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# Dependence of tectal neuron differentiation on optic innervation in teleost fish

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## SUMMARY

The dependence of optic tectal development, especially differentiation of the stratum griseum periventriculare, on optic innervation is analysed in teleost fish in a comparative anatomical and experimental embryological study.

Tectal development of the blind cave fish, *Astyanax hubbsi*, lacking optic nerves hence having non-innervated tecta, is compared to its river relative, the normal-eyed *A. mexicanus*. In the embryonic tectum of cave fish there is less white and grey matter, with smaller sized nuclei in the subventricular zone; in the cave adult the tectum is hypoplastic with poorly differentiated neurons in the stratum griseum periventriculare.

Extirpation of eye *Anlage* in river fish embryos produced changes in the tectum comparable to the differences between embryonic tecta of cave and river fish.

One-eyed catfish, *Ictalurus nebulosus*, with a relatively fibre-poor optic nerve from an underdeveloped retina on the eyed side, showed better differentiated cells in the periventricular layer of the innervated side than in the non-innervated side. Periventricular neurons in the tecta of normal catfish were still better differentiated, demonstrating that differentiation of these cells is 'dosage-dependent' upon the amount of optic innervation.

Embryo zebrafish, *Brachydanio rerio*, subjected to extirpation of eye *Anlage* before optic nerve outgrowth, showed the dependence of differentiation of tectal subventricular cells on optic nerve ingrowth as early as the third day of development.

Zebrafish raised in the dark compared to those raised in light showed no discernible tectal differences under light microscopy.

Lack of differentiation of the neurons in the stratum griseum periventriculare in cave fish, and in eye-extirpated fish, has not been previously emphasized. Differentiation of these neurons appears largely dependent upon interaction with ingrowing optic axons, independent of visual function.

## INTRODUCTION

Numerous studies have shown the dependence of development of nerve centres upon their peripheral innervation, and have implied that the afferent fibre, transneuronally, activates genes of the competent neuroblast to produce further differentiation. Among these studies are those dealing with the interaction between optic neurons and neurons of the optic tectum in animals in which the retino-tectal fibres cross completely. Unilateral extirpation of an optic *Anlage* produces a non-innervated tectum which can be compared to the innervated contralateral tectum serving as a control.

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Extirpation of an eye *Anlage* before, or after, the optic nerve grows out, results in diminution in development of the contralateral optic tectum, as shown in amphibia by Steinitz (1906), Dürken (1913), Harrison (1929), Larsell (1929, 1931), Twitty (1932), Kollros (1947) and Eichler (1971); in fish by White (1948), Pflugfelder (1952) and Leghissa (1955); and in the chick by Bondy & Margolis (1969), Filogamo (1969) and LaVail & Cowan (1971*a*, *b*). While these investigators report on changes in a variety of parameters in the non-innervated tectum – hypoplasia of white and grey matter; reduction in cell number, cell size and differentiation in the superficial layers; and decrease in mitotic rate – none refer specifically to changes in the morphology of cells in the subventricular layer in embryos, or in the stratum periventriculare in adults.

Deficiency in optic lobe development has been reported in blind cave fish: *Amblyopsis spelaeus* (Ramsey, 1901), *Phreatobius cisternarum* (Reichel, 1927), *Troglichthys rosae* (Eigenmann, 1904), *T. rosae* and *T. eigenmanni* (Charlton, 1933), *Typhlogarra widdowsoni* (Marshall & Thines, 1958), *Typhlichthys subterraneus* (Poulson, 1963), and in *Anoptichthys jordani* now known as *Astyanax hubbsi* (Stefanelli, 1954; Bath, 1962). None of these studies refer to lack of development of the neurons in the periventricular layer.

This study further probes the question of the dependence of differentiation of a neuron on a developmental stimulating effect of its primary afferent contact. The lack of differentiation of the cells of the periventricular layer of the optic tectum in fish not having retinal ganglion cell afferent connexions is shown as it occurs in nature in blind cave fish and one-eyed catfish, and experimentally, following eye *Anlage* extirpation, in normal fish embryos.

