NEURAL ACTIVITY IN THE REGENERATING OPTIC NERVE OF THE GOLDFISH

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SUMMARY

1. Retinal ganglion cells of one eye were axotomized in goldfish either by sectioning the contralateral optic tract or by ablating the contralateral lobe of the optic tectum. Between 2 and 40 days later, multiunit activity in response to diffuse light flashes was recorded from the axotomized and normal optic nerves, and from the optic tectum.

2. Two days after tract section, the amplitude of the integrated multiunit response of the axotomized nerve was normal. By 16 days it had fallen to 15% of control values, at which time visual responses carried by the regenerating tract were first recorded in tectum. Activity in the axotomized nerve then recovered gradually.

3. After ablation of one tectal lobe, multiunit responses in the axotomized nerve had not recovered by 40 days.

4. Integrated spontaneous activity in the axotomized nerve was depressed with a similar time course to the depression of light-evoked activity, both after tract section and tectal ablation.

5. Retinal ganglion cell nuclear size, a morphological indicator of the cell body reaction, varied inversely with evoked activity, whether axotomy was by tract section or by tectal ablation.

6. Electrically evoked compound action potentials of normal amplitude could be recorded from an axotomized nerve despite depressed responses to light flashes.

7. It is concluded that optic nerve axotomy in goldfish reduces the number of optic fibres carrying impulses and/or the frequency of their discharge. The effect is closely linked to morphological changes occurring in the retinal ganglion cell bodies. Recovery of impulse activity and morphology depends upon the regenerating optic fibres innervating an appropriate target.

INTRODUCTION

The ability of the optic nerve in fish and anurans to regenerate has made the retinotectal system in these lower vertebrates a valuable model for studying factors that influence the formation of ordered neural connections. One such factor that has recently attracted attention is the role of impulse activity in the optic fibres. Although neither blocking activity with tetrodotoxin (TTX) (Schmidt, Edwards &

Stuermer, 1983; Harris, 1984) nor perturbing it with strobe light prevents optic fibres from reinnervating the optic tectum, both impair the refinement of the regenerated retinotectal map (Meyer, 1983; Schmidt & Edwards, 1983; Schmidt & Eisele, 1985; Cook & Rankin, 1986). TTX also affects axonal transport in goldfish optic nerve, raising the possibility that impulse activity influences growth and regeneration by regulating the delivery of materials to the growing axon (Edwards & Grafstein, 1983, 1984, 1986). Despite this interest in the role of neural activity in the goldfish retinotectal system, no study has been made of activity in the regenerating optic nerve. In mammalian neurones that regenerate, damage to the axon triggers a set of morphological and biochemical changes in the cell body that correlates with depressed excitability of the cell to its normal synaptic input (Kreutzberg, 1986). Consequently, a similar depression might be expected in goldfish ganglion cells, given their pronounced cell body reaction to axotomy (Murray & Grafstein, 1969).

This paper reports experiments which examined the effect of axotomy on impulse activity in the goldfish optic nerve and compared the time course of the activity changes to the morphological changes in the cell bodies of retinal ganglion cells.

METHODS

The goldfish (*Carassius auratus*) used in these experiments were 9–12 cm long from snout to base of tail, and were kept under a light regimen of 14 h light, 8 h dark at 22–24 °C. Before surgery, they were anaesthetized in a 1:5000 solution of tricaine methanesulphonate and placed between moistened foam rubber pads in a V-block holder. During surgery, the anaesthetic solution was supplied continuously to the gills through a tube in the mouth. A flap of skull was removed with a scalpel and the meninges aspirated to reveal the brain. In one group of fish, whose optic tract was to be lesioned unilaterally, the forebrain was first removed by suction to allow access to the tract. Using a fine glass suction pipette, the tract was interrupted by aspirating it just rostral to its entry to the midbrain (Fig. 1). In another group of fish, the optic nerve was axotomized by aspirating an entire tectal lobe on one side. In these cases, the forebrain was left intact. In order to control for possible differences in stimulating conditions between the two sides during the electrophysiological recordings performed later, half the fish in each group were operated on the left side, half on the right. The skull flap was then replaced and secured with a small amount of cyanoacrylate cement, and the animal revived from anaesthesia.

Fish were prepared for optic nerve recording between 2 and 40 days after lesioning. They were anaesthetized, mounted in the V-block, and the cranium reopened. In fish with a unilateral tectal ablation, the forebrain was ablated, as had already been done in the tract-sectioned fish. Both optic nerves were exposed from eyeballs to chiasm by carefully removing the overlying bone with forceps. Finally, fish were immobilized by injecting 0·1 mg of gallamine triethiodide (Flaxedil) into the dorsal musculature, and their gills were perfused thereafter with aerated water.

The eyes were stimulated with flashes of white light from an optical system consisting of a tungsten–halogen bulb, heat filter, shutter, neutral density wedge and a lens projecting a collimated beam that could be directed to either eye by means of mirrors. Diffuse illumination of the retina was achieved by placing diffusers of tracing paper (Fig. 1) over the corneas. The illumination of each eye was equalized by adjusting the neutral density wedge until the same readings were obtained from identical photodiodes situated close to each eye. During the experiment, the eyes were adapted to an ambient luminance of 20 lm m^{-2} sr^{-1}. While the shutter was open, the stimulus luminance was 1200 lm m^{-2} sr^{-1}.

