POSTEMBRYONIC GROWTH OF THE OPTIC TECTUM IN GOLDFISH

II. Modulation of Cell Proliferation by Retinal Fiber Input¹

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Abstract

The proliferation of cells in the germinal zone of the optic tectum of adult goldfish was studied following unilateral optic nerve crush or removal of one eye. Dividing germinal cells were labeled with [³H]thymidine, which was injected at various times (0 to 30 days) following surgery; fish were sacrificed after short (48 hr) survival times. The numbers of labeled nuclei in the tectal germinal zones were compared on the two sides (intact and denervated). We show that permanent removal of optic input (by enucleation) resulted in a sustained *depression* of [³H]thymidine incorporation in the tectal germinal zone on the denervated compared to the intact side. Temporary denervation (by optic nerve crush) initially had a similar effect; however, upon reinnervation of the tectum by regenerating optic fibers, proliferation was *enhanced* on the experimental side compared to the intact side.

Because cells in the germinal zone are known to produce new tectal cells, neurons as well as glia, in the normal growing adult brain (Raymond, P. A. and S. S. Easter, Jr. (1983) *J. Neurosci.* 3: 1077-1091), some of the proliferating cells may have been generating neurons. This inference is supported by the observation that in two fish whose right eye had been removed more than 2 years earlier, there were fewer neurons in the denervated tectum than in the intact tectum. Thus, it is likely that the observed decrease in incorporation of [³H]thymidine by cells in the germinal zone of the denervated optic tectum resulted in a slower rate of addition of new tectal cells on the affected side.

We conclude that cytogenesis in the germinal zone of the growing optic tectum of adult goldfish is regulated by optic fiber input. This mechanism may be important in matching the rates of growth of retina and tectum in the normal brain of the growing adult fish.

Neuroembryologists have long been concerned with the question: To what extent do peripheral organs exert an influence on the development of associated structures in the central nervous system? The eye is a classic choice in experimental tests of this problem, and numerous

studies have demonstrated that retinal input exerts a profound influence on embryonic development of the optic tectum (reviewed on pp. 264-270 in Jacobson, 1978). Enucleation during early development leads to atrophy of the contralateral tectum in all vertebrates that have been studied (see for example, Terry and Gordon, 1960; Kelly and Cowan, 1972; Schmatolla and Erdmann, 1973). Although the term "hypoplasia," i.e., decreased cell number, has frequently been used to describe the atrophy, it has not always been convincingly shown that the affected tectum actually contains fewer cells. The best evidence for genuine hypoplasia comes from studies on embryonic and larval amphibians where it has been shown that mitotic activity is depressed and fewer cells are produced on the denervated side following eye removal (Larsell, 1929, 1931; Kollros, 1953, 1982; McMurray, 1954; Terry and Gordon, 1960; Eichler, 1971). There is, however, disagreement as to whether the dividing cells that are

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affected produce neurons or just glial cells (Currie and Cowan, 1974; Kollros, 1982).

In goldfish the optic tectum continues to grow by addition of new cells, neurons as well as glia, for an extended period into adult life (Meyer, 1978; Raymond and Easter, 1983). For instance, more than 100,000 new neurons are added to the tectum in adult fish during a 4-year period, from 1 to 5 years of age (Raymond and Easter, 1983). Thus, in goldfish the interval over which ingrowing retinal fibers could potentially influence the genesis of tectal cells is very prolonged. Moreover, the new optic fibers, numbering about 50/day, enter the tectum at its outermost edges adjacent to the germinal zone of postembryonic neurogenesis (Easter et al., 1981), a location well situated to influence proliferation. In this study, we ask whether cell proliferation in the tectal germinal zone is influenced by retinal output. A preliminary report has appeared elsewhere (Johns et al., 1977).

Materials and Methods

Surgery. To determine what influence optic fiber innervation might have on proliferative activity of cells in the germinal zone of the optic tectum, the optic input was surgically removed, either permanently, by removing the eye, or transiently, by crushing the optic nerve. After a delay, the crushed nerve regenerates, and abnormally large numbers of optic fibers grow into the tectum, thus permitting assessment of the effects of hyperinnervation.

