Descending optic nerve degeneration in primates

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We cut the optic nerve at the orbital apex in squirrel monkeys to study the descending degeneration of optic nerve axons and their ganglion cell bodies. We could not detect progressive disintegration of the axon from the site of injury back to the cell body. Instead, the entire length of individual axons seemed to degenerate simultaneously as early as 3 weeks and as late as 6 weeks after injury, as judged both by ultrastructural integrity and by continued slow axonal transport, a reflection of local physiologic function. We could not relate the time of degeneration to the distance of the injury from the cell body. Evidently there is a signal of injury to the cell body after axotomy, though the nature of the signal and the mechanism by which it leads to cell death are unknown.

Key words: optic nerve, descending degeneration, optic atrophy, axonal transport, retinal ganglion cells.

In all vertebrate neurons, a nerve fiber separated from its cell body does not survive. Some neurons, however, are able to regenerate a new fiber from the portion of the axon still attached to the cell body. Other neurons are incapable of regeneration and most commonly suffer cell death after axotomy. In the central nervous system, the ability to regenerate after injury depends on such things as species, age, and nerve cell type. For example, goldfish retinal ganglion cells respond to optic nerve transection by complete regeneration of their fibers, and full visual recovery occurs within one month.² In the primate visual system, injury to axons in the retinal nerve fiber layer, optic nerve, chiasm, or tract leads to ganglion cell death, and this is called descending degeneration.³

We have produced experimental descending degeneration in primate ganglion cells by transection of the orbital optic nerve. The initial aim of the study was to establish whether injured optic neurons degenerate progressively backward toward the cell body or whether the entire length of the axon dies at nearly the same time following injury. This question was raised by a previous study² and the present experiments are designed to make this determination by light and electron microscopic examination of serial segments of

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the optic nerve. At the same time, the physiologic function of the axon was judged by studying slow axonal transport at stages during degeneration. In making detailed observations of degenerating optic nerve, we noted that fibers in the central area of the nerve maintained normal axonal transport longer than peripheral fibers. These data allow conclusions about differences in degeneration between ganglion cells from various retinal areas with regard to their distance from the site of injury.

Methods

One optic nerve was transected at the orbital apex in nine squirrel monkeys (Saimiri sciurea). In five monkeys the contralateral eye and optic nerve were used as controls; one of these was sham operated. With pentobarbital anesthesia, the nerve transection was performed between 6 and 8 mm. behind the globe through a lateral orbitotomy requiring removal of the lateral orbital bone and the lateral rectus muscle. The nerve was cut with fine scissors and the muscle and skin incisions closed with silk suture. Intravenous fluorescein angiography immediately after each procedure showed that in two of the nine animals the central retinal artery was compromised. These animals are not included in the data. In the other seven eyes, there were no clinical or histologic signs of retinal or choroidal vascular damage. During the postoperative period the intraocular pressure was measured several times in some animals with the pneumotonomograph. In no case was there a significant difference (>5 mm. Hg) between eyes operated upon and normal fellow eyes. After nerve transection, animals were sacrificed at 2 weeks (two animals), 3 weeks (two animals), 4 weeks (two animals), and 6 weeks (one animal).

For the study of slow axonal transport, an intravitreal injection of $^3$H-leucine (L-leucine-4, 5-$^3$H(N), 30 to 50 Ci./mmol., New England Nuclear, 100 µCi in 0.1 ml./eye) was made 1 week prior to sacrifice in the seven nerve-sectioned and four control eyes. One week after injection coincides with the passage of the slow phase of orthograde axonal transport through the nerve in the area of the transection. Prior to each injection, 0.1 cc. of fluid vitreous was aspirated to prevent elevation of intraocular pressure and the consequent reflux of injected material through the needle tract.

