POSTEMBRYONIC GROWTH OF THE OPTIC TECTUM IN GOLDFISH

I. Location of Germinal Cells and Numbers of Neurons Produced¹

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Abstract

The growth and morphology of the optic tectum of adult goldfish were studied with light and electron microscopy and with thymidine radioautography. The tectum is roughly hemispheric in shape, with a smaller radius of curvature rostrally than caudally. A narrow region containing proliferating cells (the germinal zone) is found along two-thirds of the rim of the tectal hemisphere but is absent rostrally, adjacent to the tectal region which receives input from the rostral visual field. New cells generated in the germinal zone are added to the tectum appositionally in crescent-shaped increments; there was no evidence of migration of new cells into the rostral region which lacks a germinal zone.

Some of the new cells added to the adult tectum were shown to be neurons on the basis of cytological and ultrastructural features. Counts of tectal neurons likewise demonstrated that new cells were added with growth of the tectum; large goldfish (25 cm long) had 27% more tectal neurons than did small fish (4 cm long). Spreading apart of existing cells also contributed to overall growth of the tectum.

These results confirm and extend those of R. L. Meyer ((1978) *Exp. Neurol.* 59: 99-111). The topological dissimilarity of the patterns of growth of retina (which adds cells appositionally around its entire perimeter) and tectum supports the suggestion that retinotectal terminals must continually move (Gaze R. M., M. J. Keating, A. Ostberg, and S. H. Chung (1979) *J. Embryol. Exp. Morphol.* 53: 103-143). Our estimates of cell numbers and tectal areas lead to predictions about the directions and magnitudes of these displacements.

Teleost fish continue to grow as adults, and at least part of the increase in size of the brain can be attributed to the accumulation of new neurons. Kirsche (1967) has reviewed the subject of postembryonic cytogenesis in the vertebrate brain and has summarized much of the evidence for continuing neurogenesis in adult teleosts. In certain areas of the brains of fishes, there are clusters of undifferentiated, mitotically active cells, thought to be precursors of both neurons and glia. These proliferating cells are packed into restricted, sharply delineated regions, usually adjacent to the ventricular surface. These regions have been called *matrixzonen* (Kirsche, 1967;

Richter and Kranz, 1970), because they are thought to be similar to and indeed derived from the matrix zones of the embryonic neural tube (Fujita, 1963, 1964). We prefer the more descriptive term *germinal zone*, which more accurately reflects the mitotic activity which takes place there; we will use the latter term throughout the rest of this paper.

The germinal zones of the adult teleost brain have been identified and described in standard histological preparations (Segaar, 1965; Kirsche, 1967), and proliferation of the germinal cells has been demonstrated with [³H]thymidine radioautography (Rahmann, 1968). The most active region of proliferation is in the caudal tectum, and there are adjoining germinal areas on the medial and lateral edges of the tectal hemisphere. Meyer (1978) has shown with [³H]thymidine radioautography that these same germinal zones are the source of new cells which are added during normal growth of the tectum in juvenile and adult goldfish. He suggests that the new cells include neurons because they are found in all tectal laminae and because they are too numerous to be accounted for by

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glia alone. No histological or ultrastructural evidence of the identity of the new cells has yet been presented, however.

Our goals in the present study were five: (1) to identify and describe at the ultrastructural level the cells in the tectal germinal zone of adult goldfish; (2) to compare them with embryonic neuroepithelial germinal cells and with neuroepithelial germinal cells in the retina of adult goldfish; (3) to determine the precise shape and extent of the tectal germinal zone; (4) to count the new tectal cells added with growth; and (5) to identify the differentiated progeny. Preliminary results of some of this work have been published in abstract form (Johns and Easter, 1979). In the second paper of this series (Raymond et al., 1983) we investigate the role played by ingrowing new optic fibers in modulating proliferative activity in the tectal germinal zone.

Materials and Methods

Common goldfish (*Carassius auratus*, "Comets") obtained from Ozark Fisheries (Richland, MO) or Grassy Forks Fisheries (Martinsville, IN) were housed in filtered, aerated tap water in 10-gallon aquaria at room temperature (20 to 24°C). The fish were fed dry fish food (TetraMin) daily and were exposed to a combination of natural daylight and standard laboratory fluorescent illumination. The fish ranged in overall length from 5 to 30 cm and were, therefore, approximately 1 to 5 years old (Johns and Easter, 1977).

Histology. For light microscopy, fish were anesthetized in tricaine methanesulfonate and were then killed by decapitation. The cranium was opened to expose the brain, and the head was fixed in buffered aldehydes overnight at 4°C. The brain was then dissected out of the skull, trimmed, dehydrated in graded ethanols, and embedded in glycomethacrylate (Polysciences JB-4 or Sorvall Embedding Medium).

For electron microscopy, the brains were fixed by immersion overnight at 4°C in a mixture of 2 to 2.5% glutaraldehyde, 1 to 2.5% paraformaldehyde, 1 mM CaCl₂, and, in some cases, 0.1% picric acid, in 0.067 M phosphate buffer at pH 7.4. Some fish were first perfused through the heart with the aldehyde mixture, and the brains were then dissected out and put into fresh fixative overnight. The next day, the optic tecta were trimmed into small blocks and postfixed in 1% OsO₄ in the same buffer for 2 hr at 4°C. Following dehydration in cold, graded methanols, the tissue was embedded in Epon or Spurr's (Polysciences). Semithin sections were stained with toluidine blue for light microscopy. Ultrathin sections stained with uranyl acetate and lead citrate were viewed with a Philips (300 or 400) electron microscope.

