Retrograde Axonal Transport in the Central Nervous System

Abstract. *When horseradish peroxidase is injected into the optic tectum of a chick, axons of ganglion cells transport it centripetally to their cell bodies in the retina at a rate of about 72 millimeters per day. After intraocular injections in the young chick, the peroxidase is transported centripetally along efferent axons, and is concentrated in cell bodies within the isthmo-optic nucleus. This retrograde movement of protein from axon terminal to cell body suggests a possible mechanism by which neurons respond to their target areas.*

Differentiating neurons may respond to changes in their peripheral fields by undergoing atrophy or hypertrophy (*1*), but the mechanisms by which the neuron cell body recognizes its target field are poorly understood. One possible mechanism is by the uptake of material by the axon terminal, and the transport of it in a centripetal or retrograde direction to the cell body. Although anterograde axonal transport has received considerable attention (*2*), less information is available concerning the possibility of a reverse transport. Recently, investigators have described the retrograde movement of protein from the area of nerve terminals in a muscle, along peripheral nerves, to the cell somas (*3, 4*); however, a similar process has not been recognized within the central nervous system. We report here the uptake and retrograde transport of horseradish peroxidase by axons of two different neuronal populations in the central nervous system of the chick: from terminals in the retina to their cell bodies in the isthmo-optic nucleus (ION), and from terminals in the optic tectum to retinal ganglion cell bodies.

In the avian visual system, the completely crossed projection of retinal ganglion cells to the contralateral optic tectum (*5*) facilitates the experimental manipulation of one side with the other side as control. The source of efferent axons to the retina, the ION, has been identified both anatomically and physiologically in the avian midbrain (*6*). Within the retina, these efferent axons terminate on amacrine and displaced ganglion cells in the inner nuclear layer (*7*), and are clearly separate from the ganglion cell layer. Similarly, in the midbrain, ION cell bodies are separate from the termination of ganglion cell axons in the optic tectum. Therefore, we could distinguish the results of retrograde axonal transport from those of anterograde axonal transport by examining the discrete localization of the enzyme after the transport, despite the wide diffusion of peroxidase from the injection site (*8, 9*).

Seven chicks (*10*), one of 3 days of age, and two each of 8, 21, and 34 days of age, were anesthetized with chloral hydrate. Horseradish peroxidase [molecular weight, about 40,000 (*10*)] (2 to 5 mg in 0.05 ml of Ringer solution) was injected into the left eye of each chick. After 23 to 30 hours, the animals were perfused with a fixative containing 1.25 percent glutaraldehyde and 1 percent formaldehyde (*11*) in 0.1*M* phosphate buffer at *p*H 7.4. The following day the brains and eyes were removed, and the diameter of the eyes and the length of the retino-tectal tracts were measured. The tissue was stored overnight in phosphate buffer containing 5 percent sucrose. Serial sections, 40 μm thick, of the optic tecta and the midbrains were cut on a freezing microtome, and every tenth section was stained with 0.1 percent toluidine blue in benzoate buffer. The remaining sections were incubated for 5 to 10 minutes in a medium containing hydrogen peroxide and 3,3′-diaminobenzidine tetrahydrochloride (*12*). The sections were then mounted in gelatin on slides (*13*).

The 3-day-old chick injected with peroxidase in its left eye was killed 24 hours after the injection; retrograde transport of the enzyme from the retina centripetally to the cell bodies of the right ION was demonstrated in this specimen. Sections of the left retina were stained dark brown, and both fiber and nuclear layers were filled with peroxidase product. The right retina showed no evidence of peroxidase presence. Dark granules were distributed within the cell bodies of the right ION (Fig. 1b), although the left ION was free of enzyme (Fig. 1a). Peroxidase was absent in the outer laminae of the optic tectum 24 hours after the intraocular injection of the enzyme; this rules out a rapid anterograde transport of the preformed protein along axons of retinal ganglion cells, even though protein synthesized from labeled amino acids is transported in this way (*2*).

Table 1. Ages and survival times for chicks in which peroxidase was injected into the left optic tectum. One animal was examined at each time point unless otherwise indicated by the number in parentheses.

Age (days)	Dose (mg)	Survival (hours)	Enzyme in right retina
5	2	0.5	−
5	2	1	−
5	2	2	−
5	2	3 (3)	−
5	2	4 (3)	−
5	2	5 (3)	+
7	2	1	−
7	2	2	−
7	2	4	−
7	2	6	+
7	2	8	+
14	4	26	+
21	2–4	8	+
21	2–4	12	+
21	2–4	16	+
21	2–4	24	+

The 8-day-old chicks in this series of experiments also had a concentration of peroxidase in the cell bodies of the contralateral ION after injection of the peroxidase into one eye, but the 21-day-old and 34-day-old chicks did not have such a concentration. This age difference in the transport of material along axons of the ION neurons is unexplained, but agrees with the findings of Kristensson *et al.* (*4*), who demonstrated retrograde transport in hypoglossal neurons of suckling mice, but found peroxidase in only a few neurons after similar injections in older mice.

In a second series of experiments, the surface of the left tectum of 22 chicks of various ages (Table 1) was injected with 2 to 4 mg of horseradish peroxidase. At a selected time, each chick was perfused, and its eyes, optic tectum, and midbrain were sectioned and processed as described above. In addition, slices of retinas and optic tecta were incubated in the same medium, fixed with OsO_4, and embedded for study by electron microscopy.

