entity with defined physiological changes and concomitant diagnostic signs (*1*) which are usually ameliorated by cessation of exposure to lead and chelation therapy (*2*). However, it has been extremely difficult in both humans and laboratory animals to define the effects

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