Goldfish, 9 to 11 cm long, tip to tip, and about 2 to 3 years old (Ozark Fisheries, Richland, MO) were anesthetized in 0.05% tricaine methanesulfonate (Ayerst). The conjunctiva of the right eye was slit, and the eyeball was pulled outward and downward to expose the optic nerve, which was crushed repeatedly with a pair of curved forceps. All surgical procedures were performed by one investigator (J. A. B.), who attempted to repeat the crushing operation identically in all fish. Care was taken to avoid damaging the ophthalmic blood vessels, which could usually be seen loosely apposed to the optic nerve. If intraorbital bleeding occurred, the animal was discarded. Fish were kept at 30°C in aerated, filtered, 10-gallon aquaria for at least 2 weeks before surgery and for the duration of the experiment.

Radioautography. Each fish with a crushed right optic nerve was injected intraperitoneally with 10 μ Ci/gm of body weight of [3 H]thymidine (New England Nuclear; 51 to 62 Ci/mmol). Twenty-four hours later a second, equivalent, injection was given, and after a further 24 hr, the fish were killed by decapitation. The first injections were administered at one of the following: 0, 1, 2, 3, 6, 9, 12, 15, 18, 21, 24, 27, or 30 days after the surgery. The results described are from 24 animals, two at each survival time in most cases.

A second group of 23 fish was treated identically to the first, except that, instead of crushing the optic nerve, the right eye was enucleated. They were similarly injected with [3 H]thymidine beginning 0 to 30 days later. Three unoperated fish, also injected with [3 H]thymidine, provided normative data.

Brains were fixed by immersion in Bouin's, embedded in paraffin, and serial, 10- μ m, transverse sections were

processed for radioautography (Kopriwa and Leblond, 1962) and poststained with cresyl violet.

Labeled cells were counted on every fifth section through the full extent of the optic tectum at $\times 250$ magnification. Thirty to 50 sections from each brain were counted. The counts were restricted to the germinal zone at the dorsomedial border of the tectum (Raymond and Easter, 1983). Only heavily labeled nuclei, those with more than 10 silver grains overlying the nucleus, were included. Counts were made by two of us (P. A. R. and J. A. B.); each of us counted a full series of survival times, with similar results. Duplicate counts on the same brain by the two authors differed by no more than 20%.

The level of labeling varied greatly among individual fish, probably because of variability in the amount of isotope injected and/or incorporated. To avoid the complication of variability in label, and thus facilitate inter-animal comparisons in the graphical representations of the data, we divided the mean number of labeled nuclei per section in the left tectum by the mean number per section on the right side. Because the optic nerve in teleost fish crosses completely at the optic chiasm (Kappers et al., 1936; Ebbesson, 1968; Sharma, 1972; but see Springer and Landreth, 1977; Springer, 1981), the *left* tectum in both experimental groups was *denervated* by our surgical procedures on the right optic nerve, whereas the *right* tectum remained intact and served as an internal control. Hence any ratio significantly different from 1.0 indicates an effect of the surgical manipulation on tectal cell proliferation.

Morphometry. To determine the long-term effects of denervation on tectal growth and cell addition, the right eye was enucleated in two small fish (about 5 cm in length) which were then allowed to survive for 2 years and 4 months. The fish were kept at about 21°C in aerated, filtered, 10-gal aquaria, with combined natural light and standard laboratory fluorescent illumination. When they had grown to about 10 to 11 cm in body length (comparable in size to the animals in the radioautographic series), they were decapitated, and their brains were fixed by immersion in buffered aldehydes and embedded in glycomethacrylate. Tectal cells were measured and counted as described previously (Raymond and Easter, 1983). One other fish, comparable in size to the experimental animals when they were enucleated but with both eyes intact, was kept for the same period of time under conditions equivalent to the experimentals, and its tecta were then examined histologically.

Results

Proliferation of cells in the optic pathway. Damage to retinal fibers, produced either by enucleation or by crushing the optic nerve, resulted in increased labeling of cells in the ipsilateral optic nerve, contralateral optic tract, and *stratum opticum* of the contralateral optic tectum beginning in the first few days following surgery. This proliferative response lasted for 2 to 3 weeks, after which an increased cellularity of the optic pathways on the experimental side persisted, although incorporation of [3 H]thymidine was no longer elevated. A similar pattern was seen in both groups of experimental animals. The

dividing cells were undoubtedly glia. Stimulation of mitotic activity in glial cells, gliosis, is a well known response to nerve fiber damage (reviewed by Korr, 1980; Skoff, 1980); it is not the subject of our report. We mention it here because it was so striking and so that we might contrast it with the proliferative response in the tectal germinal zone.