Results from the 14-day-old chick that was killed 26 hours after being injected are typical of the results in this series. The injection penetrated the left optic tectum at the level of the tectal commissure; the needle passed as far as the stratum album centrale, but only injured incoming retinal fibers over a relatively restricted area. The enzyme activity was mainly concentrated near the injection site, but was also spread across the midline, and into the deeper regions of the contralateral tectum. The peroxidase activity in the right retina was found in the perinuclear cytoplasm of the ganglion cells and in the optic fiber layer; no other cellular or fiber layers in the neural retina displayed

peroxidase activity (Fig. 2b). In ultrathin sections of the retina, the electron-opaque enzyme product was found to be localized in membrane-bound vesicles in the ganglion cell somas, and within their axons in the optic fiber layer. These vesicles appeared similar to those seen after peroxidase uptake by neurons of both the peripheral and the central nervous systems (*8, 14*). Peroxidase activity was not demonstrated in the retinas of any of the 12 chicks surviving 4 hours or less, but was present in all of the ten chicks surviving 5 hours or more (Table 1). Sections of the left retina contained no peroxidase reaction product (Fig. 2a); thus, the possibility of an extracellular or vascular route of transport seems unlikely.

In a few cases the peroxidase was transported after single injections to virtually every ganglion cell in all regions of the contralateral retina, including those in the superior and posterior quadrants. The injections into the lateral surface of the tectum did not injure the axons that originate from ganglion cells in the superior and posterior retina quadrants, and which innervate the inferior and anterior tectal regions, respectively (*15*). This evidence, in addition to the finding of retrograde transport in cells of the ION after intraocular injections, indicated that the uptake and transport were not related exclusively to pathological interruption of axons.

The rate of retrograde transport was estimated from the experiments involving tectal injections. The shortest axon path, from a ganglion cell in the postero-superior retinal quadrant to an area in the antero-inferior tectal region (*15*), was estimated to be 15 mm in the 5-day-old chick (*16*). Since peroxidase activity was first identified with certainty in the retinal ganglion cells after 4 to 5 hours (Table 1), the maximum rate of retrograde transport appears to be at least 72 mm/day. This estimate is based on the assumption of a negligible time for diffusion and uptake of the peroxidase (*17*). The rate agrees reasonably well with that described by Bodian and Howe (*18*) who found poliovirus transported centripetally at about 2.4 mm/hour, or 58 mm/day, along the sciatic nerve in the monkey. A rate of about 120 mm/day was reported for the transport of the fluorescent marker Evans blue, bound to bovine albumin, after its injection into the tongue of a rabbit, and the subsequent detection of the marker in the hypoglossal nucleus (*4*). Several factors might account for this difference in transport rates, including different neuronal systems, species, markers, and ages. Similar ranges in rates have been reported for the anterograde axonal transport of protein (*19*).

The use of horseradish peroxidase as a neuroanatomical method for mapping pathways from axon terminals to cell bodies of origin must be viewed with caution because of the spread of the protein after injection into the central nervous system. Intraocular injections are more informative because there is a more restricted spread of the enzyme as compared to tectal injections.

Our work extends the findings of retrograde transport in the peripheral motor system by demonstrating retrograde transport in two different pathways within the central nervous system. The fact that axons can sample macromolecules in their local environment, and then transport them to the cell body, suggests a possible mechanism for several regulatory reactions, including the phenomena of neuronal chromatolysis after axon injury (*20*), growth regulation according to the size of the peripheral field (*1*), retrograde transsynaptic changes (*21*), and acute glial reaction (*22*).

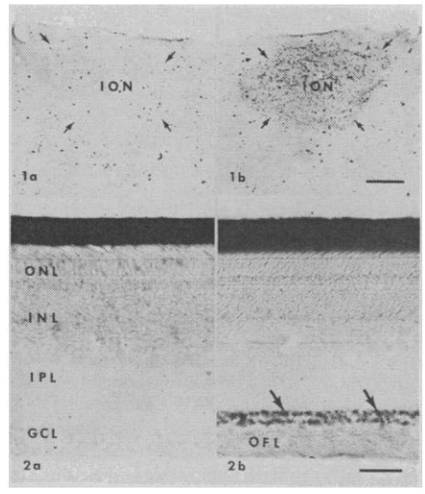

Fig. 1 (top). The left (a) and right (b) isthmo-optic nuclei (*ION*) in the midbrain of a 3-day-old chick that was killed 24 hours after peroxidase was injected into its left eye. The peroxidase is restricted to the cells of the right ION. Arrows indicate the outer margin of the ION. Unstained sections; marker, 250 μm. Fig. 2 (bottom). Retinas from the left (a) and right (b) eyes of a 14-day-old chick that was killed 26 hours after peroxidase was injected into its left optic tectum. The enzyme was localized in the ganglion cell layer (arrows) of the right retina (*GCL*, ganglion cell layer; *INL*, inner nuclear layer; *IPL*, inner plexiform layer; *OFL*, optic fiber layer; *ONL*, outer nuclear layer; unstained sections; marker, 25 μm).

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16. A minimum distance of 8 mm was estimated for the length of the optic nerve fibers within the eye from the postero-superior region of retina to the optic nerve head (optic disk); the 7-mm average length of the optic nerve and tract was added to this. As small quantities of the peroxidase may be taken up along the length of unmyelinated axons in the central nervous system, the estimated rate was based on transport from the anterior pole of the tectum.
17. Horseradish peroxidase was seen throughout the injected tectum in chicks that lived only 30 minutes after the injection; by that time, the enzyme product was observed by electron microscopy both in membrane-bound vesicles and diffusely within some axons of the stratum opticum of the injected tectum.
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