Thymidine radioautography. Goldfish, 8 to 11 cm long, were in general injected intraperitoneally with [³H]thymidine, 10 to 20 μCi/gm, specific activity: 40 to 60 Ci/mmol (New England Nuclear, Boston, MA). One to 3 days later, brains were embedded in glycomethacrylate and processed for radioautography as described previously (Johns, 1982). Sections were poststained through the emulsion with methylene blue. Short survival times such as these insured that the mitotically active germinal cells were labeled.

To learn the eventual fate of the new cells, whether they became neurons or glia, a longer survival time was used to allow the cells to differentiate; those which differentiated with few or no further mitotic divisions would retain the tritium-labeled DNA in their nuclei. Fish were sacrificed 18 to 28 days after injection, and the optic tecta were dissected out and prepared for electron microscopy as described above, except that they were not chopped into small blocks. Instead, the entire optic tecta were serially sectioned transversely at 5 μm and 2 μm on dry glass knives with a JB-4 microtome (Dupont-Sorvall). The sections were flattened with xylene vapors and heat mounted onto acid cleaned, gelatin-chrome alum subbed slides, which were then dipped in NTB-2 emulsion (full strength or diluted 1:1 with distilled water), exposed for 4 weeks at 4°C, and developed for 2 min in D-19 (Eastman Kodak). Some slides were examined without further staining, and others were stained with 1% toluidine blue in 1% borate. Coverslips were applied with glycerin, and selected sections with well labeled cells were photographed. These sections were then re-embedded for electron microscopic examination as follows. The coverslips were removed, the glycerin was cleaned off with ethanol, and the slides were thoroughly dried. The pointed end of a Beem capsule was cut off, leaving an open-ended cylinder, one end of which was dipped into Epon and applied to the slide, centered over the selected section. The slide plus cylinder was then placed into an oven at 60°C for several hours to polymerize the resin. The cylinder, now firmly attached to the slide, was filled with Epon and polymerized overnight at 60°C. To remove the block, the undersurface of the slide was heated briefly over an ethanol flame, and the Beem capsule together with the section was quickly snapped off the slide. The block was retrimmed to include only the selected region containing the labeled cell(s) of interest. Ultrathin sections were mounted on Formvar-coated slot grids. With photomicrographs of the radioautographs in hand, the grids were scanned in the electron microscope and the labeled cells were identified by their size, shape, and position with respect to other cells and recognizable landmarks, such as blood vessels and nerve fiber bundles.

Three-dimensional reconstructions. The optic tectum and its germinal zone were reconstructed from serial, transverse sections of three brains of fish 10 to 12 cm long. In one brain (embedded in glycomethacrylate) two small holes, about 200 μm in diameter, were drilled into the block at locations ventral to the tectum, parallel to its rostrocaudal axis but at different distances from the midline. These holes facilitated later alignment of the sections during the reconstruction. Serial, transverse sections were cut alternately at 2 μm and 6 μm through the entire optic tectum and were stained with toluidine blue. Twenty-seven 6-μm sections at precisely known distances from the rostral pole of the optic tectum, spaced 80 μm apart, on average, were traced at ×47 magnification using a projecting microscope. The outlines of the tectal surfaces, the ventricles, and the alignment holes were drawn, and each 6-μm section, along with the adjacent 2-μm section, was examined in a compound microscope. The tectal germinal cells have unique cytological characteristics which distinguish them from other tectal cells (see

"Results"), and, if the section under inspection contained germinal cells, their positions were marked on the tracing.

Similar reconstructions of the optic tecta and germinal zone were also made from the brains of two fish injected with [^3H]thymidine; one survived for 24 hr following injection and one survived for 18 days. The former was embedded in paraffin and the latter in Epon; in both cases a complete set of serial sections was saved and prepared for radioautography. The outlines of the tectal surface were drawn at low magnification ($\times 47$), and the positions of labeled cells were noted after the sections were inspected at high magnification ($\times 400$ to 500). The anatomical midline was used to align the sections in the transverse plane; alignment in the horizontal plane was judged by matching adjacent sections to produce the smoothest possible contour.

Reconstructions of the tectum and germinal zone were generated by hand and with computer-aided graphics. For the latter, a digitizing tablet (Summagraphics) was used to enter information from the drawings, and the reconstructed images were displayed on a Megatex graphics terminal linked to a PDP 11/34 computer. The computer-aided reconstructions were useful for rotating the images to obtain various views of the tectum and its germinal zone.

Tectal size and cell number. Goldfish, 5 to 30 cm long, were used for a morphometric analysis of tectal growth. We showed previously (Johns and Easter, 1977) that size and age are roughly correlated in goldfish, that is, larger fish are older than smaller ones. In the analysis that follows we, therefore, assume that our measurements of tectal size and cell number differ between small and large fish because of growth-related changes in the brains.