In contrast to the increased labeling in optic fiber pathways, the number of labeled nuclei in the tectal germinal zone was smaller on the denervated (left) side than on the intact (right) side during the first week after surgery in both groups of experimental animals. In enucleated animals, the left/right ratio of labeled cells in the germinal zone remained below 1.0 for 30 days following surgery (Fig. 1). In animals with a crushed optic nerve the left/right ratio was less than 1.0 initially, but it remained so only up to 6 days postcrush; from 9 to 30 days the number of labeled cells on the left was as much as 4 times greater than on the right (Fig. 2). The change

Figure 1. The ratio of the average number of labeled nuclei per section in the dorsomedial germinal zone of the denervated (left) and intact (right) optic tecta is plotted as a function of the number of days postsurgery on which [^3H]thymidine injections began. The right eye was enucleated on day 0. Note that the ordinate has a logarithmic scale. Each point represents one fish.

Figure 2. Conventions are as in Figure 1. In these fish, the right optic nerve was crushed on day 0.

TABLE I
Number of labeled nuclei in left and right optic tecta after removal of the right eye

Enucleations. Each line of the table represents data from one fish; each animal is coded according to the number of days postsurgery at which [^3H]thymidine injections began. The letters (e.g., "X" and "Y") refer to different animals at the same post-surgical day. The mean number of labeled nuclei per section in the dorsomedial region of the germinal zone in the left (denervated or experimental, E) and right (intact or control, C) optic tecta are given, along with the ratio L/R (left/right). Thirty to 50 sections were counted for each brain. Pairwise comparison of counts of labeled nuclei in the left and right tecta of each section was used to calculate the t statistic. N.S., not significant.

Fish	Left (E)	Right (C)	Ratio L/R	Paired t Statistic	Significance
0X	4.64	5.15	0.90	-1.12	0.271 (N.S.)
0Y	2.46	2.86	0.86	-1.45	0.156 (N.S.)
1X	5.03	6.31	0.80	-3.43	0.002 (R>L)
2X	4.11	5.92	0.69	-4.04	<0.001 (R>L)
2Y	4.32	6.06	0.71	-2.86	0.008 (R>L)
3X	2.37	3.71	0.64	-3.84	<0.001 (R>L)
3Y	2.94	3.97	0.74	-2.82	0.008 (R>L)
6X	3.28	4.93	0.67	-4.69	<0.001 (R>L)
6Y	3.77	7.49	0.50	-6.48	<0.001 (R>L)
9X	2.78	4.84	0.57	-5.75	<0.001 (R>L)
9Y	6.27	8.59	0.73	-3.84	<0.001 (R>L)
12X	2.93	4.35	0.68	-5.79	<0.001 (R>L)
12Y	4.32	5.05	0.86	-1.65	0.108 (N.S.)
15X	2.17	3.54	0.61	-4.05	<0.001 (R>L)
15Y	2.70	4.55	0.59	-4.84	<0.001 (R>L)
18Y	6.76	8.63	0.78	-2.32	0.027 (R>L)
21X	3.61	5.70	0.63	-4.38	<0.001 (R>L)
21Y	6.82	7.08	0.96	-0.39	0.698 (N.S.)
24X	1.26	2.71	0.46	-3.72	<0.001 (R>L)
27X	3.70	5.58	0.66	-6.48	<0.001 (R>L)
27Y	5.19	7.33	0.71	-4.41	<0.001 (R>L)
30X	5.32	7.84	0.68	-4.61	<0.001 (R>L)
30Y	3.82	6.18	0.62	-4.69	<0.001 (R>L)

between 6 and 9 days correlates with the arrival of regenerating optic fibers in the tectum at about 7 days postcrush in goldfish kept at 30°C (Springer and Agranoff, 1977).