Because the aim was to assess overall activity in the optic nerves, glass-insulated platinum–iridium (Pt–Ir) electrodes with large exposed tips (*ca.* 250 μm) were used to sample multiunit activity at several points in each nerve. The signal from the electrode was amplified 10000 times, filtered to accept spikes (3 dB down at 0·3 and 7 kHz), full-wave rectified and smoothed by a leaky

integrator with a time constant of 28 ms. The use of a short time constant (0·3 ms) in the amplifier stages preceding the integrator had the effect of discriminating against slow-wave responses (e.g. electroretinogram), as well as ensuring that increases of input pulse width above 0·75 ms produced relatively small increases (about 18 %) in the integrator output. Thus, for pulse widths comparable to those of action potentials, the integrator's output was a function primarily of pulse amplitude
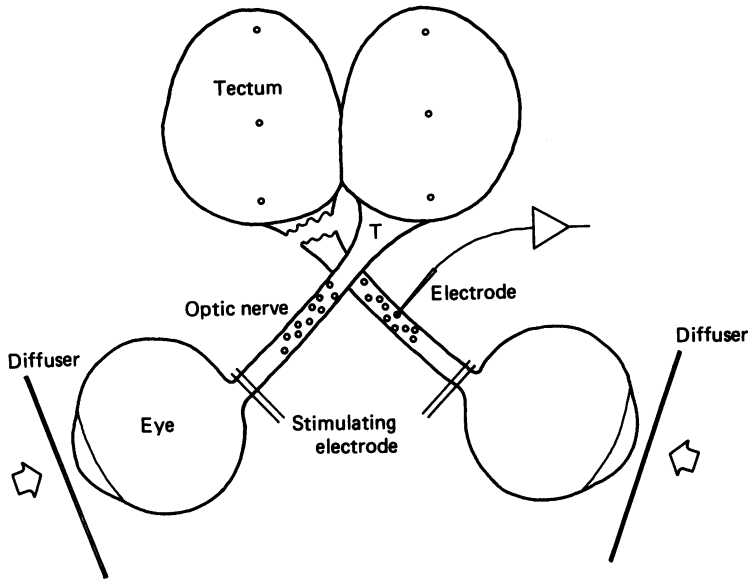

Fig. 1. Diagram of the dorsal view of the goldfish retinotectal system, showing the optic tracts (T) severed on one side, and exposed optic nerves. Nerve activity was recorded by a Pt–Ir electrode from ten sites in each nerve, and from at least three in each tectal lobe. Approximate recording sites are shown circled. Light flashes were delivered to either eye through tracing paper diffusers. In some experiments, stimulating electrodes were placed on the nerve behind the eyeball, and the compound action potential was recorded from the same sites used to record light-evoked multiunit activity.

and frequency. The integrator output, together with a photocell signal showing the occurrence of the stimulus flash, was fed to the A/D converter of a PDP 11/10 computer.

The response obtained from a given electrode penetration was analysed as follows. The computer sampled the output of the integrator every 5 ms. Ten samples were taken before the stimulus flash and these were averaged to form a base level reading. The shutter was then opened for 100 ms, and sampling continued for a further 350 ms to capture both the 'on' and 'off' responses. The mean of the seventy samples after the stimulus onset, minus the base level, was taken as the integrated 'flash response'. When the statistical significance of a response was required, the computer collected 'dummy responses' in the same way, except that the shutter was not opened. Every recording of a flash response was followed by a recording of five dummy responses, this sequence of flashes and dummies being repeated ten times. The mean flash response could then be expressed in terms of Student's t values.

Each experiment was begun by recording from the superficial layers of the tectum to assess the progress of tectal reinnervation. At least three penetrations were made: one each in rostral, central and caudal tectum. A tectal point was considered responsive if it yielded a flash response significant at $P < 0.001$ with a one-tailed test. Recordings were then made from ten different sites in both left and right optic nerves, changing sides every second penetration. The electrode was inserted into the nerve under visual control, with successive penetrations progressing from chiasm to eye (Fig. 1).

In fourteen fish with optic tract or tectal lesions, the responses to both electrical and photic stimulation were recorded at each electrode site in the optic nerves. For this purpose, stimulating electrodes (Fig. 1), consisting of a pair of fine Pt–Ir wires, were placed across the nerve just behind

the eyeball. The stimulus shock, 0·2 ms in duration, was delivered with a constant-current stimulus isolation unit (Grass Instruments, PSIU6) at a strength about 20 % higher than required to elicit a maximal compound action potential. A reference electrode for differential recording was placed on the connective tissue overlying the chiasm. The high- and low-pass cut-offs of the pre-amplifier (Grass Instruments, P15) were set at 1 Hz and 10 kHz, and the rectifier and leaky integrator were bypassed. The resulting signal was sampled by the computer every 40 μs. Because recording sites were only 1–2 mm from the stimulating electrodes, it was necessary to ensure that the stimulus artifact did not distort the recordings of compound action potentials. By delivering double shocks, and reducing the interval between them, it was easy to see the relative contribution of the artifact as the nerve became progressively refractory to the second shock.

At the end of a recording session, both retinas were fixed in ethyl alcohol–formalin–acetic acid for at least 5 days, embedded in paraffin, sectioned at 10 μm, and stained with Weigert's haematoxylin. Cells in the ganglion cell layer were examined using a 100 × oil-immersion objective and drawn with a camera lucida. A total of forty cells were sampled from each retina by the method of Yip & Grafstein (1982) and drawn. Initially, cytoplasmic profiles, nuclear profiles and nucleoli were all drawn, and their areas measured with a computerized planimetry system. Since the cytoplasm stained weakly, particularly in normal retinas, the analysis of the morphologic changes has been based on nuclear areas and nucleolar frequencies.

RESULTS

Since goldfish optic nerves decussate almost completely at the chiasm, a lesion made post-chiasmatically will induce axon regeneration and the cell body reaction in the ganglion cells of the contralateral eye. Lesioning the optic tract or ablating one tectal lobe avoids damage to the eye and retinal blood supply, and leaves the entire prechiasmatic nerve intact for recording (see Fig. 1).