Animals were sacrificed under deep pentobarbital anesthesia by perfusion of 4 percent phosphate-buffered paraformaldehyde retrograde through the aorta. The globes, optic nerves, chiasm, and tracts were dissected free en bloc, with paraformaldehyde fixation for 24 hours. The optic nerves were severed from the globe at the sclera. The globes were divided horizontally through the optic disc and pupil. One half was embedded in paraffin for autoradiography and light microscopy. The other half was divided into small samples of optic disc and retina, postfixed in 2 percent osmium tetroxide in phosphate buffer, and embedded in epoxy resin for electron microscopy. The optic nerves including the transection and chiasm were divided horizontally in half with a razor blade. One half was embedded in paraffin and serially sectioned for autoradiography. The chiasmal area was examined in each case for the presence of uncut fibers which would carry radioactive label beyond the transection. The other half was divided vertically into five 1 mm. segments from just behind the globe through the transection area. These were postfixed in osmium, embedded in epoxy resin, and examined both in thick section by light microscopy and by electron microscopy.

Autoradiographs were prepared with the use of Kodak NTB-2 emulsion with 1 week exposure. They were developed in Kodak D19 for 2 minutes, fixed in Rapid Fix for 2 minutes, and stained with hematoxylin. The pattern of grains was examined by low-power dark-field microscopy and, in selected specimens, grain counts were performed with the use of the mean number in 50 fields of a 100 square micron area viewed under 1,250× oil immersion.

Results

Axonal transport. In the control optic nerves, there was a relatively uniform density of transported radioactivity from globe to chiasm 1 week after injection (Fig. 1). In two experimental eyes from animals sacrificed 2 weeks after nerve transection, the amount and pattern of slow axonal transport were not different from normal. Labeled material passed uniformly from ganglion cells to the transection site (Fig. 1). Small clumps of grains could be seen near the cut area.

In both animals sacrificed 3 weeks after nerve injury, the pattern of slow axonal transport was abnormal (Fig. 1). There was a considerable decrease in radioactivity in the fibers traveling in the peripheral portion of the nerve cross-section. In fibers
Fig. 1. Autoradiographs of longitudinally sectioned optic nerve during slow phase of axonal transport. In each pair of photographs, the globe was at the left in the left-hand picture and the right-hand picture shows the nerve at the orbital apex. These autoradiographs are reproduced in dark-field illumination, with grains representing transported material appearing as small white dots. Glial cell nuclei appear as larger white dots. C: Control nerve with normal slow transport. Right photograph includes beginning of chiasm where unlabeled fibers from contralateral nerve dilute the pattern of white grains. 2W: Two weeks after orbital nerve transection, the pattern of grains at left resembles the control. The transport continues up to the nerve cut in the right photograph. 3W: Three weeks after transection, there is reduced grain concentration in the nerve periphery but normal density in the center of the nerve. The pattern is maintained down to the transection shown at right. 4W: Four weeks after transection, the transport is uniformly reduced throughout. 6W: Six weeks after transection, the only white dots seen in most of the nerve are glial nuclei. There was a peripheral area of uncut fibers (arrow) showing normal transport. (All x30.)
in the central one fifth of the nerve cross-section, the amount of label was similar to that in control nerves. To confirm this impression, quantitative grain counts were compared over central fibers compared to fibers in the periphery of the nerve at the same distance from the globe. Whereas in normal nerves the ratio of center to periphery nerve radioactivity was 1.04, in the 3 week experimental specimens this ratio had increased to 2.03. However, in both central and peripheral fibers there was no major decrease in grain concentration from the area near the globe to the area near the transection. The retrobulbar zone/transection zone grain density ratio was 1.06 in 3 week posttransection nerves and 1.14 in normal nerves.

Four weeks after nerve transection, the amount of slow axonal transport was reduced even further (Fig. 1). There was no retained normal transport in the central fibers of the nerve as seen at 3 weeks. The grain concentration was uniformly decreased from the globe to the transection site.

Six weeks after transection, there was very little slow transport evident (Fig. 1). In a small area of the nasal periphery of the nerve there was a dense concentration of grains in fibers which could be seen in serial sections to have been missed in the nerve cut, since they transported label well into the chiasm. These fibers provided an unintentional internal control in this specimen, showing how depressed the slow transport was in transected fibers at six weeks.

**Histological changes.** We will describe the course of descending degeneration in the retinal ganglion cells and the portion of the optic nerve attached to the globe after transection.