## MATERIALS AND METHODS

Five varieties of fish were used in this study: (1) *Brachydanio rerio* (*Hamilton-Buchanan*), also known as the zebra danio, whose developmental stages have been described by Hisaoka & Battle (1958). This fish has a well-developed visual system with full optic lobes, since its behaviour largely depends upon visual acuity. (2) *Astyanax hubbsi*, the Mexican blind cave fish, formerly known as *Anoptichthys* (Hubbs & Innes, 1936). It has regressed optic development with hypoplastic optic lobes. In the stock used in this study, a normal lens or functioning eye never developed. A fibre-poor optic nerve was present only during the first 4 or 5 days of development and then degenerated completely. (3) *Astyanax mexicanus*, the Mexican river fish with normal eyes and full optic lobes, whose behaviour relies much on visual acuity. The close relatedness of the two *Astyanax* varieties is shown by the ease in which hybridization can take place between them (Sadoglu, 1957; Kosswig, 1965). The embryological development of both these fish has been described by Cahn (1958) and Frank (1961). (4) *Ictalurus nebulosus*, the common brown catfish, manifesting relatively poor eye and optic lobe development, reflecting the fact that this fish does not depend

upon visual acuity for its survival (Polyak, 1957; Bath, 1962). (5) One-eyed *I. nebulosus* from Dog Lake, Oregon, whose non-innervated optic lobe is less developed. Weisel & McLaury (1964) have described 14 of these fish taken from this lake in 1962. Six had only a left eye, seven had no external evidence of eyes, and one had apparently normal eyes.

*Spawning methods.* Early embryos were required for eye *Anlage* extirpation. The two varieties of *Astyanax* were spawned by the methods of Lüling (1954), and the zebrafish by the method of Legault (1958).

*Microsurgical technique.* Eye *Anlage* extirpations were performed on stage 21 in zebrafish (Hisaoka & Battle, 1958), and stage 13 in the river and cave fish (Cahn, 1958). These stages are comparable in development, and are just prior to optic nerve outgrowth. Since this age is well before hatching, the eggs must first be dechorionated under a dissecting microscope, freehand, using sharpened steel needles. The dechorionated embryo was then transferred to an agar-based Petri dish containing MS 222, 1:5000, in full-strength Holtfreter's solution to paralyse the embryo and prevent spontaneous motion. The embryo was partially buried under a flap of agar so that it would remain in a fixed position during the manipulations of surgery. Extirpations were done using transillumination under a stereoscopic dissecting microscope at $\times 90$, with glass needles made on a microforge. Two micromanipulators were used: a Cailloux manipulator, a pneumatic type which gives sensitive control in three dimensions with one lever, placed on the right-hand side of the microscope for the main movements of dissection; and a two-lever micromanipulator, DP10/S10 (Research Instruments Ltd, Middlesex, U.K.) providing separate controls for the $X-Y$ axis, and for the $Z$ axis, on the left, as an assisting instrument.

Excision of the optic cup was accomplished by making a slit in the overlying ectoderm, cutting the optic stalk, freeing the eye *Anlage* by cutting around its periphery, and delivering it through the ectodermal slit. This operation was accomplished with little trauma to the neural tube. Embryos were then transferred to full-strength Holtfreter's solution for 1 h to promote healing, and finally placed in half-strength Holtfreter's solution at 28 °C. Infection was not a problem.

*Histological procedures.* Whole embryos, larvae and adult brains were fixed in Bouin's solution or 10% formalin. After routine paraffin embedding they were cut in serial transverse sections at thicknesses of 4–6 $\mu$m, and stained by either Heidenhain's haematoxylin, Delafield's haematoxylin and eosin, cresyl violet, or Bodian's silver method. Great care was taken to see that specimens were oriented in a true anterior–posterior axis, so that transverse sections would be symmetrical.

