Retinal Projections in Blind Snakes

Abstract. *Following unilateral enucleation of blind snakes, serial sections of the brains were stained by the Fink-Heimer procedure; the sections revealed terminal degeneration in the lateral geniculate nucleus of the thalamus bilaterally, nucleus posterodorsalis of the pretectum bilaterally, and superficial layers of the contralateral optic tectum. The stained degenerating fibers in the tectum were considerably less dense than in the thalamus.*

After observing that the eyes of snakes differ in structure from the eyes of lizards, Walls suggested that "early snakes were subterranean and their eyes underwent wholesale degeneration into a condition fairly well represented by the modern Amphisbaenidae. . ." (*1*). Studies (*2–4*) of retinofugal projections in snakes indicate that the central connections of retinal ganglion cells reflect, not the wholesale degeneration of the eye postulated by Walls, but rather a more conservative reduction and simplification, which was suggested by Underwood (*5*). Northcutt and Butler (*3*) and Halpern and Frumin (*4*) independently compared snakes with other reptiles and observed a diminutive nucleus rotundus and concluded that the retinotectal-rotundal pathway was diminished with no apparent compromise in the retinogeniculate system. I have sought further evidence in support of this point of view through study of the retinal projections of species, such as the burrowing or worm snakes, belonging to the families Typhlopidae and Leptotyphlopidae which are believed to be closely related to the ancestors of the modern "higher" snakes.

