course. The results with knife cuts suggest that many, though perhaps not all, of the relevant fibers may terminate within the ventrolateral mesencephalon, probably within the substantia nigra. Collectively, our findings are consistent with the course and distribution of the strionigral pathway which terminates predominantly within the zona reticulata of the substantia nigra (*16*). They challenge the hypothesis that the strionigral projection functions solely as a feedback pathway onto DA-containing cells. Instead, our data support the hypothesis that this fiber system plays a role as an output pathway from the neostriatum.

Our results may also help to elucidate the contribution of basal ganglia circuitry to other behaviors. Ventrolateral diencephalic electrocoagulations similar to those effective in blocking rotation have also been shown to prevent the restoration of feeding and drinking by L-dopa or apomorphine in rats with bilateral damage to the ascending DA-containing neurons (*17*). It now seems likely that many aspects of the syndrome of behavioral impairments seen in rats with lesions centered in the lateral hypothalamus can be attributed to a spread of the damaged region to include both ascending dopaminergic axons and neostriatal efferent fibers.

Finally, we anticipate that the approach outlined in this paper should be useful in determining the basal ganglia circuitry involved in the expression of clinical movement disorders, for example, the tardive dyskinesias or Huntington's chorea. With modifications, the approach might also prove useful in determining neural systems underlying DA-related psychotic states.

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8. Electrocoagulations were made by current from a radio-frequency (Siemens Radiotom) or direct-current (Grass) lesion maker through the tip of a stainless steel insect pin, insulated except for a 0.5-mm tip.
9. Transections were made with the knife designed by R. M. Gold, G. Kapatos, and R. J. Carey [*Physiol. Behav.* **10**, 813 (1973)].
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12. The reduction of rotation was calculated as follows: $1 - (\text{mean postlesion rotation score} \div \text{mean prelesion rotation score}) \times 100$. Only turns to the right were considered in the analysis, as turning to the left was negligible in all experiments. (Injections of 6-OH-DA were made in the left hemisphere.) In instances in which postlesion rotation exceeded prelesion scores, the percent blocking score was recorded as 0 percent.
13. Similarly, near-total destruction of the ascending dopaminergic neurons with 6-OH-DA appears necessary before marked behavioral impairments are manifest [M. J. Zigmond and E. M. Stricker, *Science* **177**, 1211 (1972); G. R. Breese, R. D. Smith, B. R. Cooper, L. D. Grant, *Pharmacol. Biochem. Behav.* **1**, 319 (1973)].
14. This score for each square was calculated as the mean reduction in rotation for each animal in which the lesion encroached upon that square. Those rats in which the lesions did not damage a particular square were not used in determining its contribution. For example, if a particular square was damaged by the lesions of four rats in which rotation was blocked by 0, 10, 10, and 20 percent, it would receive a score of 10 percent. Only those squares damaged by three or more lesions contributed to the analysis.
15. The rostral cuts were made by positioning the tip of the knife holder 4.0 mm in front of the earbar (level skull), 3.3 mm lateral, and 4.3 mm above the earbar. The knife was extended 2.0 mm medially and lowered stereotaxically to 1.8 mm above the earbar. The caudal cuts were made in a similar manner, with the knife holder positioned anterior 1.5, lateral 2.5, and 3.6 mm above the earbar, and lowered to 1.0 mm above the earbar. The rostral cuts were situated at the anterior border of the zona reticulata, averaging 1.5 mm in their mediolateral and dorsoventral extent. The caudal cuts were of a similar size, situated at the posterior tip of the zona reticulata. With each type of transection, the damage to the underlying crus cerebri was variable.
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Clockwise Growth of Neurites from Retinal Explants

Abstract. *When retinal explants from goldfish are grown on a polycation substratum, a marked tendency for directionality of neurite outgrowth is observed. While the direct relevance to nerve growth in vivo is not known, the phenomenon is interpreted as reflecting an inherent helicity of the neurites.*

Whereas cells in culture frequently migrate, neurons characteristically do not but rather send out processes which seem, by a number of criteria, to represent their natural axonal and dendritic extensions. There has been considerable speculation regarding the possible factors that influence the nature of this outgrowth and their relation to mechanisms in vivo whereby growing nerve fibers selectively reach their predetermined targets. Possible intrinsic mechanisms mediated by the nature of the neuron itself, and possible extrinsic factors such as chemical and electrical gradients, as well as the interaction of the growing neurite with its substratum or with neighboring neurites, have been proposed as playing a part in determining observed growth patterns in culture (*1*, *2*). We report here that both collagen film– and polycation-coated glass or plastic surfaces support neurite extension in goldfish retina explants, but that on the polycation substrata, a marked directionality in fiber outgrowth is invariably observed.