*Methods of evaluating results.* Measurements of tectal size and the amounts of its grey and white matter in the embryos were done by preparing paper cutouts from tracings made using a Zeiss camera lucida projector at $\times 400$. After preliminary study it became apparent that the tectal length was not significantly

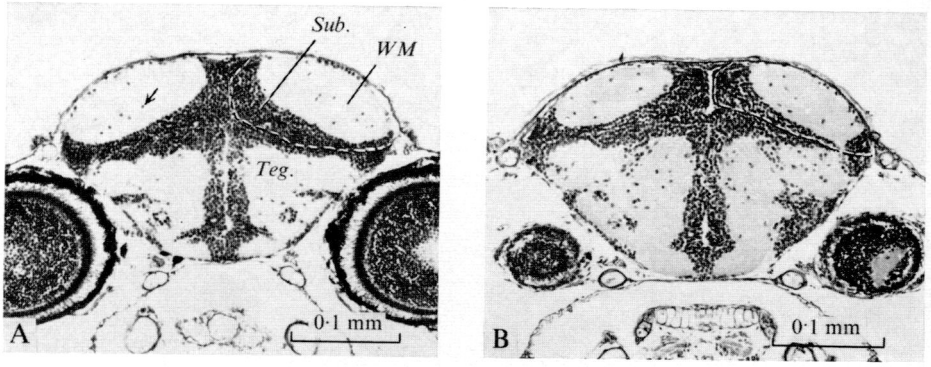



Fig. 1. *Astyanax* embryos, 1 week old. Cross-section through midtectum. A, *A. mexicanus* (river fish); B, *A. hubbsi* (cave fish). Tectum outlined by dotted lines. *Sub.*, subventricular zone; *WM*, tectal white matter, arrow points to marginal cell; *Teg.*, tegmentum. Note smaller size of cave fish tectum. Haematoxylin and eosin.

affected by the experimental conditions. It was therefore considered that the weight of a paper tracing of a transverse section through the tectum at its greatest thickness would be a valid measurement of relative tectal size. Most often the greatest thickness of the experimental tectal side corresponded to the greatest thickness of the control side in the same section. In a few instances, the greatest thicknesses were in different sections. The same stock and manufacture of paper was used throughout the study. Variation in the weight of the paper, and the accuracy of cutting out, was checked and found to vary by less than 0·15 %/unit area. This variation was considered insignificant relative to the degree of differences encountered in the weights in the experimental groups. Expressions as 'tectal weight' or 'midbrain section weight' are referred to without units of measurement, since they are derived from relative weights of paper cutouts.

Measurements of the longest diameter of subventricular nuclei were made from anonymous coded photomicrographs of embryonic tecta at × 1000. Two methods were used. Close to 1500 nuclei were measured with a Zeiss TGZ3 Particle Size Analyzer, and 950 nuclei were measured directly with a millimetre ruler from the photographs. There was close agreement in the results from both methods.

### *Nomenclature*

Since results in both embryonic and adult tecta are reported, it is necessary to clarify terminology. The teleost optic tectum during the first week of age, when the observations in embryos are reported, is not differentiated into the familiar adult layers. Consistent with the terminology agreed upon by the Boulder Committee (Angevine *et al.* 1970), it consists of a grey-matter zone occupying the inner (ventricular) half of each tectal lobe – equivalent to the ventricular and subventricular zones; a white-matter outer zone – equivalent to the intermediate and marginal zones; and a scattering of (marginal) cells,

relatively few in number in the white matter, thought to have migrated from the subventricular zone (Fig. 1).

In the adult teleost the optic tectum has separated from the underlying tegmentum and has differentiated into a number of recognizable layers. These have been described in detail by Cajal (1904), Edinger (1908), Kappers, Huber & Crosby (1936), Leghissa (1955), and by Wawrzyniak (1962) who thoroughly reviews the architectonics of the teleost tectum. This paper utilizes the nomenclature of Leghissa (1955) in which he distinguishes seven main layers. These are from the inner layer outward: I, stratum griseum periventriculare; II, stratum fibrium album profundum; III, stratum griseum centrale; IV, stratum plexiforme internum; V, stratum griseum externum; VI, stratum plexiforme and fibrium album externum; and VII, stratum fibrium marginale.