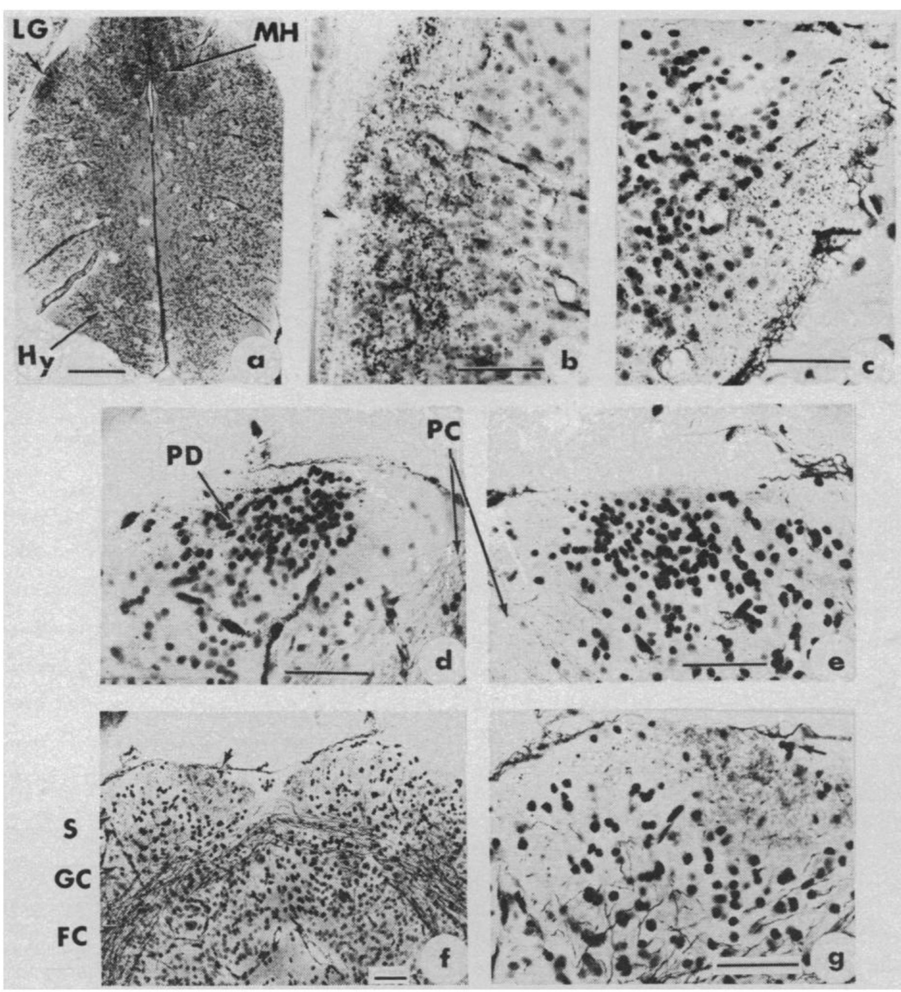

Fig. 1. Selected coronal sections of the brain of a blind snake surviving 14 days. The top of each figure is dorsal, the bottom is ventral. (a) Diencephalon at level of lateral geniculate nucleus. The side contralateral to the enucleation is on the left (scale, 500 μm). (b) Higher magnification of lateral geniculate nucleus illustrated in (a) (scale, 100 μm). The arrow points to imperfection in pial surface, which can be seen at end of arrow point in (a) leading from the *LG* label. (c) Ipsilateral lateral geniculate nucleus (scale, 100 μm). (d) Contralateral nucleus posterodorsalis (scale, 100 μm). (e) Ipsilateral nucleus posterodorsalis (scale, 100 μm). (f) Low magnification view of the optic tectum. The arrow points to three cell nuclei which can be seen in (g) at the end of the arrow point to aid in orientation. (g) Higher magnification of optic tectum contralateral to enucleation. Abbreviations: *FC*, stratum fibrosum centrale; *GC*, stratum griseum centrale; *LG*, lateral geniculate nucleus; *MH*, habenula medialis; *Hy*, hypothalamus; *PC*, posterior commissure; *PD*, nucleus posterodorsalis; and *S*, stratum griseum et fibrosum superficiale.

Klauber (*6*) and Underwood (*5*) report that specimens of the families Typhlopidae and Leptotyphlopidae are "light shy and responsive to light falling on the eyes" (*5*, p. 247). Thus these animals may reasonably be expected to have a functional visual system capable of processing limited photic information.

Three snakes of the genus *Typhlops* were unilaterally enucleated under Fluothane anesthesia by removing one eye spot and covering scale. The snakes were then housed for 10, 14, and 23 days at 22° to 24°C. Frozen coronal sections were cut and then stained by the Fink-Heimer (*7*) method for degenerating axons and terminals.

Degenerating axons cross the midline in the optic chiasm and course in a thin fascicle dorsocaudally as the optic tract along the lateral wall of the thalamus. In the thalamus degenerating fibers and terminals fill the lateral geniculate body completely (Fig. 1, a and b) on the side contralateral to the enucleation. The lateral geniculate nucleus is a group of cells with small perikarya organized in a nucleus which is rounded in contour. The ipsilateral optic tract also shows signs of degeneration, and the corresponding lateral geniculate nucleus contains a substantial amount of a degeneration (Fig. 1c).

Caudal to the lateral geniculate nucleus, the optic tract continues dorsocaudally into the pretectum. The most prominent retinofugal recipient in the contralateral pretectum is the nucleus posterodorsalis (Fig. 1d). Degeneration in the ipsilateral nucleus posterodorsalis is consistently less dense (Fig. 1e) than in the contralateral nucleus.

The optic tectum of *Typhlops* is much smaller and is cytoarchitecturally less well developed than in other snakes (*8*). The tectal layers receiving optic terminals are narrow and the degeneration in the tectum is sparse [Fig. 1, f and g; and compare to illustrations of tectal degeneration in (*4*)]. Only the contralateral tectum receives retinofugal fibers.

No degeneration was observed along the ventrolateral surface of the caudal diencephalon and midbrain where a basal optic tract and nucleus have been described in other snakes (*2–4*).

These observations of the retinal efferent system in blind snakes differ in several respects with those reported for "higher" snakes (*2–4*). In *Typhlops* the ipsilateral contribution to the lateral geniculate nucleus is greater than in other snakes. The prominent nucleus lentiformis mesencephali, nucleus geniculatus, pretectalis, basal optic tract, and basal optic nucleus of "higher" snakes are not recognizable as distinct structures in *Typhlops*. The most striking difference is, however, the paucity in *Typhlops* of retinotectal connections which are abundant in "higher" snakes.