The length of the optic tectum from rostral to caudal poles was measured with a Vernier caliper on 26 brains fixed in Bouin's solution. The numbers of tectal cells were estimated from the brains of three large (21.2 to 28.4 cm long) and four small (4.5 to 4.6 cm long) goldfish, fixed by immersion and embedded in glycomethacrylate. Transverse $2\text{-}\mu\text{m}$ sections were cut and stained with toluidine blue. Typically, 10 to 15 sections, equally spaced rostrocaudally, were examined in more detail. The vast majority (about 96%) of the neurons in the optic tectum of goldfish are in the periventricular layer (Meek and Schellart, 1978). The periventricular neurons were recognized by their round nuclei and speckled chromatin. First, camera lucida drawings were made at low magnification ($\times 40$), and the length of the periventricular layer was measured on a digitizing tablet (MOP-III, Zeiss). Then, at $\times 1000$ magnification, 15 to 20 nuclei in the periventricular layer were drawn and their diameters measured on the digitizer. Finally, at $\times 500$ magnification, all nuclei in selected segments (50 or $100\text{ }\mu\text{m}$ long and separated by roughly $300\text{ }\mu\text{m}$) were counted, excluding blood cells, endothelial cells, and radial ependymal glia; the latter were recognized by their lobulated nuclei (Stevenson and Yoon, 1981, 1982). The mean of the nuclear diameters, measured in all sections, and corrected for the underestimation resulting from the inclusion of partial nuclei in the measured sample (Konigsmark, 1970), was used to calculate the number of nuclear centers per $2\text{-}\mu\text{m}$

section (Abercrombie, 1946). This number was multiplied by the number of sections between the sampled section and the next most rostral sampled section to yield the number of nuclear centers per slab where a "slab" is the portion of tectum between two sampled sections and including one of them. This approximation is quite acceptable over most of the tectum, where transverse sections cut radially through the tectal cortex, but it is unacceptable over the rostral portion, where transverse sections are oblique or tangential to the tectal surface. For slabs from this region a geometrical correction was made, for which the rostral pole was approximated as a partial sphere.

Results

Size and shape of the optic tecta. As juvenile and adult goldfish grow, the brain enlarges along with the body. Figure 1 compares the brains of two fish, a small one (5 cm long) and a large one (17 cm long). The optic tecta are the largest lobes of the brain. They are oblong in shape and somewhat elongated in the rostrocaudal dimension (Fig. 1A); the tectal "length", measured along the rostrocaudal axis in dorsal view is approximately 20% greater than either the "width" (mediolateral) or the "depth" (dorsoventral). In lateral view it can be seen that the long axis of the oblong tectal hemisphere is tipped, rostral end downward, by about 40° (Fig. 1B). The shape and orientation of the tecta do not change appreciably with the growth of adult fish.

The amount of growth of the optic tectum during adult life was estimated by measuring the length of the optic tecta in fish of various sizes. The linear regression of tectal length in millimeters (y) to body length (tip to tip) in centimeters (x) was $y = 0.16x + 1.59$; correlation coefficient, $r = 0.88$. The brains of older, larger fish are *relatively* smaller than those of younger animals: in the largest fish the tectal length was 2.4% of the animal's length, whereas in the smallest fish the comparable value was 4.6%. This negatively allometric relation between enlargement of neural structures and somatic growth has been noted previously in studies of the growing eyes of goldfish (Johns and Easter, 1977) and other fish (Muller, 1952).

Each tectal lobe is a somewhat distorted hemisphere, the rim of which lies approximately within a single plane. With respect to the sagittal plane, the plane of the tectal rim is rotated about both the rostrocaudal and mediolateral axes. The ventral edge of the plane is rotated laterally by 35° ; the caudal edge is rotated dorsally by 20° . The tectal hemisphere bulges rostrally; that is, the radius of curvature is much smaller rostrally, where the tectal sheet curves sharply underneath itself, than caudally, where the tectum is flatter, almost planar. The shape suggests a cap, such as a beret. The optic tract approaches the tectum rostrally and splits into two brachia, one dorsomedial and the other ventrolateral. The rostral tectal pole fits tightly within the fork produced by the two brachia.

Numbers of tectal cells. Neuronal cell nuclei in the periventricular layer (Fig. 2) were counted in four small and three larger goldfish. The results are given in Table I. These counts show that the large fish had 27% more

Figure 1. Comparison of freshly dissected brains from two adult goldfish, a large animal and a smaller one. *A*, Dorsal view. *B*, Lateral view. The left optic tecta (*OT*) are labeled in *A* and enclosed by *black lines* in *B*. The *calibration bar* is 5 mm.

Figure 2. Photomicrograph of a transverse glycomethacrylate section at the dorsomedial border of the optic tectum; dorsal is up and medial is to the right. Ventricular spaces (V) underlie the tectum. Bundles of optic fibers course through the tectum in the stratum opticum (SO). The tectal germinal zone (G), shown in the *inset*, is at the medial end of the periventricular layer (PVL), adjacent to the torus longitudinalis (TL). A bundle of commissural fibers (*arrow*) penetrates the germinal zone and runs through the periventricular layer parallel to the tectal surface. The tectal germinal zone is in reality separated from the torus longitudinalis by a ventricular cavity, but in this section the space has been obliterated during histological processing. The flattened ependymal cell layers which line the ventricular surfaces at the apposed tectum-torus interface are indicated by the *arrowhead* in the *inset*. The *calibration bar* is 100 μ m.