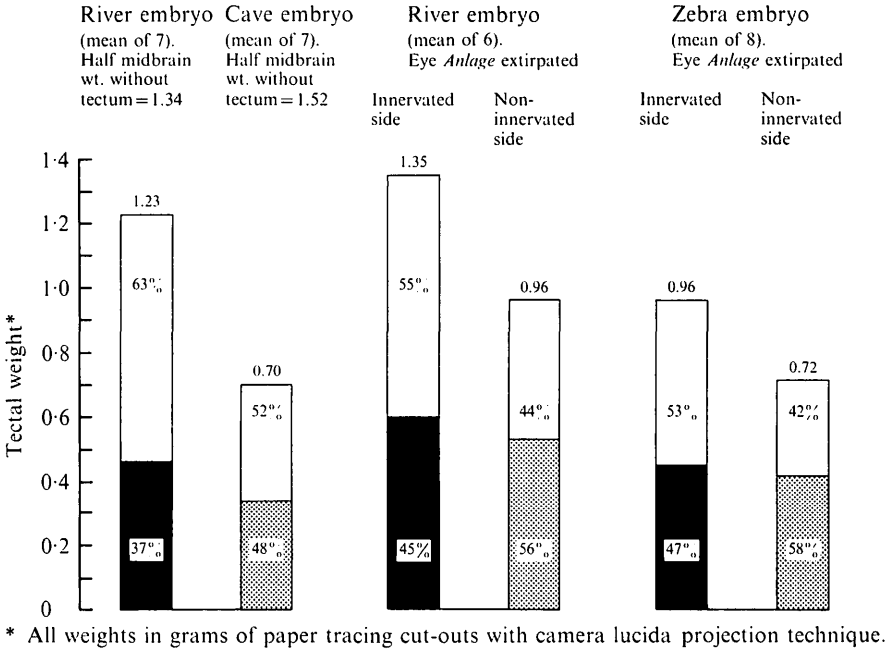
The periventricular layer (I) contains the greatest concentration of neurons in the tectum, which in Golgi preparations have long dendrites ascending as high as layers V and VI. It is into these layers that the optic afferent axons descend from the outer portion of layer VI, in which they run, to synapse with dendrites from the stratum periventriculare, and to a lesser degree with neurons in the central and external grey zones. Layer II consists chiefly of efferent fibres (tectofugal), largely from the periventricular neurons, but also in part from neurons in the central zones. Layers III and V contain pyramidal, fusiform and multipolar neurons acting as associative neurons thought to synapse with afferent optic, olfactory and gustatory fibres in the neuropil of layer IV.

## RESULTS

*Embryonic tectal development* – A. mexicanus (*river fish*) *versus* A. hubbsi (*cave fish*). Seven river fish and seven cave fish embryos, 1 week old, were studied (Fig. 1 A, B). The mean weight of the largest midbrain sections was 5·15 for the river fish embryos, and 4·45 for the cave fish embryos. The mean tectal weight (both tecta) was 2·47 in the river, and 1·40 in the cave embryos, so that the weight of the underlying (non-tectal) midbrain was 2·68 in the river embryos and 3·05 in the cave embryos, indicating that the cave fish embryonic tegmentum is not smaller.

In the river fish there was 63 % white matter, and 37 % grey matter in a 1·23 weight tectum; the cave embryo white matter was 52 %, and its grey matter 48 % of a 0·70 weight tectum (Fig. 2). The cave fish white matter was decreased by 53·3 %, and the grey matter by 26·1 %.

Measurements of nuclear size in the subventricular zone, and in the underlying tegmentum, were done in these two groups of embryos. Tectal nuclei in the cave fish appeared smaller than in the river embryos, and than nuclei in the tegmentum of both varieties. Actual measurements confirmed the visual impression (Fig. 3, columns 1, 2). The mean diameter of tectal nuclei in the river fish was $9{\cdot}60 \pm 0{\cdot}52$ S.D., as compared to $9{\cdot}85 \pm 0{\cdot}67$ for the underlying tegmental nuclei




* All weights in grams of paper tracing cut-outs with camera lucida projection technique.