Figure 2 is a typical set of multiunit recordings of light-evoked responses recorded in the normal and axotomized optic nerves of a goldfish that received a lesion of the left optic tract 16 days earlier. The recordings from ten sites in each nerve are shown superimposed. On the control side (left nerve), a brisk discharge to both the onset and offset of the stimulus was obtained at nearly every site sampled. On the axotomized side (right nerve), only a few sites yielded significant responses, and these were of low amplitude and longer than normal latency. However, even at this post-operative interval, when depression was maximal, it was possible to find a few recording sites that yielded activity.

In fourteen goldfish with lesions of the optic tract or tectum, responses to both light flash and electrical stimulation were recorded at the same site in the nerve. Figure 3 shows examples of both types of response in the control and lesioned nerves of a goldfish 10 days after axotomy by tectal hemiablation. The upper records are compound action potentials to supramaximal shocks to the nerve behind the eyeball. Each row presents electrical responses of comparable amplitude with their corresponding flash responses displayed below. It can be seen that a given amplitude of electrical response is associated with a smaller flash response in the axotomized nerve. The electrical response of the axotomized nerve generally exhibited a slower waveform than did the normal nerve.

The dissociation of photic and electrical responses caused by axotomy was examined further by sampling both types of activity at several points along an electrode penetration. The results for two fish with tectal hemiablations are shown in Fig. 4. Within a given penetration of a normal nerve, a rough proportionality existed

between the amplitudes of the photic responses and electrical responses. Thus when all the data from five penetrations in the control nerve were combined for one of the fish (Fig. 4C), a highly significant correlation (0·742, $P < 0.001$) was obtained. Although the combined data for the control nerve of the other fish (Fig. 4A) failed to show a significant correlation (0·178, $P > 0.05$), proportionality was still evident in
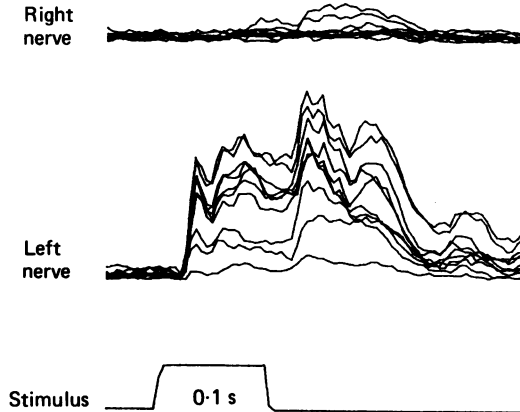

Fig. 2. Examples of multiunit activity recorded from the left and right nerves 16 days after section of the left optic tract. Each trace is the mean response to ten light flashes presented to the corresponding eye. Traces are shown superimposed from the ten electrode positions sampled in each nerve. The 0·1 s light flash stimulus is shown by the photocell trace.

individual penetrations. The main point of Fig. 4 is that for a given amplitude of electrical response, lesioned nerves (B and D) yielded a much weaker photic response than did control nerves (A and C).

In order to study the time course of the depression of impulse activity, a group of fish was given unilateral optic tract lesions and recorded at various post-operative intervals. Another group was given unilateral tectal ablations to examine the effects of removing the site of termination of regenerating optic axons. In Fig. 5B the integrated flash responses of axotomized nerves are plotted as a percentage of the control nerve values at different times after optic tract section. At 2 days after surgery, the mean response was not significantly different from normal, but thereafter it fell, rapidly at first, to a minimum of about 15% at 16 days. Responsiveness recovered more slowly, achieving 80% of normal by 40 days. The recovery phase appeared to start after about 16 days, at which time statistically significant visual responses were first recorded from the tectal lobe normally innervated by the axotomized nerve. This is shown in Fig. 5A, representing the percentage of responsive tectal points sampled in that lobe. Figure 6 shows that in nerves axotomized by tectal hemiablation, there was no recovery between 16 and 40 post-operative days. No significant light-evoked responses were recorded in the remaining tectal lobe via the ipsilateral eye by 40 days, although that lobe was responsive to visual stimulation of the contralateral eye.

The question of whether axotomy affects the level of spontaneous activity, that is activity in the absence of changing visual stimulation, was examined by comparing
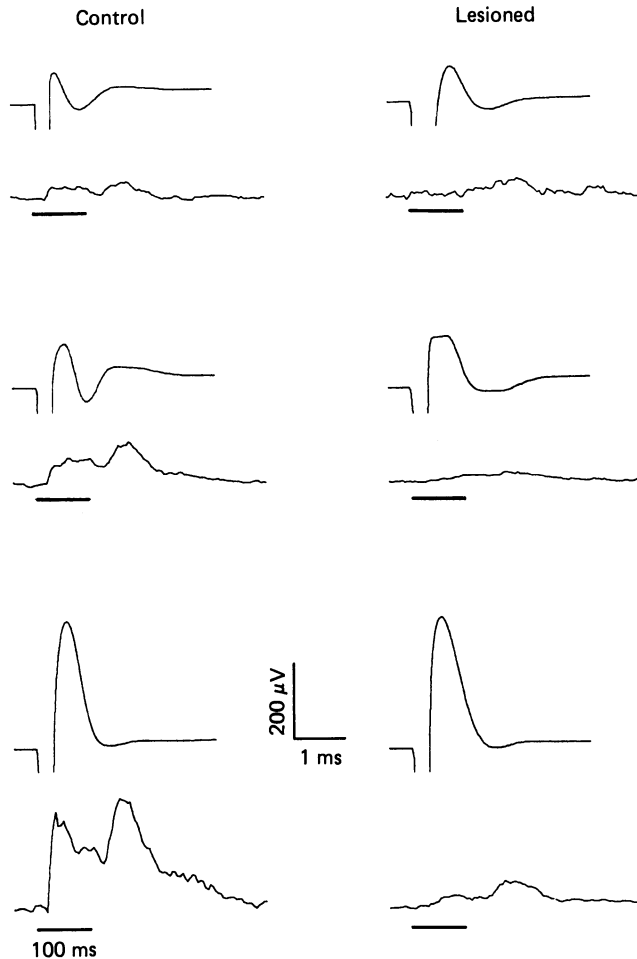
Fig. 3. Electrical and light-evoked responses from three sites in a normal nerve (control), and three sites in a nerve axotomized (lesioned) by hemiablation of the tectum 10 days earlier. The upper traces in each row, showing responses to supramaximal electrical stimulation (0·2 ms duration), are approximately matched in amplitude (1st peak to 1st trough). See the 200 μV, 1 ms calibration bars. The lower trace in each row shows the multiunit responses to the light flash recorded immediately after the electrical response and without moving the electrode. The bars below signal the occurrence of the 100 ms light flash.