Figure 3. Electron micrograph of germinal cells (*) and periventricular neurons (n). Abundant ribosomes can be seen in the germinal cells. Note the elaborate Golgi complex in one of the PVL neurons (*arrowhead*). The *calibration bar* is 1 μ m.

cells than the smaller ones ($636,000 \pm 19,700$ and $501,000 \pm 60,400$, means $\pm$ SD). In the four fish in which cells were counted in both tectal lobes, the numbers on the two sides differed by only 1% to 8%. The smallness of the differences supports the inference that the big tecta did actually contain more cells. Statistical evaluation of the data (Student's *t* test) reveals a significant difference between the two groups ($p < 0.05$).

Table I also gives the thickness, area, volume, and density of cells in the periventricular layer. Notice that the periventricular layer in large fish is only half the thickness in small fish and that the tectal area increases nearly 5-fold, whereas the density of cells decreases about 4-fold. Thus, the tectum has grown by both spreading the pre-existing cells over a larger area and by adding new cells. Concurrent, similar changes occur in the growing retina (Johns and Easter, 1977; Johns 1981; Easter et al., 1981).

We now turn to the source of new tectal cells—the germinal zone.

Tectal germinal zone—cytology. In transverse sections, the ends of the periventricular layer at the rim of the tectal hemisphere are in some places bounded by a tightly packed cluster of distinctive cells (Fig. 2). They have elongated, lobulated nuclei and densely basophilic cytoplasm. Ultrastructurally, their cytoplasm is rich in free ribosomes; the nucleus often contains a prominent nucleolus, and the nuclear chromatin is somewhat condensed near the nuclear membrane (Fig. 3). These cells are easily distinguished from the adjacent periventricular neurons, which have round or oval nuclei with relatively more euchromatin (Fig. 3). The characteristics of the cells at the tectal margin are quite similar to previous descriptions of embryonic neuroepithelial germinal cells in other animals (Tennyson, 1970; Hinds and Hinds, 1974; Graziadei and Monti-Graziadei, 1978) and the cells in the circumferential germinal zone in the growing retinas of postembryonic goldfish (Johns, 1981, 1982).

Like the latter two cell types, the cells in the tectal germinal zone are proliferating, as demonstrated by both

the presence of mitotic figures and the incorporation of [3 H]thymidine into their nuclei. A representative radioautograph of the tectal germinal zone from a fish injected intracranially with [3 H]thymidine 24 hr before sacrifice is shown in Figure 4. These labeled nuclei are at the dorsomedial edge of the periventricular layer. Most labeled nuclei associated with the germinal zone were at the junction between the cluster of elongated germinal cells and the adjacent rounded periventricular nuclei; many, but not all, were at or near the ventricular surface.

Labeled nuclei were seen in other locations, especially along the ventricular surface underneath the tectal lobe and around the neighboring *torus longitudinalis*, a paired tubular structure which lies between and parallel to the medial edges of the optic tecta (Figs. 2 and 4). These other proliferating cells have been studied by Stevenson and Yoon (1981), who identified them as radial glial and ependymal cells. A few additional labeled nuclei which were scattered throughout the tectum could be identified as endothelial cells or glia associated with fiber tracts (Fig. 5).

Tectal germinal zone—morphology. Histological reconstructions from sectioned brains provided information about the size and shape of the tectal germinal zone, which was recognized by the presence of labeled nuclei (in a [3 H]thymidine-injected fish) or the presence of cells with the characteristic cytology described above (in an unlabeled animal). The results were similar in the two cases, confirming that the distinctive cytology of the germinal cells allows them to be identified independently of the radioactive label.

Figure 6 shows a set of representative camera lucida drawings with the positions of the germinal cells indicated. We would emphasize three points that are illustrated by this figure. First, germinal cells are found only at the ends of the periventricular layer. Second, the germinal zone is crescent shaped. Germinal cells are absent from the rostral tectum, and their density increases in the caudal direction on both the dorsomedial and ventrolateral edges of the tectum. The two arms are

TABLE I
Tectal size and cell number

Data from seven fish are presented; in three cases either the right (ROT) or left (LOT) tecta were analyzed; in the other four cases, both tecta were used. The size of the fish is indicated in the first three columns: the overall length (from tip of nose to tip of tail), the standard length (from tip of nose to base of tail), and the weight. The number of cells counted and the average nuclear diameters are given. The thickness, area, and volume of the periventricular cell layer (pvl) are listed, followed by the planimetric density of cells and the total number of cells in the periventricular layer of the tectum.