The major thalamic nucleus receiving tectal efferents in reptiles and birds is the large nucleus rotundus (*9*). The nucleus rotundus of the snakes *Natrix sipedon* (*3*) and *Thamnophis sirtalis* (*4*) is extremely small and is just barely distinguishable in some blind snakes (*8*). Thus it would appear that in these blind snakes the retinogeniculate and pretectal projections are sufficient to handle the limited reactivity to light which they demonstrate (*5, 6*).

In view of the supposedly primitive phylogenetic status of the blind snakes, the limited development of the retinotectal projection is notable since the tectum has been described as the dominant area for visually guided behavior in nonmammalian vertebrates (*10*). Perhaps in view of the above findings, the retinothalamic projection deserves greater attention in these forms.

The blind snake is potentially valuable as an example of a naturally occurring experiment in specialization. It would be of considerable interest to know whether the absent and reduced retinal projections correspond with absent or reduced features of visually dependent behavior and whether the retained projections contribute to functions which are held in common by other vertebrates.

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References and Notes

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Application of the Elution Centrifuge to Separation of Polynucleotides with the Use of Polymer Phase Systems

Abstract. *A flow-through centrifuge without rotating seals enables the use of polymer phase systems for chromatographic separation of macromolecules. The capability of the technique is demonstrated on partition of polynucleotides.*

In recent years we have developed a number of arrangements for high-efficiency countercurrent separations. Best results were obtained by using a centrifuge to set up a multistage phase partition system in a coil containing thousands of turns of thin tubing. The flow-through coil planet centrifuge (*1*) and elution centrifuge (*2*) have both been shown to give high-efficiency separations of small molecules such as dinitrophenyl amino acids.

The versatility and usefulness of polymer phase systems for partition of macromolecules and particulates have been well documented (*3–5*). However, application of these systems to conventional liquid chromatography becomes difficult because of their aqueous/aqueous phase composition. Hence, the partition technique at present relies upon the countercurrent distribution method, which requires a relatively long separation time and results in a considerable dilution of the sample. Below we report separation of polynucleotides by using polymer phase systems in conjunction with our elution centrifuge. Advantages of the method are higher efficiency, shorter separation time, and minimum solute dilution. Stepwise or gradient elution with continuous monitoring and fractionation can be used as in conventional chromatographic systems.

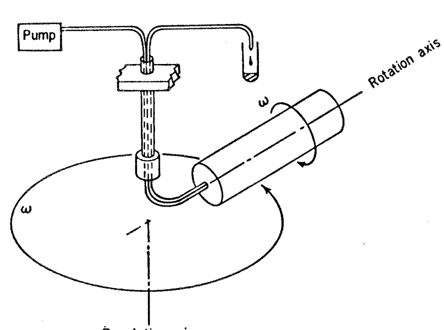

Fig. 1. Principle of the elution centrifuge. A cylindrical holder, carrying a separation column, revolves around the central axis (revolution axis) of the apparatus while it rotates about its own axis (rotation axis) to prevent twisting the flow tubes.

The principle of the elution centrifuge is illustrated in Fig. 1. A cylindrical container, carrying the separation column, revolves in the horizontal plane while it rotates about its own axis at the same angular velocity to prevent twisting the flow tubes. Thus the system allows the effluent to move in and out through the running column without rotating seals. The centrifugal force produced by the planetary motion retains the stationary phase in a fine helical column while the mobile phase is steadily eluted. Consequently, solutes introduced locally are subjected to an efficient partition process resulting in chromatographic separation without influence from a solid support.

The polymer phase systems used in the present study have been introduced by Albertsson (*4*), consisting of 5 percent (by weight) dextran T 500 (Pharmacia), 4 percent (by weight) polyethylene glycol 6000 (Union Carbide), and 10 m*M* sodium phosphate of various compositions indicated in parentheses below. The phase mixture is gently degassed by suction and equilibrated at 18°C. The same temperature is maintained during the elution by cooling the apparatus.

Three column configurations, each made of Teflon tubing 5 m long and 0.55 mm in inside diameter, are examined for the operational requirements of countercurrent chromatogra-