pre-stimulus base levels recorded in control and axotomized nerves. Although the base level was comprised of an arbitrary d.c. offset in addition to spontaneous impulse activity, comparison between the two nerves of a given animal is valid because the same electrode and integrator offset were used. The differences in base levels (axotomized − control), averaged from the ten penetrations of each nerve have been normalized as Student's t values and plotted as a function of time in Fig. 7. In the tract-sectioned fish (circles), the base level of the axotomized nerves was depressed with a time course roughly similar to that of light-evoked activity. From the 5% probability level ($t = 2\cdot1$, 18 d.f.) shown by the dotted horizontal line in Fig. 7, it is clear that on average, base levels in the axotomized nerve had declined significantly

at 4 days after tract section, and remained depressed for at least another 24 days. In nerves axotomized by tectal hemiablation (squares), however, base levels had not recovered by 40 days.

The morphological manifestations of the cell body reaction of retinal ganglion cells were assessed from measurements of nuclear area and nucleolar frequency in the
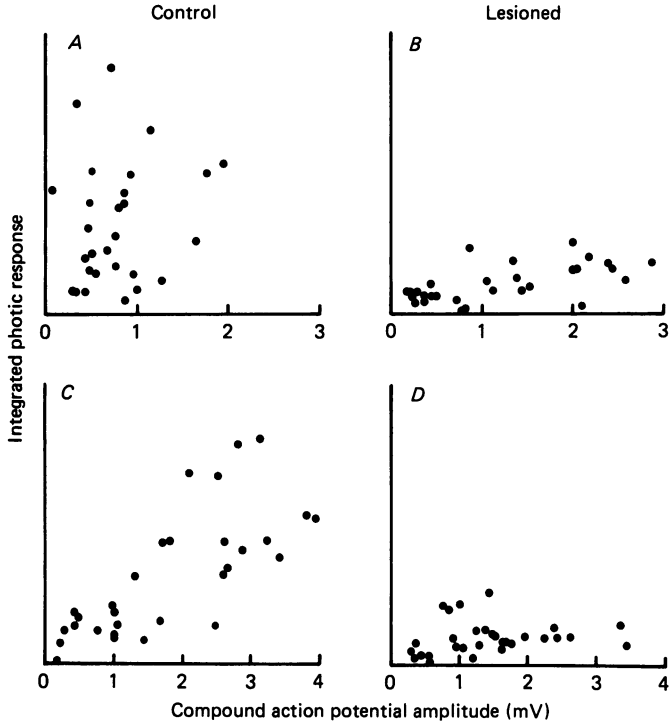

Fig. 4. Light-evoked *vs.* electrically-evoked responses in normal (control) and axotomized (lesioned) optic nerves of two goldfish. The upper graphs (*A* and *B*) are results from the normal and axotomized nerves of a fish 17 days after left tectal ablation; the lower graphs (*C* and *D*) from a fish 18 days after right tectal ablation. The ordinates show the integrated multiunit response to light flashes in arbitrary units; the abscissae the amplitude in millivolts of the compound action potential (1st peak to 1st trough) elicited by maximal electrical stimulation of the nerve. Each point represents a single site from which both photic and electrical responses were recorded.