Fish	Overall Length	Standard Length	Weight	No. of Cells Sampled	Mean Nuclear Diameter	pvl Thickness	pvl Area	pvl Volume	pvl Cells/mm ² × 10 ⁵	Total pvl Cells × 10 ⁵
	cm	cm	gm		μm	μm	mm ²	mm ³		
262 LOT	4.5	2.9	1.1	2726	6.1	65	1.8	0.117	2.29	4.13
263 ROT	4.5	2.9	1.1	2681	6.8	63	2.0	0.126	2.40	4.80
264 LOT	4.5	3.0	1.1	3534	6.0	62	2.0	0.127	2.53	4.99
ROT				3851	6.0	62	2.1	0.130	2.57	5.39
265 LOT	4.6	2.9	1.0	3146	6.2	71	2.2	0.157	2.60	5.72
ROT				2471	6.2	71	2.2	0.158	2.64	5.80
257 LOT	28.4	14.4	80	2344	6.8	36	9.0	0.331	0.72	6.47
ROT				2374	6.8	36	9.4	0.338	0.68	6.36
266 LOT	24.2	13.7	73	2058	6.5	29	9.0	0.261	0.73	6.53
267 ROT	21.2	13.6	72	1795	6.6	32	11.0	0.352	0.55	6.09
LOT				1161	6.6	32	10.8	0.346	0.54	5.86

joined at the caudal pole, where the density of germinal cells is maximal. Third, in regions of tectum which support a germinal zone, ventricular spaces underlie the periventricular layer. The nomenclature is confusing here, as the so-called periventricular layer is not everywhere close to the ventricle. Where the periventricular layer of tectum directly overlies tegmentum, with no intervening ventricular space, it is not bounded by germinal cells (Fig. 6). We believe this to be an important point and will return to it later in our discussion of the embryonic origins of the adult germinal zone. These observations confirm those of Kirsche and Kirsche (1961) and Meyer (1978).

When the tectum was reconstructed using computer graphics and tilted so that the germinal zone lay in the plane of the screen, the result is shown in Figure 7. This shows the rostralateral bulge, where the radius of cur-

vature is smaller than elsewhere. It also illustrates the rostral gap in the germinal zone. With respect to the center of the circle which best fit the caudal rim, the rostral gap subtended between 100° and 140° .

Progeny of the germinal zone. At longer survival times after injection of [^3H]thymidine, label was seen over nuclei in the periventricular layer adjacent to the germinal zone. Figures 8 and 9 show two tectal sections from a fish injected 18 days previously with [^3H]thymidine. These are semithin sections that were prepared for light microscopic radioautography and then remounted and thin sectioned for standard electron microscopy (without radioautography). In Figure 8A, a light microscopic radioautograph, a cluster of labeled nuclei in the periventricular layer is seen adjacent to the germinal zone at the dorsomedial edge of the right optic tectum. We infer that this band of labeled nuclei near the germinal zone at

Figure 4. Radioautograph of a transverse section through the germinal zone (*G*) at the dorsomedial tectal border, from a fish injected with [^3H]thymidine 24 hr before sacrifice. Four labeled nuclei (*arrowheads*) are located at the edge of the germinal zone; three of them are near the ventricular surface (the ventricular cavity between optic tectum and torus longitudinalis (*TL*) has again been obliterated during histological processing). The calibration bar is 50 μm .

Figure 5. Radioautograph showing two labeled glial cell nuclei (*arrows*) in the stratum opticum (*SO*) of the optic tectum following [^3H]thymidine injection. An endothelial cell nucleus (*arrowhead*) is also labeled. Pial surface is up, ventricle down. The calibration bar is 20 μm .

Figure 6. Camera lucida drawings of seven selected transverse sections through the left optic tectum of a goldfish injected 24 hr earlier with [^3H]thymidine. Rostral is toward the lower left and caudal is toward the upper right. The arrows point to labeled nuclei in the tectal germinal zone (*shaded*), which is located at the dorsomedial and ventrolateral ends of the tectal periventricular layer (PVL). The germinal zone extends across the tectum in the caudalmost section, where it is continuous with another germinal zone in the midbrain tegmentum (*t*) underlying the ventricle. Ventricular spaces are *hatched*.

longer survival times represents the progeny of those dividing cells in the germinal zone that incorporated [^3H]thymidine. Thus, increments of new tissue are added along the tectal boundaries that support a germinal zone as the brain grows. At higher magnification in the *inset*, the labeled nuclei are numbered 1 to 8. In the low magnification survey electron micrograph of a thin section from the block prepared from this semithin section (Fig. 8B), nuclei 1 through 8 are identified (by reference to size, shape, and position) and numbered. Several of the labeled nuclei (2, 6, and 8) clearly show neuronal

features: the nuclei are rounded with dispersed chromatin, and the cytoplasm is abundant and electron lucent. These cells match previous descriptions of periventricular neurons in the teleost optic tectum (Ito, 1970; Stevenson and Yoon, 1982). One of the labeled nuclei (7) certainly belongs, not to a neuron, but to a cell that has remained in the germinal zone (Fig. 8B). Note the lobulated, dense nucleus and dense cytoplasm with numerous free ribosomes.

Figure 9 illustrates another section from the same fish. In this case, there is only one heavily labeled nucleus in

Figure 7. A computer-aided reconstruction of the right optic tectum of a goldfish. The view is as if looking from "inside" the brain, onto the plane of the tectal rim (see the text). The cross-hatched region represents the tectal germinal zone, which is along the rim of the tectal hemisphere, represented by the inner contour.

the light microscopic radioautograph (Fig. 9A), and it lies at the superficial border of the periventricular layer. The cells at this level of the periventricular layer have been described previously as pyriform neurons with apical dendrites, and this labeled cell conforms to that description (Ito, 1970). Even in the light micrograph, at higher magnification (Fig. 9A, *inset*), the apical dendrite is clearly seen. The cell is easily identified in the corresponding thin section (Fig. 9B) by its large, round, pale nucleus with very dispersed chromatin and its apical dendrite. The perikaryon contains rough endoplasmic reticulum, mitochondria, and Golgi apparatus and the apical dendrite contains abundant microtubules (Fig. 9C). Two vesicle-containing profiles are apposed to the soma at what may be immature synaptic junctions; note the regularity of membrane separation, flocculent material in the cleft, and some suggestion of a postsynaptic membrane density associated with the profile on the left.