The mean numbers of labeled nuclei in the germinal zone at the medial edge of the optic tectum on each side for each animal are given in Tables I, II, and III, along with the values and significance levels of the t statistic for comparison of the two sides. Table I shows that there were significantly fewer labeled nuclei in the germinal zone of the denervated tectum than in the germinal zone on the intact side in 19 of 21 fish injected with [^3H]thymidine beginning 1 day or more after enucleation. In the two animals injected on the day of surgery (0Y and 0X), the difference between the two sides was not significant. Table II shows that the numbers of labeled nuclei were significantly lower on the denervated side in six of seven fish injected with [^3H]thymidine beginning 0 to 6 days after optic nerve crush. The mean ratio (L/R) for these seven fish is 0.72. In fish injected between 9 and 18 days, the numbers of labeled cells were not significantly different on the two sides in five of seven cases; in two cases there were more labeled nuclei on the affected side than on the intact side. The mean ratio (L/R) for these seven fish is 1.32. In fish injected 21 or more days after surgery, there were significantly more labeled

nuclei on the affected side in six of eight cases; the mean ratio (L/R) for these eight fish is 2.25. Table III shows that in all three normal fish, the numbers of labeled cells did not differ significantly between the two sides; the mean ratio (L/R) is 1.04.

There is a large variability in absolute numbers of labeled nuclei in the germinal zone among fish in the same experimental group (Tables I and II) and even among normal animals (Table III); the numbers of labeled nuclei per side per section range from 0.32 to 14.07 (the minimum and maximum are both in the crushed group). Several possible reasons might account for this individual variation. A malpositioned needle or leakage of the radioactive solution during injection might result in variable levels of circulating radioactive precursor and thus affect the amount of incorporation of [^3H]thymidine and the observed numbers of labeled nuclei. Another possibility is that there are real differences in proliferative rate in the tectal germinal zones of different fish. It is known that individual fish grow at markedly different rates, and some evidence suggests that the mitotic activity of neuronal germinal cells is greater in rapidly growing goldfish than in slower growing individuals (P. A. Raymond, unpublished observations).

In summary, an absence of retinal input resulted in

TABLE II
Number of labeled nuclei in left and right optic tecta after crushing the right optic nerve^a

Fish	Left (E)	Right (C)	Ratio L/R	Paired <i>t</i> Statistic	Significance
0B	1.76	2.41	0.73	-2.90	0.006 (R>L)
1A	3.74	4.07	0.92	-0.81	0.420 (N.S.)
2A	2.95	4.19	0.70	-4.31	<0.001 (R>L)
3A	1.77	2.60	0.68	-2.56	0.014 (R>L)
3B	3.12	5.15	0.61	-4.63	<0.001 (R>L)
6B	4.23	6.65	0.63	-7.10	<0.001 (R>L)
6C	11.09	14.07	0.79	-2.61	0.013 (R>L)
9A	3.58	3.17	1.15	1.48	0.147 (N.S.)
9B	10.44	7.41	1.41	3.75	<0.001 (L>R)
12A	6.31	5.37	1.18	1.42	0.164 (N.S.)
12B	15.41	6.94	2.23	9.70	<0.001 (L>R)
15A	2.05	1.69	1.21	0.93	0.359 (N.S.)
15B	3.55	3.10	1.15	0.76	0.454 (N.S.)
18B	3.57	3.81	0.94	-0.40	0.690 (N.S.)
21B	2.53	0.70	3.78	5.77	<0.001 (L>R)
21C	1.12	0.32	3.47	4.23	<0.001 (L>R)
24A	4.46	3.44	1.30	2.52	0.016 (L>R)
24B	4.09	3.71	1.10	1.11	0.277 (N.S.)
27A	0.53	0.35	1.50	1.10	0.280 (N.S.)
27B	4.86	3.24	1.50	3.04	0.005 (L>R)
30A	2.08	0.60	3.47	6.18	<0.001 (L>R)
30B	1.95	1.05	1.86	2.99	0.005 (L>R)

^a Conventions are as in Table I.

TABLE III
Number of labeled nuclei in left and right optic tecta in normal fish^a

Fish	Left	Right	Ratio L/R	Paired <i>t</i> Statistic	Significance
N1	7.50	7.17	1.05	0.867	0.393 (N.S.)
N2	1.35	1.17	1.15	0.678	0.505 (N.S.)
N3	9.19	9.94	0.92	-1.22	0.232 (N.S.)

^a Conventions are as in Table I.

less labeling, and an abnormally high number of ingrowing axons resulted in more. These results support the hypothesis that cell proliferation in the tectal germinal zone is stimulated by ingrowing retinal axons.

