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31. This is Technical Paper 5329 from the Oregon Agricultural Experiment Station, Oregon State University, Corvallis 97331. We thank L. J. Hunter, J. Casteel, T. Will, and J. Hotchkiss for their technical assistance. Supported in part by grants ES00550, ES00541, ES00210, and ES01926 from the National Institute of Environmental Health Sciences and by grant CA25766 from the National Cancer Institute.

7 November 1979; revised 29 January 1980

Order in the Optic Nerve of Goldfish

Abstract. *A small amount of horseradish peroxidase, injected into the goldfish optic nerve and transported into the retina, filled an annulus of ganglion cells. Since the retina grew by annular addition of cells, this result shows that axons from cells of similar age clustered together in the nerve.*

Most nervous systems are composed of collections of nerve cell bodies connected by groups of axons. The connections are often highly ordered, and proper function depends on this order. Much of our knowledge of the formation, maintenance, and modification of such orderly connections has been derived from studies of the retinotectal system of lower vertebrates, especially fish and amphibians. Ganglion cells of the retina send axons to the tectum via the optic nerve. The axons from ganglion cells at different locations on the retina terminate on the tectal surface in a retinotopic map; that is, neighboring retinal sites project to neighboring tectal sites. Although the spatial relations between the retina and tectum have been extensively studied, relatively little information exists concerning order in the optic nerve. Recent anatomical studies have shown that axons of neighboring retinal ganglion cells are often near each other in the nerve (*1*-*3*). This result has been interpreted as evidence that the nerve is retinotopically ordered, and, in addition, that the order was instrumental in the original establishment of connections (*4*). There are, however, reasons to doubt that the nerve is so simply ordered. Electrophysiological attempts to reveal retinotopic order have failed (*5*). Also, no evidence exists for the necessary consequence of the retinotopic hypothesis: that axons from widely separated retinal sites are separated in the nerve. Scholes has suggested, from the appearance of the retinas of cichlid fish, that axons near each other in the nerve originate from ganglion cells located in annuli centered on the optic disk (*2*). We have confirmed Scholes's predictions in the goldfish and conclude that the nerve is ordered, but chronologically rather than retinotopically.

We have used the histochemical marker horseradish peroxidase (HRP) to trace clusters of optic nerve axons to their retinal origins. A small amount of HRP, injected into the optic nerve just behind the eye, was taken up by a discrete group of axons that transported it both anterogradely to the tectum and retrogradely into the retina (*6*). A longitudinal section of an optic nerve which received such an injection is shown in Fig. 1a. Axons filled with HRP reaction product form a dense cluster surrounded by relatively clear areas. Figure 1b shows the whole retina, prepared as a flat mount, which was attached to this optic nerve. The HRP reaction product filled axons radiating from the optic disk and an annulus (arrow) of ganglion cell bodies. Filled axons were not found peripheral to the annulus, nor were filled ganglion cell bodies found either central or peripheral to it. (The other dark areas in Fig. 1 are blood vessels, most prominent peripheral to the annulus, and clumps of pigmented epithelium which stuck to the retina near the optic disk and margin.) Figure 1c is a view of the central edge of the annulus; individual ganglion cell bodies, some dendrites (single arrow) and axons leaving the annulus (double arrow) are filled with HRP reaction product.

In six cases in which HRP injection was confined to part of the optic nerve, one retina had a complete annulus of stained ganglion cells and five had partial annuli ($< 360°$ in circumference). The annuli varied in width and in distance from the optic disk. In one retina, two partial annuli at different distances from the disk were filled. Since the goldfish retina grows by adding new cells at the margin (*7*), the cells in each annulus are of similar age. Hence the annular labeling pattern indicates that axons of similar age cluster together in the optic nerve (*8*). The HRP injections tended to be confined by glial boundaries in the nerve. Since glial compartmentation of the optic nerve appears to be random (*9*, *10*), we suggest that each partial annulus resulted when the HRP was confined to a region which contained only a fraction of an age-related cluster of axons. The filled ganglion cells were always tightly grouped rather than scattered around the annulus, implying that within an age-related cluster of axons, those from neighboring ganglion cells were relatively near one another. This inference is consistent with previous reports that axons from neighboring places on the retina tend to stay together in the optic nerve of goldfish (*1*).

The results of our HRP injections do not allow us to determine if neighboring clusters of axons in the optic nerve arise from adjacent retinal annuli, although the ultrastructural appearance of the nerve itself suggests this. The optic nerves of small (young) goldfish contain a cluster of nonmyelinated axons. This cluster always lies adjacent to clusters containing slightly larger axons surrounded by thin myelin sheaths. Axons in more distant clusters are larger still and surrounded by even heavier myelin sheaths (*10*). We suggest (i) that this gradient of axon size and myelination results from the addition of new (nonmyelinated) axons near others recently added and (ii) that myelination of the axons has proceeded further in the older clusters. These suggestions are supported by several pieces of evidence. (i) Only a few scattered nonmyelinated axons are found in the optic nerves of large (old) goldfish (*10*); the absence of a cluster of nonmyelinated axons rules out their existence as a stable population and suggests that they are not efferent axons since efferents exist in both large and small goldfish. [In addition, Schmidt has recently shown that efferent axons in the goldfish optic nerve are large, myelinated axons (*11*).] (ii) Lesions of the peripheral retina disrupt only axons in the nonmyelinated cluster and in nearby clusters of thinly myelinated axons (*12*). This evidence strongly suggests that nonmyelinated axons are from ganglion cells at the peripheral margin of the retina. Lesions that damage axons from both peripheral and more central (older) retina disrupt axons in the nonmyelinated and thinly myelinated clusters

0036-8075/80/0418-0311$00.50/0

and also axons in clusters containing more heavily myelinated axons (*12*); the disrupted axons are not scattered around the optic nerve but are in contiguous clusters. The latter evidence supports our hypothesis that older axons (from more central retina) are myelinated and that axon clusters add to the optic nerve near axon clusters of similar age.