In other sections (not illustrated) labeled nuclei were correlated with periventricular cells which, upon ultrastructural examination, were found to contain basal bodies and, in some cases, cilia. Cilia have been described previously in neurons of the central nervous system, and they are especially abundant in neurons of the periventricular layer in the teleost optic tectum (Ito, 1970). Similar results (not illustrated) were obtained from another fish which survived 28 days after injection.

We did not see labeled neuronal nuclei anywhere in the tectum except in association with the germinal zones. Moreover, there was no indication that the newly produced neurons migrated away from their site of origin, for instance, into the rostral regions of tectum which lack a germinal zone, because there were germinal cells adjacent to all labeled neuronal nuclei.

Discussion

We have described a germinal zone in the optic tectum of young adult goldfish (we estimate their ages at approximately 1 to 5 years), and we have demonstrated

both by counting cells and with thymidine radioautography and electron microscopy that new neurons are produced in the germinal zones as the tectum grows. The existence of sites of neurogenesis that persist postembryonically has long been known, or at least assumed, in the brains of fishes and amphibians (Kirsche, 1967). Kirsche believes that these postembryonic germinal zones are remnants of the matrix layer of proliferating cells in the ventricular epithelium of the embryonic brain because of (1) the similar cytological appearance of germinal cells in the postembryonic germinal zones and cells in the embryonic matrix layer, (2) the strict association of germinal cells with ventricular spaces, and (3) the location of germinal zones at sites toward which gradients of maturation coalesce during embryonic development. In the developing embryonic optic tectum, for example, there is a pronounced rostral-to-caudal gradient of maturation, such that the first cells to differentiate are at the rostromedial pole and the last ones are at the caudomedial edge, in chicks (Cowan et al., 1968) and amphibians (Straznický and Gaze, 1972) as well as in fish (P. A. Raymond, unpublished observations). The germinal zone in the adult goldfish tectum is located precisely in the youngest, or last-formed, region at and around the caudal tectal borders and is everywhere adjacent to the ventricle.

We have shown that neurogenesis occurs only in the germinal zone at the rim of the tectum. This agrees with previous studies of tectal regeneration (cf. Kirsche, 1960; Kirsche and Kirsche, 1961; Richter, 1968; Richter and Kranz, 1977), which showed that the germinal zone is required for tectal regeneration, because its removal prevents the renewal of tectal tissue. Our cytological evidence at the electron microscope level is the first direct demonstration that some new cells generated in the germinal zone during normal tectal growth are neurons. Detailed studies by Stevenson and Yoon (1981, 1982), which included thymidine radioautography with variable survival periods up to 51 days and electron microscopic examination of labeled cells, failed to provide evidence for production of long lasting cells, other than periependymal radial glia, in tectal regions *interior* to the germinal zones in adult goldfish. Our results confirm that cells which divide in the tectal interior are glia or glial progenitors or vascular elements.

We do not claim that the germinal zone produces *only* neurons; rather we suggest that complete blocks of tectal tissue, including all of its cellular components, are built at the growing borders. Neurons, ependymal cells, and periependymal radial glia, as well as the glia of the superficial fiber layers, or more precisely the progenitors of all these cells, ultimately derive from the germinal zone. Moreover, at least some mitotic divisions of germinal cells result in progeny which fail to differentiate but remain in the dividing population (see Fig. 8). This is necessary, of course, if the germinal zone is to remain productive.

Sliding connections? The germinal zone is the *growth zone* of the tectum, where increments of new tissue, crescent shaped like the germinal zone itself, are added as the tectum enlarges. Meyer (1978) concluded similarly in a previous light microscopic radioautographic study of tectal growth in juvenile goldfish. This mode of apposi-

Figure 8. A, Light microscopic radioautograph of a transverse section through the dorsomedial edge of the optic tectum of a fish injected with [^3H]thymidine 18 days before sacrifice. A cluster of eight labeled nuclei, numbered in the *inset*, are located in the periventricular layer (PVL) adjacent to the germinal zone (G). The calibration bar is 50 μm . B, Low power electron micrograph of the same region as in the *inset* in A. The nuclei which contained the thymidine label are identified by shape and position and numbered 1 through 8. The calibration bar is 5 μm .