same animals that were used for the electrophysiological recordings. In normal retinas, one nucleolus was seen on average in twenty nuclei sampled. At 2 days after axotomy, the frequency of nucleoli seen increased sharply to about one per nucleus, with many nuclei containing multiple nucleoli. In the tract-sectioned fish, nucleolar frequency declined after 16 post-operative days. The size of nuclei, as measured by nuclear area and plotted in Fig. 8, increased more gradually with time, but it too started to return to normal values after 16 days. The time course of the latter measure of the cell body reaction mirrored the depression of light-evoked activity in the optic nerves. In fish with tectal hemiablations, there was no significant recovery up to 40 days in either nucleolar frequency or nuclear area.
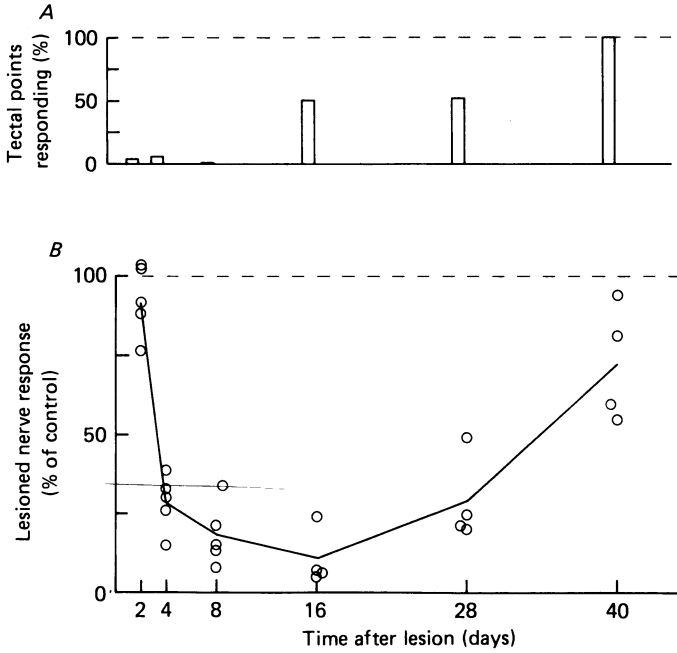
Fig. 5. A, percentage of tectal points sampled that yielded statistically significant light-evoked responses at different times after optic tract section. B, the light-evoked multiunit response of lesioned nerves at the same times after optic tract section. Each circle shows for a single animal the mean of ten integrated responses of the lesioned nerve as a percentage of ten responses from the control nerve. Same animals as in A.
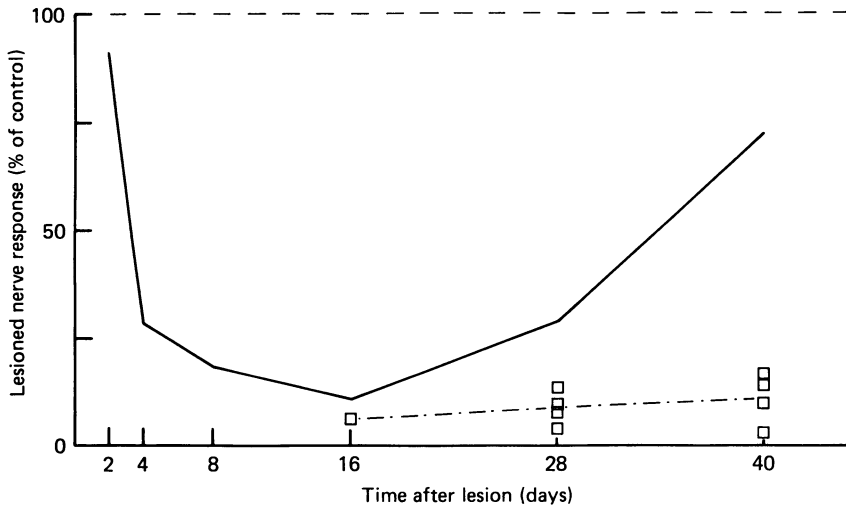

Fig. 6. The light-evoked multiunit responses of axotomized nerves at different times after optic tract section (continuous line from Fig. 5) or after tectal ablation (squares).
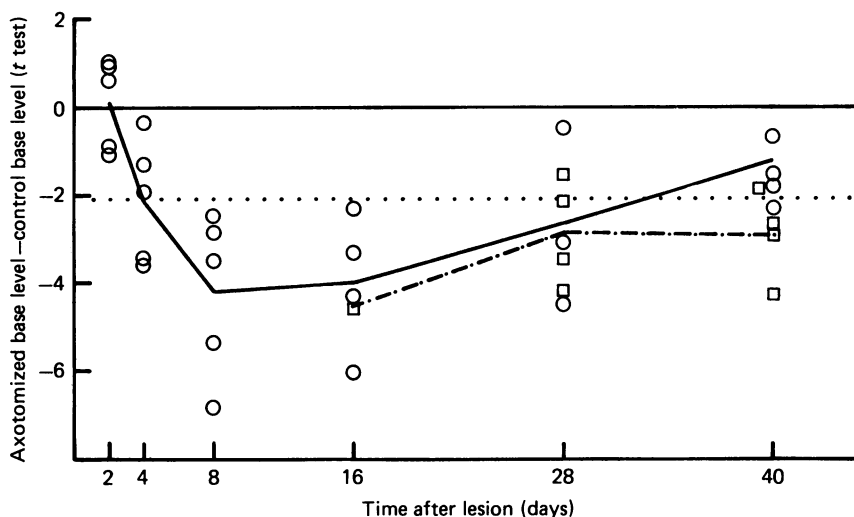
Fig. 7. Time course of base level activity after axotomy by optic tract lesion or tectal hemiablation. Symbols show for each animal the base level recorded in the axotomized nerve minus the base level in the control nerve, the difference being expressed on the ordinate in terms of Student's *t* test. Same animals as in Figs 5 and 6. Circles and continuous line: optic tract lesions. Squares and dot–dash line: tectal hemiablation. Horizontal dotted line shows 5% level of significance, two-tailed *t* test, 18 d.f.
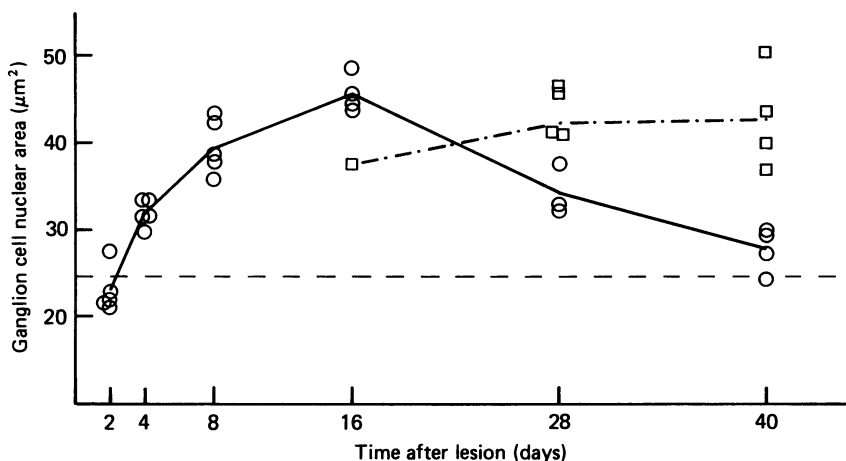

Fig. 8. *A*, retinal ganglion cell nuclear area (square micrometres) measured in the axotomized retina at different times after tract section (circles) or tectal hemiablation (squares). The horizontal dashed line shows mean values from control retinas. Data taken from the same animals used for electrophysiology.