Tectal cell numbers. We now turn to the question of how the accumulation of new tectal cells might be affected by changes in the proliferative rate. By comparing numbers of periventricular neurons in the two optic tecta of fish that had had one eye removed more than 2 years previously, we sought to discover whether the depression in [^3H]thymidine incorporation we had observed on the denervated side represented a persistent decrease in the rate of production of new tectal cells that would eventually result in fewer total tectal cells on the affected side.

The results of the tectal cell counts are presented in Table IV. The ratios of total numbers of periventricular tectal neurons on the right (control) versus left (denervated) tecta in the two enucleated fish were 1.34 and 1.13, respectively. The comparable ratio (more numerous/less numerous) for the intact animal was 1.02, and similar ratios, of 1.01, 1.02, 1.04, and 1.08, were found in four other intact animals whose tecta were examined for the first paper of this pair (Raymond and Easter, 1983). The ratios from the two experimental animals were significantly higher than those of the five controls ($p < 0.05$, one-sided Mann-Whitney test). No differences in cell size or thickness of the periventricular layer existed between intact and denervated tecta. These results imply, therefore, that over a period of 2 years and 4 months, during which time the tectum grew, fewer new neurons were added to the denervated tectum than to the normal (innervated) tectum in the same animal. This atrophy of the denervated side was readily apparent in histological sections (Fig. 3), and reconstruction of the tecta showed that the denervated lobes were smaller in area and volume than the control lobes in the same animals (Table IV). These results are consistent with the proposal that the rate of cell production in the germinal zone is depressed on the denervated side.

There are some inconsistencies that complicate the interpretation of these data, however. First, the absolute numbers of tectal cells varied substantially among fish. For instance, the laboratory-reared control fish (#256), which was smaller than the three large pond-reared fish whose tecta were examined in the previous paper (Raymond and Easter, 1983), had more cells (730,000) than any of the latter. (The total tectal cell number for the large fish in the previous paper, which were 18 to 22 cm long, ranged from 586,000 to 653,000.) Thus, the size of the fish is not a very good predictor of tectal cell number. Second, the results of cell counts in the two enucleated fish do not lead to an unambiguous conclusion about the quantitative effect of long term denervation on tectal cell addition. One of the enucleated fish (#255) had such a large number of cells on *both* sides (more than 600,000) that a great many new cells must have been added since the enucleation, if we assume that there were about 500,000 cells in the tectum when the eye was removed. (This assumption derives from the fact that the total cell number in the small fish from the previous paper ranged from 413,000 to 580,000; the experimental animals were

about this size at enucleation.) The other enucleated fish (#254) had an experimental tectum with about the *same* number of cells (437,000) as in the small fish and a normal tectum with a number *intermediate* (585,000) between small and large fish. This would imply an absence of further cell addition in the germinal zone on the denervated side following enucleation. Therefore, because we could not count tectal cells in the same fish both before enucleation and 2 years later, we cannot be sure of the magnitude of the effect of denervation on the addition of tectal cells.

Discussion

We have shown that proliferative activity in the germinal zone of the optic tectum of adult goldfish can be influenced by ingrowing retinal fibers. This conclusion relies on results from two separate analyses: (1) radioautography to demonstrate incorporation of [^3H]thymidine into dividing cells in the germinal zone and (2) estimations of the numbers of tectal neurons produced.

Removal of optic input depresses cell proliferation in the tectal germinal zone. Removal of one eye of an adult goldfish results in decreased cell proliferation in the germinal zone of the optic tectum on the denervated side compared to the control side, which receives an uninterrupted retinal input. Moreover, there were fewer tectal neurons on the denervated side in long-term enucleates. Both results are consistent with the hypothesis that loss of retinal input slows down the rate of addition of new tectal cells.

Other reports have similarly concluded that removal of an eye results in decreased mitotic activity and/or fewer cells produced in the developing tectum, in embryonic killifish (*Fundulus heteroclitus*: White, 1948) and in larval amphibians (*Rana* sp.: Kollros, 1953, 1982; Terry and Gordon, 1960; Eichler, 1971; *Hyla regilla*: Larsell, 1929, 1931). Still other reports dispute the claim that tectal neurogenesis is affected by early deafferentation, in embryonic fish (zebrafish, *Brachydanio rerio* and *Astyanax mexicanus*: Schmatolla, 1972; Schmatolla and Erdmann, 1973) and in larval amphibians (*Rana pipiens*: Currie and Cowan, 1974). The conflicts between these reports stem largely from the issue of whether the dividing cells are producing neurons or glia. The earlier reports, which demonstrated a decrease in the number of mitotic cells in the ependymal layer of the tectum on the denervated compared to the intact side in larval amphib-