Figure 9. *A*, Another section from the same brain as illustrated in Figure 8. One nucleus at the superficial border of the periventricular layer is labeled with $[^3\text{H}]$ thymidine (arrow in inset). Note the pyramidal shape and long apical process. The calibration bar is 50 μm . *B*, Low magnification electron micrograph of the same cell. The apical dendrite (arrowheads) extends into the tectal neuropil. The calibration bar is 5 μm . *C*, the same neuron at higher magnification from an adjacent section. Two vesicle-filled profiles, possibly presynaptic terminals (*) are apposed to the neuronal soma.

tional growth also occurs in the amphibian tectum (Gaze and Watson, 1968; Straznicky and Gaze, 1972). In the retina new cells are also added appositionally, except that the retinal germinal zone surrounds the entire circumference (adult goldfish: Johns, 1977; Meyer, 1978; larval amphibians: Straznicky and Gaze, 1971; Beach and Jacobson, 1979). From the well known organization of the retinotectal projection, we can infer that the gap in the germinal zone in rostral tectum corresponds topographically to the rostral visual field (temporal retina), but we would like to know more precisely what region of the retinal germinal zone does not have a matching tectal germinal zone. That is, retinal fibers from which of the newly produced retinal ganglion cells must innervate nongrowing regions of tectum? In order to estimate this, we have assumed: (1) that the electrophysiologically defined receptive field of the tectum, mapped with the eye in water (Meyer, 1977), coincides with the optical field of the eye (Easter et al., 1977) and (2) that the visuotectal map of the horizontal meridian, obtained from Schwassman and Kruger (1965), is the same as the horizontal meridian of the optical field, determined using the criteria of Easter et al. (1977). With those assumptions, we estimate that the rostral 140° of the perimeter of the visual field (the temporal retinal perimeter) projects to the portion of the tectum in which growth by cell addition does not occur. This is illustrated in Figure 10, in which the visual field of one eye is represented as a circle. The narrow annulus which surrounds the circle is *hatched* along a portion of its length; the *hatched area* corresponds to the visuotopic projection of the tectal germinal zone. Thus, new retinal fibers which originate from the region of the retinal perimeter bounded by the *unhatched region* project to the nonproliferating tectal rim; note that about one-third of the retinal germinal zone is affected.

Figure 10. The visual field of one eye of a goldfish is represented by a circle, and vertical and horizontal meridians are shown. The *small circle* in the dorsal-rostral quadrant is the projection of the optic disc. The tectal germinal zone projects to the boundary of the visual field on the caudal side (*cross-hatched*); 140° of the rostral perimeter (*unhatched*) corresponds topographically to the rostral tectal rim which lacks a germinal zone.

The topological dissimilarity of retinal and tectal germinal zones was first noted by Gaze and his collaborators in amphibians (Gaze et al., 1974). They also noted that the retinotectal maps were conformal in small and large animals. From these observations, they suggested that the retinal terminals must shift connections continually during growth (Gaze et al., 1974). This suggestion has been supported in two independent ways. The first was the electrophysiological demonstration that functional synapses were formed in young tectum by the earliest retinal fibers (Chung et al., 1974). The second was the anatomical demonstration of degenerating retinal terminals in that region of the tectum where the hypothesis predicted (Gaze et al., 1979). Our contribution to this topic derives from related and more quantitative observations in goldfish. We have confirmed Meyer's report that the retina and tectum grow in topologically dissimilar ways (Meyer, 1978), and we also note that the retinotectal maps are similar in small and large goldfish (5 to 9 cm length: Meyer, 1977; 12 to 18 cm length: Schmidt et al., 1978). Moreover, our cell counts and measurements of the area of the tectal surface enabled us to estimate the relative importance of "stretch" and neurogenesis in the growth of the tectum, and we concluded that stretch was by far the more important, quantitatively. Of the roughly 5-fold increase in tectal surface, about 80% was a result of stretch, and only 20% was due to the addition of new cells.

Previous treatments of the hypothesis of sliding connections have not dealt directly with the morphological consequences of growth by stretching. However, this is an important consideration because, if retinal terminals are simply displaced passively along with the underlying tectal cells, then the connections of individual terminals need not be altered with growth. Figure 11 is intended to illustrate the process of tectal growth. The curved tectal surface is represented as a disk. In all three panels, the rostral pole of the tectum is at 0. In Figure 11A, the *small disk* enclosed by the circle *x*, centered at *X*, represents the small tectum. It grows, by stretching, into a larger surface (Fig. 11B) enclosed by the circle *y*, centered at *Y*, which represents that part of the new tectum which is made up of "old" cells. The entire large tectum is represented by the *large solid line* which incorporates parts of the circles *y* and *z* (centered at *Z*). We have made the assumption, supported by our observations, that the planimetric density of cells (number of cells/mm²) is uniform throughout the tectum, and, therefore, the area of a region is proportional to the number of cells in it. The sketches in Figure 11 are drawn to scale and incorporate three of the observations made in this paper: (1) the new cells are absent over the rostral third of the tectal perimeter; (2) the new cells represent about 20% of the total; and (3) the surface of the large tectum is about 5 times the surface of the small one.

To appreciate the consequences of tectal stretch on the retinotectal map, consider the point, *X*, which corresponds to the center of the original small tectum (Fig. 11A). The cells there receive terminals from central retina. As the tectum stretches (Fig. 11B), the same central retinal cells would be expected to project to the center of the larger tectum. If their terminals were carried along passively as the tectum stretched, they would be