DISCUSSION

In the electrophysiological experiments, both spontaneous and light-evoked integrated impulse activity in the optic nerve were depressed after axotomy, but recovered when the optic fibres were allowed to reinnervate their normal target, the contralateral tectum. Moreover, the activity depression was correlated with the morphological changes seen in the retinal ganglion cell bodies after axotomy.

Multiunit recording is a simple and convenient means of sampling the overall impulse activity within the nerve. However, by itself the method cannot distinguish between the following effects of axotomy in accounting for the activity depression: (1) a reduction in the firing rate of optic fibres, (2) a reduction in the number of optic fibres discharging impulses, (3) diminished extracellular currents set up by action potentials, and (4) a relative inability to record those currents. The results of electrical stimulation, however, tend to rule out the last two explanations because compound action potentials of normal amplitude could be obtained from axotomized nerves, even when photic responses recorded at the same site were depressed (Figs 3 and 4). Nevertheless, it is possible that membrane properties change in an axotomized nerve, reducing the spontaneous and photic activity that can be recorded from it. Evidence for one such change is the slowing of action potentials found in the electrical stimulation experiments (Fig. 3), an effect similar to that found in mammalian motoneurones whose conduction velocity decreases after axotomy (Kuno, Miyata & Muñoz-Martinez, 1974; Mendell, Munson & Scott, 1976). However, slower action potentials could not alone account for the depression; if anything, they would slightly increase the integrator's output (see Methods). While it is possible to conceive of changes in the electrical properties of axotomized axons that could weaken the recording of photic discharges but not of the synchronous volleys elicited electrically, the most parsimonious explanation is that axotomy reduces the total impulse traffic in the optic nerve.

If traffic is reduced, it was never completely abolished; even in the most depressed nerves, some sites yielded appreciable light-evoked responses. One explanation is that unilateral tract section or tectal hemiablation failed to cut all fibres in the contralateral optic nerve because a small number of fibres do not decussate in the chiasm (Springer & Gaffney, 1981). Another possibility is that the cyprinid retina contains a variety of ganglion cell types (Kock & Reuter, 1978; Hitchcock & Easter, 1986), some of which may be less sensitive to axotomy, or react with different time courses.

The depression of impulse activity probably has its origins in the retina. There is an extensive literature on the effects of axotomy in mammalian motoneurones and autonomic neurones showing that alterations at the level of the cell body reduce excitability to synaptic input. These changes include shrinkage of dendrites (Sumner & Watson, 1971), loss of postsynaptic membrane specialization (Sumner, 1975), reduced sensitivity to transmitter substances (Brenner & Martin, 1976), loss of synaptic terminals (Blinzinger & Kreutzberg, 1968; Purves, 1975), and changes in the activity of enzymes related to neurotransmitter biosynthesis or degradation (Ross, Joh & Reis, 1975; Rotter, Birdsall, Burgen, Field, Smolen & Raisman, 1979). Although similar changes could be occurring in goldfish retinal ganglion cells to depress light-evoked and spontaneous activity, there is as yet no direct evidence for them. Nevertheless, the biochemical changes that have been described, principally increased protein synthesis and axonal transport (Murray & Grafstein, 1969; McQuarrie & Grafstein, 1982), are consistent with a diversion of cell resources to axonal regeneration.

The biochemical changes induced by axotomy are accompanied by conspicuous morphological changes in the ganglion cell body that were described in detail by

Murray & Grafstein (1969). The first to appear, an increase in nucleolar size and frequency, is evident at 2 days after optic tract section, followed a day or two later by swelling of the cell body. Although nuclear size was measured in the present experiments, it changed with a time course similar to that of cell body size reported previously (Murray & Grafstein, 1969). Thus nuclear size had risen by 4 days and started to fall between 16 and 28 days after tract section (Fig. 8). Moreover, nuclear size, like cell body size (Burmeister & Grafstein, 1985), failed to recover when the regenerating fibres were deprived of their normal tectal target. What is striking about these morphological changes is that their time courses, so different with and without tectum, are closely mirrored by the changes in both evoked and spontaneous impulse activity in the nerve (compare Figs 5 and 6 with Fig. 8). Apparently, the syndrome of biochemical and morphological events called the cell body reaction includes processes that reduce the ganglion cell's excitability to synaptic input.

The mechanism of recovery from the cell body reaction has been discussed by Burmeister & Grafstein (1985), who also found that the morphological changes were greatly prolonged after tectal ablation. The implication of these experiments is that recovery starts when the ganglion cell bodies receive a signal from the tectum. It is unclear which event during regeneration gives rise to such a signal, but it is probably not merely the arrival of optic fibres within tectal tissue because fibres are present in tectum well before recovery starts. This is true after tract section, when regenerating optic fibres were found in tectum 7–10 days later (Murray, 1976), but especially after tectal hemiablation, when the remaining tectal lobe is invaded by optic fibres from the ipsilateral retina several weeks before any cell body recovery takes place (Lo & Levine, 1980; Springer & Cohen, 1981; Burmeister & Grafstein, 1985). Figure 5 indicates that the recovery process starts around 16 days after tract section when statistically significant multiunit visual responses were first recorded from tectum. Since such activity correlates with the restoration of tectally mediated behaviour (Northmore & Masino, 1984), synaptogenesis is likely to be the critical event, especially as this is also the time that the first postsynaptic activity can be recorded in tectum (Schmidt *et al.* 1983). Tectal hemiablation experiments provide stronger evidence that synaptogenesis is responsible for recovery of the cell body reaction, for under these circumstances the delayed recovery matches the timing of two other phenomena that suggest delayed synaptogenesis. The first is that no visual activity in tectum driven by the ipsilateral eye was seen in these experiments before 40 days, and in other studies such activity took as long as 10–12 weeks to appear (Sharma, 1973; Levine & Jacobson, 1975). The second is that visual behaviour mediated by an anomalous ipsilateral projection was also delayed by 10–12 weeks, compared with about half the time when the ipsilateral projection was allowed to innervate the same tectal lobe without a competing contralateral projection (Davis & Schlumpf, 1984). It is also at these longer times that segregation of the terminals of the two retinal inputs takes place (Springer & Cohen, 1981), apparently by a process of competitive interaction (Constantine-Paton & Law, 1982).