ians, assumed that the progeny of the dividing cells were neurons (Kollros, 1953; Terry and Gordon, 1960; Eichler, 1971). Currie and Cowan (1974) disputed this assumption, claiming instead that the dividing cells were glial progenitors. In a recent rebuttal to the criticisms of Currie and Cowan (1974), Kollros (1982) provides further evidence that following removal of one eye, mitotic activity is reduced in the corresponding tectum of *Rana pipiens* larvae even at early stages in larval development when neurogenesis is proceeding vigorously. But he still provides no direct evidence that the production of neurons is affected. In our work with adult goldfish we have documented that the dividing cells in the optic tectum whose mitotic activity is depressed are, in fact, neuronal progenitors (Raymond and Easter, 1983) and that fewer neurons are produced in the denervated tectum compared to the intact side as the fish grows (the present paper). Thus, the criticism that the effect of denervation is solely on glial cell proliferation does not apply to our study.

Our results are in disagreement with those of Schmatolla (1972) and Schmatolla and Erdmann (1973), who report that unilateral enucleation at early embryonic stages, prior to outgrowth of the first optic fibers, does not alter formation of neurons in teleost fish. They found equal (or possibly *increased*) numbers of [^3H]thymidine-labeled nuclei in the tectal germinal zone on the denervated side and equivalent total numbers of tectal cells on both sides when the tecta were examined histologically at 6 days after enucleation. While it might be argued that embryonic tectal development in teleosts differs from later stages of growth, the conflicting results are most reasonably explained by a species difference. Zebrafish differ from goldfish in that tectal neurogenesis in the former is apparently restricted to the first 10 days of age (Schmatolla and Erdmann, 1973), and mitotically active germinal zones are not found in the optic tectum of juveniles or adults (Rahmann, 1968). Thus zebrafish might be more similar to chicks, in which tectal neurogenesis is also restricted to a relatively brief, early period, and the presence or absence of retinal input has no effect on tectal cell numbers (Cowan et al., 1968; Kelly and Cowan, 1972).

Regenerating optic fibers stimulate cell proliferation in the tectal germinal zone. The results of the experiments in which one optic nerve was crushed give support to the notion that ingrowing optic fibers might stimulate

TABLE IV
Tectal size and number of cells in enucleated fish

Measurements of the size and cellular content of the periventricular layer (PVL) are compared between the right (ROT) and left (LOT) optic tecta of three fish, two of which had the right eye (RE) removed. Numbers are rounded to the nearest thousand.

Fish	Treatment	No. of Cells Sampled	Mean Nuclear Diameter	PVL Thickness	PVL Area	PVL Volume	PVL Cells (per mm^2) $\times 10^5$	Total PVL Cells $\times 10^5$	Ratio (L/R)
			μm	μm	mm^2	mm^3			
LOT	RE enucleated	1867	6.4	44	5.2	0.229	0.84	4.37	1.34
ROT		2205	6.8	43	6.5	0.280	0.90	5.85	
LOT	RE enucleated	2944	6.1	40	4.8	0.192	1.25	6.00	1.13
ROT		4920	6.4	41	7.1	0.291	0.95	6.75	
LOT	Intact	3203	6.8	42	6.9	0.290	1.05	7.25	1.02
ROT		3369	6.7	42	7.0	0.294	1.05	7.35

Figure 3. Camera lucida drawings of selected transverse sections through the brain of a fish (#255 in Table IV) whose right eye had been removed more than 2 years earlier. Rostral is toward the *bottom* of the figure, and caudal is toward the *top*. The periventricular layer of the tectum is indicated by *stippling*. Note that the left optic tectum (*LOT*) is substantially smaller than the right tectum (*ROT*).

mitotic activity in the tectal germinal zone. Following optic nerve crush, the proliferative response in the germinal zone was initially depressed but was later enhanced. The reversal in proliferative response was probably simultaneous with the renewed arrival of retinal fibers growing into the tectum. Earlier results of McMurray (1954) in *Xenopus* are analogous to ours in goldfish. She showed that removal of optic input by repeated crushing of the optic nerve (comparable to our

enucleations) resulted in a decrease in mitotic activity in the ependymal layer on the denervated side, and, when the optic nerve was allowed to regrow, mitotic activity returned to normal or even supranormal levels.