Ten to 20 days after the optic nerve was crushed intraorbitally, goldfish retinas were removed under sterile conditions and cut into 600-μm squares as described (*3*). Prior crushing of the optic nerve was necessary in order to obtain vigorous neuritic outgrowth (*3*, *4*). The retinal explants were placed in culture dishes coated with a collagen film (*5*) or with poly-L-lysine (*6*). Polycation-coated surfaces have been shown to promote cell adhesion (*7*) and, more recently, neurite extension (*6*, *8*). Cultures were examined with a Leitz Diavert inverted microscope. The observed image is that which would be seen by looking at the culture dish from above. After several days of growth in vitro on the poly-L-lysine substratum, we saw a marked tendency for the extending neurites to grow in a direction which we term "clockwise," relative to the observer (Fig. 1, a to d). In constrast, neuritic outgrowth on collagen films showed no such tendency (Fig. 1, e and f), even if the films had been coated with poly-L-lysine.

A number of possible explanations for the directionality were explored. The plastic surface of the poly-L-lysine–coated tissue culture dishes (Falcon) could not have mediated the observed directionality in growth, since the same pattern was observed on poly-L-lysine–coated glass coverslips. It is not likely, in fact, that any aspect of preparation of the polycation-coated surfaces introduced a template for outgrowth on the substratum. If such were the case, the nonneuronal, fibroblast-like cells (*9*), which occasionally form a monolayer around the retinal explant, might be expected to migrate out in a clockwise orientation as well. As shown in Fig. 2a, these cells have no tendency to migrate in a clockwise direction. The possibility that the chirality of the substratum (poly-L-lysine) somehow imparted directionality in growth was ruled out, because the clockwise pattern was seen in explants in dishes that had been coated with poly-DL-lysine or poly-D-lysine.

A role for external guidance factors such as electromagnetic, gravitational, or Coriolis-force fields was excluded by experiments in which the explants were grown between two coverslips. Either the lower or upper coverslip was coated with poly-L-lysine. Untreated coverslips do not support neuritic outgrowth or attachment of the explant. As before, clockwise outgrowth was observed if the lower coverslip was poly-L-lysine–coated. However, when the neurites grew out on an upper poly-L-lysine–coated coverslip, the pattern appeared counterclockwise when viewed from above (that is, remained clockwise with respect to the coated substratum). If gravity or other extrinsic factors such as magnetic (*10*) or Coriolis forces had been exerting an effect on the growth pattern, the neurites would have appeared to grow clockwise in both cases.

Since the directionality seems not to be imparted by extrinsic factors, mechanisms intrinsic to the retinal explant must be considered. Two observations suggest that the clockwise pattern is not related to an anatomical frame of reference within the explant itself or derived from the donor: (i) the outgrowth was found to be clockwise whether the explant came from the right or the left eye; and (ii) the clockwise directionality was seen whether the explants had been placed in the dish with the vitreal surface up and the photoreceptor layer against the substratum, or the reverse (*11*).

It is also apparent that the directionality is imparted entirely at the region of the growing neurite tips. The neurites do not progressively "bow out" between the point of exit from the explant and the distal end as growth progresses (Fig. 2, b and c). Under the conditions of high adhesiveness provided by the polycation-coated surface, the neurites are attached to the substratum along their length, forming, in Sidman and Wessells' terminology, a "time trace" of the path of the growth cone (*2*).

We conclude that the observed clockwise directionality is a consequence of an inherent helical property of the neurites which is expressed as the growing tip advances on a suitable adhesive planar surface. This inferred helicity may be within individual neurites, in fascicles of neurites, or both. The interaction of a right-handed helix with a planar surface could give rise to a clockwise curvature of the central axis (*12*). Whether the helicity of the neurite imparts a torque to the growth cone or whether the rotation of the growth cone imparts helicity to the neurite is not known.

Although we have not directly demonstrated helicity in the retinal explant neurites, the suggestion of their helical nature is not unprecedented. Degenerating nerves in vivo (*13*) and in vitro (*14*) have been observed to spiral or twist during retraction. Such spiraling may be conferred by the inherent structure of the nerve fiber as indicated by reports of a right-handed helicity of the neurofilaments within the giant axons of *Myxicola* and squid (*15*). Of possible importance is the undocumented report of Vinnikov in 1946 that retinal nerve fibers in explants from adult crucian carp contain "neurofibrillae" that twist in a "corkscrew fashion" (*16*).