The changes in impulse activity in the regenerating optic nerve have interesting implications for understanding the processes leading to functional recovery, especially in view of recent studies of the effects of manipulating impulse activity artificially (Meyer, 1983; Schmidt & Edwards, 1983; Cook & Rankin, 1986). Several

experiments in goldfish have examined the consequences of blocking retinofugal impulse activity by injections of tetrodotoxin (TTX) into the eye. Because the present results show a period of relative neural silence starting a few days after axotomy and lasting roughly until tectal reinnervation, one would expect TTX to be most effective outside this period, i.e. before 4 days, and after about 14 days of axotomy. The following results tend to bear out this expectation.

The first visual behaviour to recover during regeneration, the startle response, is delayed by a single intraocular injection of TTX at the time of optic nerve crush, but subsequent injections fail to delay the response any further (Edwards & Grafstein, 1983). Intraocular TTX injections also interfere with the refinement of the retino-tectal map that normally occurs after regenerating optic fibres invade the tectum. However, it is only after 14 days of optic nerve crush that TTX impairs map refinement (Schmidt & Edwards, 1983). Edwards & Grafstein (1983) have pointed out that these two periods of TTX sensitivity are times when the regenerating optic fibres are likely to be forming axonal branches. Thus, an excess of regenerating axons is found close to the lesion site (Murray, 1976, 1982), suggesting that each cut axon sprouts several branches early in regeneration. It takes only a single TTX injection at the time of the lesion to reduce this sprouting (Edwards & Grafstein, 1983). Regenerating optic fibres are also seen to branch within tectum (Murray, 1976; Fujisawa, Tani, Watanabe & Ibata, 1982). A branching mode of growth early in tectal reinnervation might be important in the reformation of an ordered retinotectal map from a disordered initial projection (Stuermer & Easter, 1984) if, as is thought, optic axons seek regions of correlated activity in which to terminate (Schmidt, 1985). Therefore, an inhibition of branching by TTX could well contribute to the impairment of map refinement observed at this phase. The present results suggest that in the normal course of regeneration, impulse activity is restored in optic axons as they innervate the appropriate tectal lobe, and in time for activity to play its part in map refinement.

A further implication of the results, given the evidence just cited, is that regenerating axons branch primarily when they carry impulse activity. If activity can influence neural growth, an idea that is gaining experimental support (Reh & Constantine-Paton, 1985; Cohan & Kater, 1986), it is possible for the suppression and subsequent reactivation of impulse activity after axotomy to regulate patterns of neural growth during regeneration.

REFERENCES

BLINZINGER, K. & KREUTZBERG, G. W. (1968). Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Zeitschrift für Zellforschung* **85**, 145–157.

BRENNER, H. R. & MARTIN, A. R. (1976). Reduction in acetylcholine sensitivity in axotomized ciliary ganglion cells. *Journal of Physiology* **260**, 159–175.

BURMEISTER, D. W. & GRAFSTEIN, B. (1985). Removal of optic tectum prolongs the cell body reaction to axotomy in goldfish retinal ganglion cells. *Brain Research* **327**, 45–51.

COHAN, C. S. & KATER, S. B. (1986). Suppression of neurite elongation and growth cone motility by electrical activity. *Science* **232**, 1638–1640.

CONSTANTINE-PATON, M. & LAW, M. I. (1982). The development of maps and stripes in the brain. *Scientific American* **247**, No. 12, 62–70.

COOK, J. E. & RANKIN, E. E. C. (1986). Impaired refinement of the regenerated retinotectal projection of the goldfish in stroboscopic light: a quantitative WGA–HRP study. *Experimental Brain Research* **63**, 421–430.

DAVIS, R. E. & SCHLUMPF, B. E. (1984). Visual recovery in goldfish following unilateral optic tectum ablation: evidence of competition between optic axons for tectal targets. *Behavioural Brain Research* **13**, 287–291.

EDWARDS, D. L. & GRAFSTEIN, B. (1983). Intraocular tetrodotoxin in goldfish hinders optic nerve regeneration. *Brain Research* **269**, 1–14.

EDWARDS, D. L. & GRAFSTEIN, B. (1984). Intraocular injection of tetrodotoxin in goldfish decreases fast axonal transport of [^{3}H]glucosamine-labeled material in optic axons. *Brain Research* **299**, 190–194.

EDWARDS, D. L. & GRAFSTEIN, B. (1986). Intraocular tetrodotoxin reduces axonal transport and transcellular transfer of adenosine and other nucleosides in the visual system of goldfish. *Brain Research* **364**, 258–267.

FUJISAWA, H., TANI, N., WATANABE, K. & IBATA, Y. (1982). Branching of regenerating retinal axons and preferential selection of appropriate branches for specific neuronal connection in the newt. *Developmental Biology* **90**, 43–57.

HARRIS, W. A. (1984). Axonal pathfinding in the absence of normal pathways and impulse activity. *Journal of Neuroscience* **4**, 1153–1162.