Fig. 2. Relative amounts of tectal white and grey matter, at 1 week of age, in cave and river *Astyanax*, and in innervated versus non-innervated (one eye *Anlage* extirpated) embryonic tecta. □, White matter; ■, grey innervated; ▦, grey non-innervated.

– this difference of 2·5 % is not considered significant. The mean diameter of the tectal nuclei of the cave fish was 8·43 ± 0·45, and 9·84 ± 0·68 for its underlying tegmental nuclei – a decrease of 14·3 %; they were 12·2 % smaller when compared to the tectal nuclei in the river fish. Statistically the differences showed highly significant $P$ values less than 0·001.

*Adult river fish* (A. mexicanus) *versus adult cave fish* (A. hubbsi) – *tectal development and differentiation.* Brains of adult river and cave fish, over 1 year old, comparable in size (ca. 6 cm long), appear quite different grossly. The river fish has full round optic lobes meeting in the midline which are approximately twice as big as the olfactory lobes; the optic nerves are correspondingly large. The cave fish brain has small optic lobes, about one-half the volume of the optic lobes of the river fish, and about the same size as the olfactory lobes. A midline gap of 0·5 mm separates the two lobes; there are no optic nerves. In cross-section under low magnification the difference in size of the optic lobes is evident (Fig. 4A, B).

The river fish tectum has well-developed layers I, II, VI and VII; the central region, layers III, IV and V, is large, but since its neurons are more or less randomly dispersed, no distinct layers are seen (Fig. 5A). In the cave fish, layer II and the large central region (III, IV, V) are reduced by approximately 50 % in thickness, and layer VI is barely discernible (Fig. 5B).