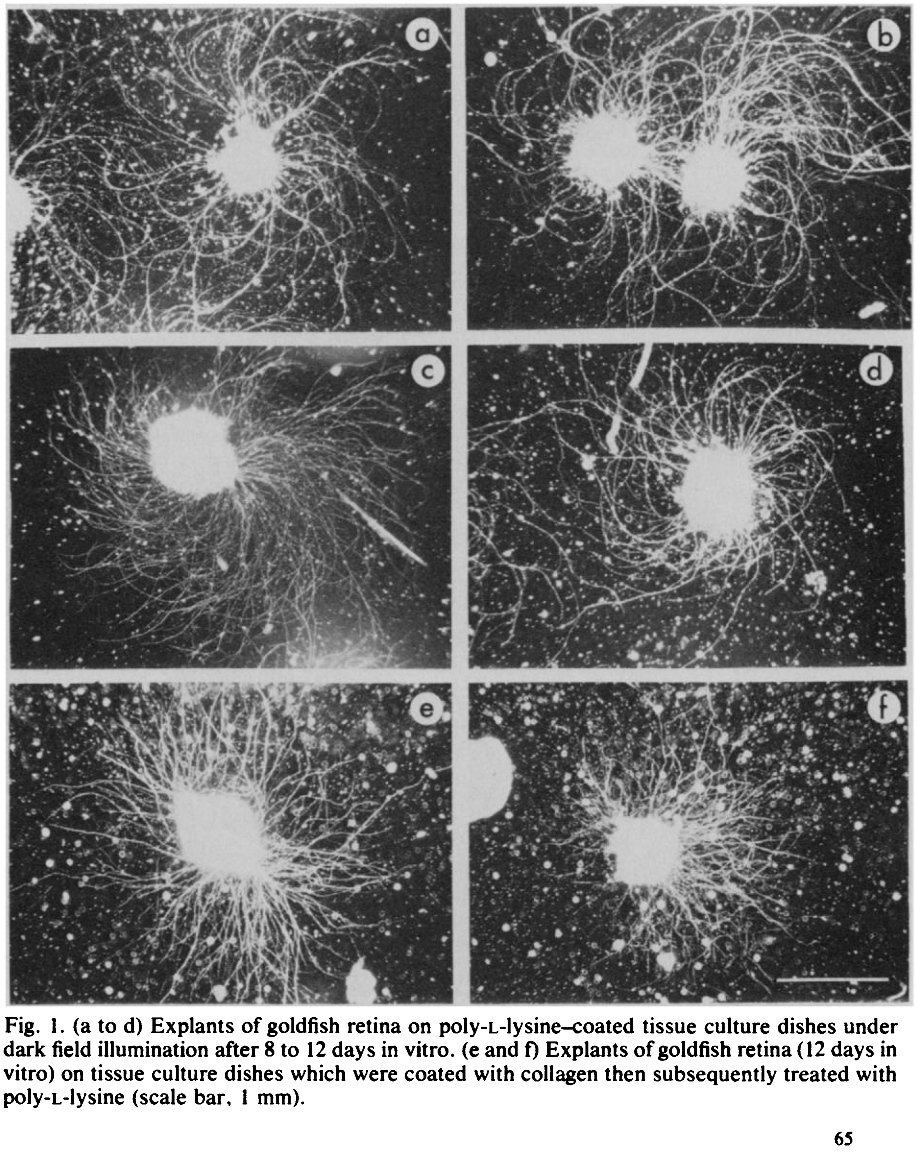

Fig. 1. (a to d) Explants of goldfish retina on poly-L-lysine–coated tissue culture dishes under dark field illumination after 8 to 12 days in vitro. (e and f) Explants of goldfish retina (12 days in vitro) on tissue culture dishes which were coated with collagen then subsequently treated with poly-L-lysine (scale bar, 1 mm).

Clockwise directionality has not been previously reported for other neural or retinal explants, although numerous observations of growth patterns in tissue culture have been made. It should be emphasized that the striking directionality seen in the present study is independent of the orientation of the explant, although the growing fiber is tethered to it. It thus differs from such observations as dorsal-root neurites growing toward a parent spinal cord explant (*17*). We have seen the clockwise growth pattern only in the goldfish retina grown out on polycation-coated surfaces. We have not succeeded in obtaining neurite outgrowth from *Xenopus* retinal explants on polycation-coated surfaces and have not seen the clockwise pattern when *Xenopus* retina is grown out on collagen films, a result in conformity with our previous studies on goldfish and *Xenopus* explants with collagen gels (*3*, *4*).