The ultrastructural observations and the results of HRP injections lead us to conclude that the optic nerve of goldfish is composed of age-related clusters of axons with each cluster near other clusters of similar age. We can only speculate about why axons associate together by age. Perhaps the new axons go wherever space is available, following residual radial glial channels (*13*). Perhaps the optic disk is impenetrable over most of its surface, and new axons exit through the only available crack (*14*). Alternatively, the new axons may be mutually adhesive, as has been suggested by experiments in tissue culture (*15*).

The age-related order cannot hold through the entire trajectory to the brain. At some point before the axons enter the tectum, the age-related clusters must split since axons from the ventral and dorsal hemiretinas enter the tectum via the medial and lateral branches of the optic tract, respectively (*16*). Axons that were together at the injection site behind the eye did not disperse in either nerve or tract (Fig. 1a). This suggests that the age-related clusters do not split until shortly before the branch point in the optic tract. The pattern of splitting of the fascicles in the optic tract in both goldfish (*12*) and cichlids (*2*) supports this view.

We believe that the optic nerve of goldfish is composed of clusters of axons from ganglion cells born at the same time and that, within each cluster, neighboring axons arise from neighboring ganglion cells. The annular pattern of retinal growth in goldfish, which may be necessary to produce the pattern of order found in its optic nerve, has also been found in some other lower vertebrates (*17*). The pattern of birth of retinal ganglion cells in mammals has not yet been studied. Although one cannot therefore generalize our result to all vertebrates, it is possible that the axons in the optic nerves of many vertebrates are ordered by age.

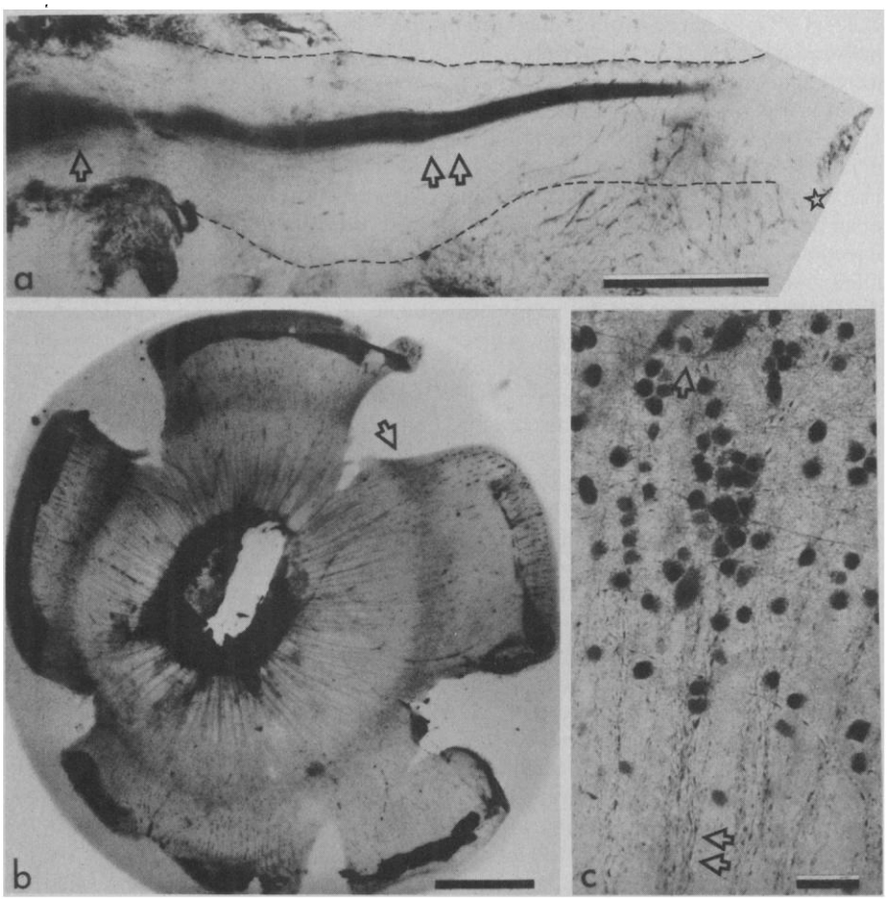

Fig. 1. (a) Longitudinal section of the optic nerve of a goldfish after HRP was injected into the nerve just behind the eye. The bundle of axons filled with HRP reaction product is seen both in the optic nerve (arrow) and in the optic tract (double arrow). The injection site is not shown. The star indicates the periventricular cell layer of the tectum. Scale, 500 μm. (b) Retina which was attached to this nerve, prepared as a flat mount. The arrow indicates the annulus of ganglion cell bodies filled with HRP reaction product. The optic disk (center) is torn. Scale, 1 mm. (c) Part of the annulus, showing filled ganglion cell bodies, some dendrites (single arrow) and axons (double arrow) leaving the annulus. Scale, 25 μm.

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18. We thank B. W. Agranoff for the use of his photomicrography facilities, M. W. Dubin and A. M. Heacock for critical reading of the manuscript, and D. Madrid for typing. Supported by NIH postdoctoral fellowship EY 05294 to A.C.R. and research grant EY 00168 to S.S.E.

5 November 1979