Two weeks after nerve section the number and appearance of the retinal ganglion cells were normal. The only abnormality of the axons was the presence of occasional focal aggregations of intraxonal organelles dispersed throughout the width of the nerve (Fig. 2). The nerve was similar in appearance near the globe and near the transection.
Three weeks after nerve section, the number of ganglion cells was beginning to decrease in the parafoveal retina. Chromatolysis of ganglion cells was not observed. The few ganglion cells which were seen to be abnormal, and presumably in the process of degenerating, had condensed nuclei and vacuolated cytoplasm. In the optic nerve, the axons had considerably more organelle accumulations and incipient myelin sheath disruptions than before (Fig. 3). These changes did not seem to be more extensive in axons in the center of the nerve compared to axons in its periphery, nor was there more degeneration in the nerve near the globe compared to near the transection.

By 4 weeks, the number of ganglion cells was considerably reduced (Fig. 4) and axonal degeneration was widespread throughout the nerve (Fig. 5). Six weeks after transection, there were few ganglion cells or axons remaining. The optic nerve contained only glial cells and degeneration products of former axons.

Discussion

In a previous histologic study, descending degeneration seemed to involve primate optic nerve axons along their whole length at once. The present study concentrated on the critical time period for axonal degeneration—the first 6 weeks after transection. Both detailed histologic examination and the pattern of cessation of slow axonal transport confirmed that in descending degeneration of the optic nerve, axons are affected along their entire length rather abruptly—some as early as 3 weeks and others as late as 6 weeks after injury. There was no sign of axons progressively dying back toward the cell body, in which case the amount of transported label would have been normal or high in the axon segment near the globe and low or absent in the
Fig. 4. Light micrographs showing foveal retina 4 weeks after nerve section (A) and normal (B). There is obvious loss of ganglion cells in A. (Both x600; phase contrast; paraphenylenediamine.)

Fig. 5. Four weeks after nerve transection there is considerable degeneration of optic nerve axons. (x6,600.)

degenerating segment near the transection (Fig. 6).

This is in keeping with the general observation that in retrograde degeneration of various neurons the direct effect of the injury may extend a short distance up the axon, but the remainder of the proximal axon stump does not degenerate unless the cell body dies. Other studies of optic nerve degeneration in lower animals have yielded similar findings. Lin and Ingram6,7 transected the optic nerve in cats and judged the degree of degeneration by light microscopic histochemistry. They suggested that the very early degenerating axons may be more affected near the transection than at the globe. However, between 2 and 3 weeks after transection, during the degeneration of most axons, the degeneration was uniform through the nerve. Cowan et al.8 studied descending degeneration by histochemistry in pigeon optic nerve centrifugal fibers, whose terminals are in the eye and whose cell bodies lie in the brain.
The degeneration began closer to the cell body than to the transection and no evidence for a progressive dying back was found.

Evidently, the cell body reacts to the injury (by disintegration) without the prior degeneration of the axon between it and the site of injury. Therefore, some signal of axon injury must reach the cell body. To understand how this kind of signaling might work, it is appropriate to consider what is known about the response of neurons in general, as represented by lower vertebrate retinal ganglion cells or neurons of the peripheral nervous system, which regenerate after injury. During the first week after injury of these cells, the cell body greatly increases ribonucleic acid and protein synthesis,\textsuperscript{1, 2, 9, 10}, a process represented morphologically as chromatolysis. In addition, the rate of slow axonal transport may increase\textsuperscript{2, 11} or decrease,\textsuperscript{11-12} perhaps depending on the evolution of the cell response. The rate of rapid transport seems to remain constant,\textsuperscript{13} though its content may change. Retrograde transport from transection to cell body continues after injury and may be normal, increased, or decreased in amount.\textsuperscript{14-16}

In regenerating neurons, it appears that the closer the injury is to the cell body, the more rapid and intense is the increased cell body synthesis (chromatolysis).\textsuperscript{9} When the regenerated axon reaches an appropriate target organ, regeneration stops and the cell body alterations subside. To explain these observations, it is hypothesized\textsuperscript{15, 16} that the target organ normally provides a substance to the cell body by retrograde axonal transport. This substance effects a feedback control over cell body synthetic activity. With axon injury, the link between the target organ and the cell body is interrupted and the cell body does not receive the hypothetical messenger substance. The decrease in this substance may be the signal for chromatolysis. Chromatolysis would be delayed as long as there is still some continued arrival of the substance from the axon stump, and this could explain the relation between the location of the injury and the onset time of chromatolysis.