There has been considerable speculation regarding the role of the extracellular matrix in controlling movement of the growing nerve fiber (*18*). The fibrous nature of the collagen substratum evidently prevented the expression in culture of the tendency of the retinal neurites to curve in a clockwise direction, while the poly-L-lysine–coated substratum apparently provided the planarity and optimal adhesiveness for its expression. Although the collagen substratum probably resembles conditions in vivo more closely than does a polycation surface, the observation of clockwise growth on the latter may nevertheless aid in our understanding of the development and regeneration of nerve fibers. The requirement for prior crushing of the optic nerve in order to obtain vigorous neuritic outgrowth from the retinal explants strongly suggests that the outgrowing neurites originate in the ganglion cell layer of the retina. An intrinsic helicity in growing optic nerve axons may be relevant to the interaction of the individual growing nerve tips with their environment and to postulated fiber-fiber interactions in the sorting of optic nerve fibers in vivo (*19*). The clockwise directionality may thus be an expression of one of many yet unknown rules that will ultimately account for the well-known observation that retinal fibers find their way to selected sites in the brain in a highly ordered fashion.

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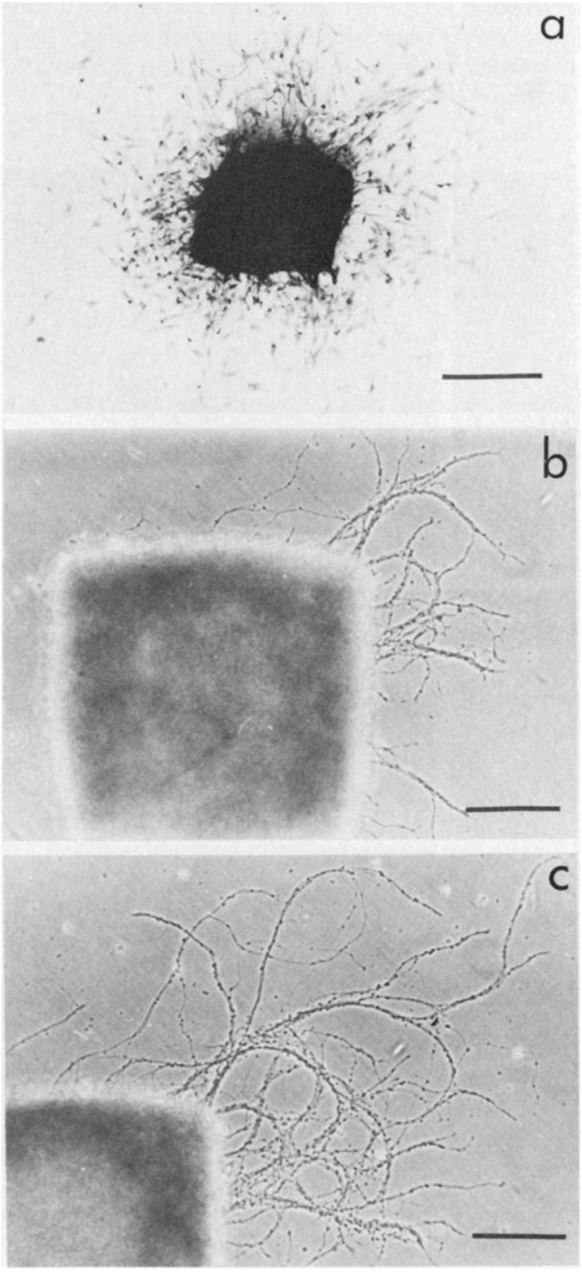

Fig. 2. (a) Bright-field photomicrograph of retinal explant stained with cresyl violet after 5 days of culture in the absence of fluorodeoxyuridine (*11*). Neuritic processes are not well stained (scale bar, 400 μm). (b) Phase-contrast photomicrograph of retinal explant after 1 day in vitro. (c) The same explant after 2 days in vitro (scale bar, 150 μm). While occasional neurites and fascicles of neurites curve counterclockwise, the net direction of the majority of fibers of an explant grown on a polycation surface is invariably clockwise, as is evident in Fig. 1.

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11. Outgrowth from the previously unoperated eye is delayed and contains a mixture of neuritic and nonneuritic elements (*4*). In these cultures, as well, a clear tendency for clockwise directionality of the neuritic elements is seen.
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