There have been other reports that regenerating optic fibers stimulate proliferation of tectal cells. In fact, our own studies were prompted in part by two earlier reports of enhanced cellular proliferation in the amphibian optic tectum during retinal fiber regeneration (Gaze and Wat-

son, 1968; Gruberg and Stirling, 1974). The dividing cells they observed were located along the ventricular surface, in the ependymal layer, and although neither study provided convincing evidence as to whether the new cells were neurons or glia, Gruberg and Stirling (1974) suggested that they might be neurons, because they later migrated away from the ventricle.

In other studies, there was no doubt that the cells being investigated were glial, however. Stevenson and Yoon (1981) showed that mitotic activity of periependymal radial glial cells is enhanced at 30 to 35 days after the optic nerve is crushed in goldfish kept at 20 to 22°C. They also observed increased labeling of nuclei (glial cells) in the superficial layers of the tectum, especially in the regions through which the optic fibers course (stratum opticum, stratum fibrosum et griseum superficiale, and stratum griseum centrale; Kappers, et al., 1936) at 5 to 35 days postcrush. The latter was very similar to what we have described as gliosis in this report. With enucleation experiments Stevenson and Yoon (1978) demonstrated that mitotic activity in the superficial layers was a response to degeneration of optic fibers, whereas proliferation of the periependymal radial glia was specifically enhanced by regeneration of new fibers. Moreover, only the radial glia produced long lasting labeled progeny, whereas labeled nuclei in the optic fiber layers disappeared with longer survival times (up to 6 months after injection). Thus, the response of the periependymal radial glial cells is analogous to that of the cells of the germinal zone; the difference is that the former only produce more of their own kind, whereas the latter, we believe, are pluripotent stem cells from which tectal cells of all kinds, both neurons and glia, ultimately derive (Raymond and Easter, 1983).

Proliferation of glial cells in other systems can also be stimulated by growing nerve fibers (Korr, 1980; Skoff, 1980). For instance, studies in tissue culture have shown that Schwann cells are stimulated to divide by neuritic outgrowth (Wood and Bunge, 1975). But myelination of regenerated optic fibers in goldfish does not occur until several weeks to months after the fibers reinnervate the tectum (Murray, 1976), so the proliferative responses in superficial tectal layers that we observed, as well as those described by Stevenson and Yoon (1978), are unlikely to be associated with division of oligodendrocytes for the purpose of myelination.

Thus, proliferation is stimulated in a number of different cell types in the tectum following degeneration or regeneration of optic fibers. However, only the increased mitotic activity in the tectal germinal zone can be associated with neurogenesis because that is the only site at which new neurons are produced in the adult goldfish optic tectum (Raymond and Easter, 1983). We conclude that the rate of production of new tectal neurons can be modulated by optic fiber input in adult fish.

A possible explanation for the influence of optic input on the germinal zone. The depression of [³H]thymidine labeling in the germinal zone of the denervated tectum in adult goldfish begins very soon (within 2 days) after optic nerve crush or enucleation. Such a short latency implies that the cells of the germinal zone probably were not responding to the absence of retinal fibers, *per se*,

because retinal fibers were still present, although degenerating, throughout the 30-day period over which the experiment was carried out (Murray, 1976; Wolburg, 1978). Perhaps the degenerating fibers in some way signaled their malaise to the germinal zone, which responded by decreasing its mitotic activity. Possible reasons for and mechanisms of such a communication are obscure, however.

A more plausible possibility is that the germinal zone is normally stimulated by the daily arrival of 50 or so new retinal axons coming from newly generated retinal ganglion cells (Johns, 1977; Easter et al., 1981). Thus when these axons disappear following crush or enucleation, the rate of tectal cell proliferation drops. When the axons become abnormally numerous, as during regeneration, proliferation accelerates. Such an interaction would allow the growth of the tectum to be directly coupled to that of the retina; this would be advantageous, because the rate of growth varies substantially among individual fish (Brown, 1957). Thus, production of new tectal cells could be modulated by the quantity of new optic input, such that in a rapidly growing animal, in which many new optic fibers reach the tectum each day, more tectal cells would be generated to accommodate them.

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