Of particular interest is that in those nerves capable of regeneration, the cell bodies disintegrate if the regenerating axons fail to reach an appropriate target organ.\textsuperscript{10} This kind of disintegration also occurs during development; neurons whose axons fail to make connection with their target organ degenerate.\textsuperscript{17} The relationship between the signal for cell death and the signal for initiation or termination of chromatolysis is by no means clear, though both are hypothesized to be carried by retrograde axonal transport. The cell body response is clearly different—in one case, cell metabolism is heightened; in the other, the cell ceases its functions.

Some neurons, such as primate retinal
ganglion cells, do not attempt regeneration. They have no chromatolysis, nor do they show marked increase in protein synthesis by autoradiography. The lack of increased protein synthesis could be responsible for the failure to regenerate, or it could be merely a sign of a more primary inability of the primate retinal ganglion cell to restore its normal structure successfully. These cells were clearly capable of developing an axon in embryologic life. We do not know whether the adult primate neuron receives no signal for chromatolysis or whether it is nonresponsive to the signal.

However, primate retinal ganglion cells do show the disintegration response after axotomy. If the signal for cell death is carried by retrograde transport, the message should reach the cell soma within the first day, if present estimates of the speed of retrograde transport are correct. Yet, the cells remain normal for 1 to 2 weeks. It must be assumed, then, that there is a cumulative effect of cessation of transport. Perhaps the signaling substance is normally continuously arriving from the axon terminal and has a certain metabolic half-life in the cell soma. With cessation of transport, the time required for the cell body to sense a critical decrease in signal concentration would depend upon the rate of disappearance. Moderately increased intraocular pressure reversibly blocks ortho- and retrograde axonal transport for short periods in retinal ganglion cells without producing cell body death. This further suggests that if transport interruption plays a role in signaling the onset of cell body death, the effect must be a cumulative one, dependent on a maintained blockade.

It has been suspected that the timing of atrophy following injury to the optic nerve depends on how far from the eye the injury occurs, much as in nerves capable of regeneration the timing of chromatolysis depends on how far away from the cell body the axon is injured. In our experiment, if descending optic nerve degeneration were initiated more rapidly by injuries closer to the cell body, then fibers of the central optic nerve would degenerate most quickly, since their cell bodies in the macula are closer to the cut than the peripheral cell bodies which send fibers into the peripheral optic nerve. Our data show a tendency in the opposite direction, with central fibers of the nerve maintaining normal axonal transport longer than peripheral fibers. This suggests that the distance of the injury from the cell body is not the most important factor in the timing of degeneration. The type of ganglion cell or its fiber diameter may be more important. In primates, the central optic nerve in mid-orbit contains more small diameter fibers than the peripheral nerve. It has been suggested that in ascending degeneration (eye to brain) fibers smaller in diameter degenerate more slowly than larger fibers. It may be that the slower degeneration of central fibers in descending degeneration is related to their smaller diameters.

It is interesting that the time of cell death after optic nerve axotomy is found to be independent of the distance from the cell body in other animal models and in man. When cat ganglion cell axons were transected either at the orbital optic nerve, along the optic tract, or at the lateral geniculate body, descending degeneration was noted from 2 to 3 weeks after each of the three lesions. In a human eye examined histologically 5 weeks after unilateral optic tract transection, hemiretinal loss of ganglion cells had occurred. Lundstrom and Frisen took serial red-free photographs of a patient with gunshot injury to the optic nerve just in front of the chiasm. The retinal nerve fiber layer began to disappear at 4 weeks and was gone by 6 weeks. In previous monkey experiments and in this study, disappearance of the retinal nerve fiber layer began to be apparent clinically at 4 weeks, as in the human cases. Yet, the monkey nerves were transected 6 to 8 mm. from the eye and...
in the two human patients the injuries were at least 30 to 40 mm. from the globe. Loss of retinal ganglion cells by descending degeneration therefore seems to be relatively independent of the distance of the injury from the cell body.

REFERENCES