HITCHCOCK, P. F. & EASTER, S. S. (1986). Retinal ganglion cells in goldfish: a qualitative classification into four morphological types, and a quantitative study of the development of one of them. *Journal of Neuroscience* **6**, 1037–1050.

KOCK, J.-H. & REUTER, T. (1978). Retinal ganglion cells in the crucian carp (*Carassius carassius*) I. Size and number of somata in eyes of different size. *Journal of Comparative Neurology* **179**, 535–548.

KREUTZBERG, G. W. (1986). The motoneuron and its microenvironment responding to axotomy. In *Neural Transplantation and Regeneration*, ed. DAS, G. D & WALLACE, R. B., pp. 271–276. New York: Springer-Verlag.

KUNO, M., MIYATA, Y. & MUÑOZ-MARTINEZ, E. J. (1974). Differential reaction of fast and slow α-motoneurones to axotomy. *Journal of Physiology* **240**, 725–739.

LEVINE, R. L. & JACOBSON, M. (1975). Discontinuous mapping of retina onto tectum innervated by both eyes. *Brain Research* **98**, 172–176.

LO, R. Y. S. & LEVINE, R. L. (1980). Time course and pattern of optic fiber regeneration following tectal lobe removal in the goldfish. *Journal of Comparative Neurology* **191**, 295–314.

McQUARRIE, I. G. & GRAFSTEIN, B. (1982). Protein synthesis and axonal transport in regenerating goldfish retinal ganglion cells. *Brain Research* **235**, 213–223.

MENDELL, L. M., MUNSON, J. B. & SCOTT, J. G. (1976). Alterations of synapses on axotomized motoneurones. *Journal of Physiology* **255**, 67–79.

MEYER, R. L. (1983). Tetrodotoxin inhibits the formation of refined retinotopography in goldfish. *Developmental Brain Research* **6**, 293–298.

MURRAY, M. (1976). Regeneration of retinal axons into the goldfish optic tectum. *Journal of Comparative Neurology* **168**, 175–196.

MURRAY, M. (1982). A quantitative study of regenerative sprouting by optic axons in goldfish. *Journal of Comparative Neurology* **209**, 352–362.

MURRAY, M. & GRAFSTEIN, B. (1969). Changes in the morphology and amino acid incorporation of regenerating goldfish optic neurons. *Experimental Neurology* **23**, 544–560.

NORTHMORE, D. P. M. & MASINO, T. (1984). Recovery of vision in fish after optic nerve crush: a behavioral and electrophysiological study. *Experimental Neurology* **84**, 109–125.

PURVES, D. (1975). Functional and structural changes of mammalian sympathetic neurones following interruption of their axons. *Journal of Physiology* **252**, 429–463.

REH, T. A. & CONSTANTINE-PATON, M. (1985). Eye specific segregation requires neural activity in three-eyed *Rana pipiens*. *Journal of Neuroscience* **5**, 1132-1143.

ROSS, R. A., JOH, T. H. & REIS, D. J. (1975). Reversible changes in the accumulation and acti-

vation of tyrosine hydroxylase and dopamine-beta-hydroxylase in neurons of nucleus locus coeruleus during the retrograde reaction. *Brain Research* **92**, 57–72.

ROTTER, A., BIRDSALL, N. J. M., BURGEN, A. S. V., FIELD, P. M., SMOLEN, A. & RAISMAN, G. (1979). Muscarinic receptors in the central nervous system of the rat. IV. A comparison of the effects of axotomy and deafferentation on the binding of [³H]propylbenzilylcholine mustard and associated synaptic changes in the hypoglossal and pontine nuclei. *Brain Research Reviews* **1**, 207–224.

SCHMIDT, J. T. (1985). Selective stabilization of retinotectal synapses by an activity-dependent mechanism. *Federation Proceedings* **44**, 2767–2772.

SCHMIDT, J. T. & EDWARDS, D. L. (1983). Activity sharpens the map during the regeneration of the retinotectal projections on goldfish. *Brain Research* **269**, 29–39.

SCHMIDT, J. T., EDWARDS, D. L. & STUERMER, C. (1983). The re-establishment of synaptic transmission by regenerating optic axons in goldfish: time course and effects of blocking activity by intraocular injection of tetrodotoxin. *Brain Research* **269**, 15–27.

SCHMIDT, J. T. & EISELE, L. E. (1985). Stroboscopic illumination and dark rearing block the sharpening of the regenerated retinotectal map in goldfish. *Neuroscience* **14**, 535–546.

SHARMA, S. C. (1973). Anomalous retinal projection after removal of contralateral optic tectum in adult goldfish. *Experimental Neurology* **41**, 661–669.

SPRINGER, A. D. & COHEN, S. M. (1981). Optic fiber segregation in goldfish with two eyes innervating one tectal lobe. *Brain Research* **225**, 23–36.

SPRINGER, A. D. & GAFFNEY, J. S. (1981). Retinal projections in the goldfish: a study using cobaltous-lysine. *Journal of Comparative Neurology* **203**, 401–424

STUERMER, C. A. O. & EASTER, S. S. (1984). Rules of order in the retinotectal fascicles of goldfish. *Journal of Neuroscience* **4**, 1045–1051.

SUMNER, B. E. H. (1975). A quantitative study of subsurface cisterns and their relationships in normal and axotomized hypoglossal neurones. *Experimental Brain Research* **22**, 175–183.

SUMNER, B. E. H. & WATSON, W. E. (1971). Retraction and expansion of the dendritic tree of motor neurones of adult rats induced *in vivo*. *Nature* **233**, 273–275.

YIP, H. K. & GRAFSTEIN, B. (1982). Effect of nerve growth factor on regeneration of goldfish optic axons. *Brain Research* **238**, 329–339.