Figure 11. The three panels show two-dimensional representations of the (left) optic tectum of a small fish (**A**) and a large fish (**B** and **C**). The point *O* is the rostral tectal pole, and dorsal is up, in all cases. The points *X*, *Y*, and *Z* are the centers of the circles *x*, *y*, and *z*. The radial distance of any point on the figure is scaled so that equal areas on the surface of the hemisphere appear as equal areas on the planar representation (Cook, 1979). **A**, Circle *x* represents the small tectum. **B**, Circle *x* (the small tectum, before it grew) is dashed. Circle *y* bounds the portion of the large tectum comprised of "old" tectal cells. The outer contour, encompassing segments of both *z* and *y*, defines the boundary of the new tectum. The origins of the arrows are at 0° , $\pm 45^\circ$, $\pm 90^\circ$, $\pm 135^\circ$, and 180° on the perimeter of the small tectum, *x*. The arrowheads are at the same angular positions on the circle *y*. Thus the arrows show the displacements of the cells on the periphery of the small tectum, relative to the rostral pole, due to growth of the tectum by stretch. The arrows also represent the displacements (due to stretch alone) of the retinal terminals (from retinal ganglion cells at the margin of the small retina) that innervate these regions. **C**, The outer contour is the boundary of the large tectum, and the solid circle is the boundary of the stretched original tectum, shown as a solid circle *y* in **B**. The arrows originate at the sites of the arrowheads in **B** and show the locations of the neighborhoods of tectal cells which received retinal terminals from the most peripheral portions of the small retina. The arrowheads point to sites on the large tectum where terminals from those same retinal ganglion cells would be located after both retina and tectum have grown. Thus the arrows show the displacements of the retinal terminals relative to the cells which they originally contacted in the small tectum.

found at *Y*. Their distance from the rostral tectal pole (*OY*) would be much larger than originally (*OX*), but this apparent movement would not have involved the acquisition of different neighboring cells. The retinal terminals would still be nested among the same tectal cells as before, because both cells and terminals were carried along together in the stretch. However, the topographies of the retinotectal projections in large and small fish are similar, which requires that the central retinal terminals be found at *Z*, the center of the large tectum (Fig. 11*B*). Thus, the movement of the terminals relative to tectal cellular neighborhoods (*YZ*) is predictable and very much smaller than the apparent movement (*XZ*), owing to the preponderance of stretch in the enlargement of the tectum. A troublesome fact is that the degree of similarity between retinotectal maps in small and large fish is not established accurately enough to permit us much confidence that this small displacement could be detected, so the case for sliding terminals is weakened by considering only the center of the retinotectal projection.

A much stronger case can be made by considering the movements of the terminals which originated from the most peripheral retinal ganglion cells in the small fish. In Figure 11*B*, the arrows show the displacements of these terminals which would be expected from stretch alone. These retinal cells had projected to the edges of the small tectum. As the retina grew by annular addition of new cells, those which previously lay on the margin were

displaced centrally, and maintenance of a constant retinotectal map requires that their terminals project to more central portions of tectum, away from the edge. Note, however, that some of the arrowheads in Figure 11*B* (those in rostral tectum) end at or very near the tectal edge, which demonstrates that passive stretch has carried them to a tectal location inappropriate to their required position in the retinotectal map. Their tectotopically appropriate positions are illustrated by the small dashed circle in Figure 11*C*. This circle is derived from two premises: (1) the planimetric density of retinal ganglion cells is uniform over the entire retinal surface in goldfish (Johns and Easter, 1977) and (2) the large fish have double the number of retinal ganglion cells of the small fish (Easter et al., 1981), and, therefore, the "old" ganglion cells will all be contained within a central retinal zone of area equal to half that of the entire retina. The ganglion cells on the edge of this retinal circle will project to a circle in the middle of tectum, as shown by the central dashed contour in Figure 11*C*. If the terminals had been carried along passively by tectal stretch, they would lie on the circle *y*, the boundary of the old stretched tectum. The arrows in Figure 11*C* show the displacements of the terminals from the location where stretch would have taken them to the location required by constancy of retinotopia.

Notice that the displacement vectors in Figure 11*C* have different magnitudes and directions. The more rostral arrows (at 0° , $\pm 45^\circ$, and $\pm 90^\circ$) all point caudally and

centrally. The caudal component is attributable to the pattern of tectal growth, as the axons sprout toward the newly formed region caudally. The central component results from the need to squeeze 100% more axon terminals onto a field of cells which has been augmented by only 27%. In more caudal tectum, the *arrows* (at $\pm 135^\circ$ and 180°) have lost their caudal component; indeed, the one at 180° is directed rostrally. In this case, the axon terminals are predicted to have moved in the sense opposite to the growing tectal border, because the pressure to squeeze more terminals into a confined space overwhelms the tendency to spread into the new territory. The number of new axon terminals to be insinuated into caudalmost tectum is greater than the new tissue can accommodate, so the terminals slide rostrally.

These displacement vectors are dependent on the quantitative details of growth in both retina and tectum. For example, using 27% and 100% as the increases in numbers of tectal and retinal ganglion cells, respectively, we have found that the rostral displacements are large and the caudal ones smaller. Had we assumed less retinal growth, the central circle would have been larger, which would diminish the rostral vectors and reverse the caudal ones. No matter what numbers are used, the exercise illustrates: (1) that terminals must be displaced to neighborhoods of new tectal cells for maintenance of the retinotectal map; (2) that some of the displacement vectors amount to substantial fractions of the tectal extent and could not easily be lost in local, age-dependent distortions of the map; and (3) that the displacement vectors depend in a complex way on both the position on the tectum and the quantitative details of retinal and tectal growth. The question of how neurogenesis in the retina and tectum are coordinated is taken up in the second paper of this series (Raymond et al., 1983).

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