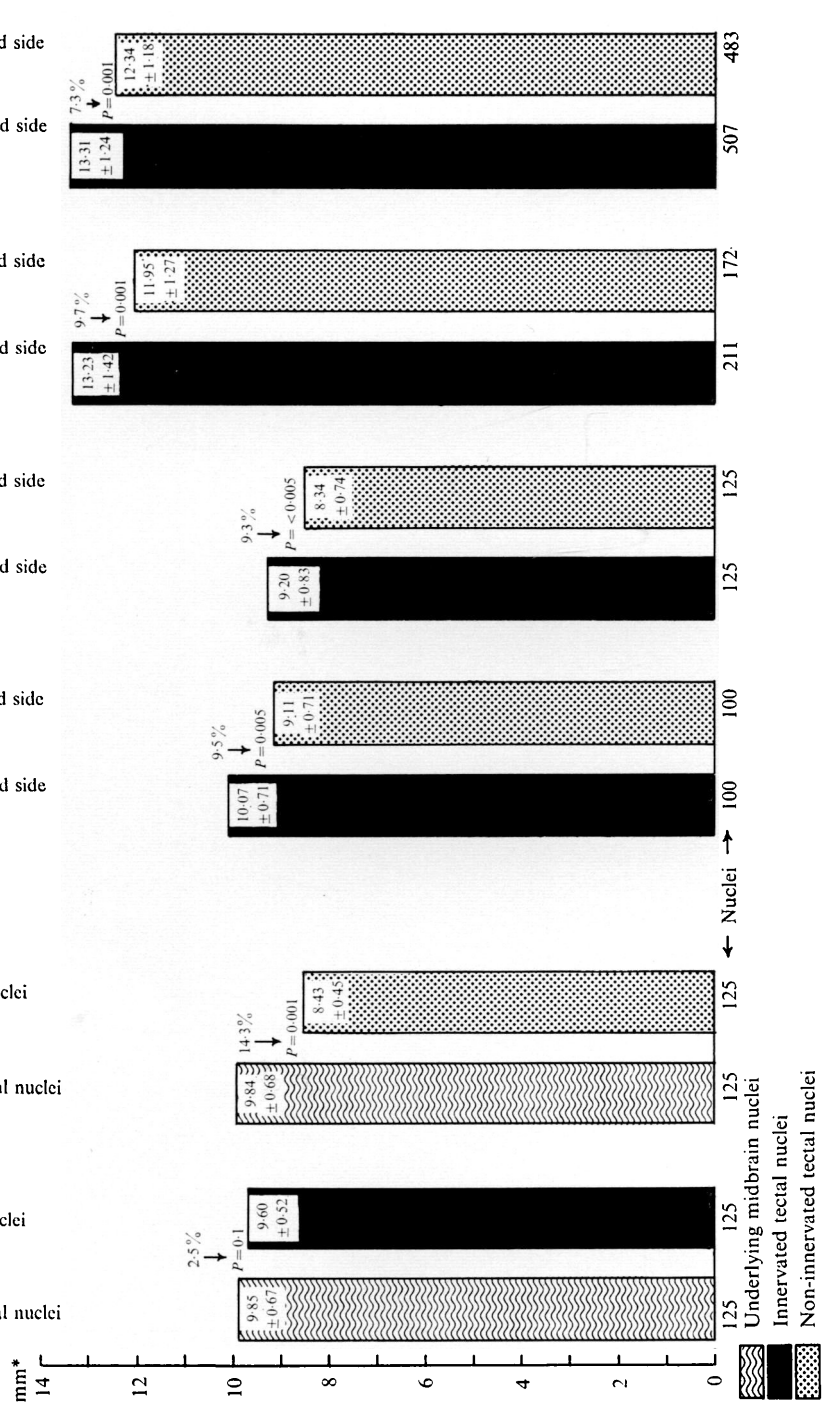
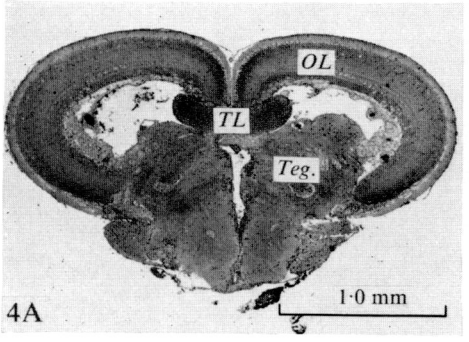
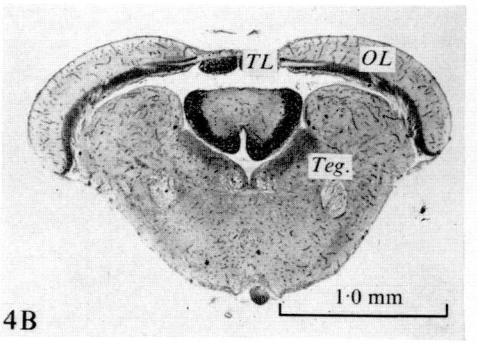
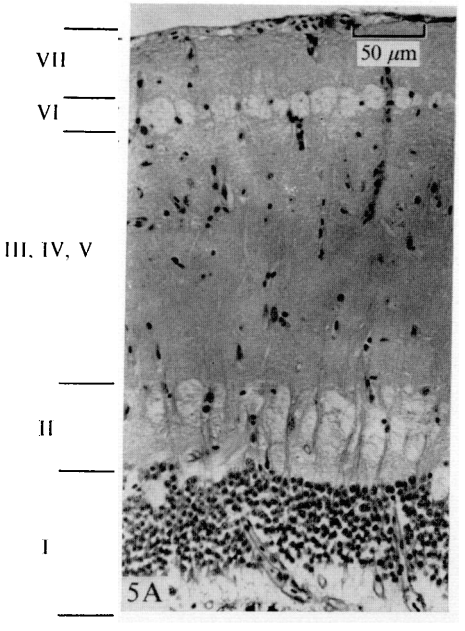



Fig. 3. Relative tectal subventricular cell nuclear size in cave and river *Astyanax* embryos at 1 week of age, and in innervated versus non-innervated tecta. * Relative sizes as measured from enlarged photomicrographs; † the sizes of these nuclei are somewhat greater since the measurements in mm were made with a Zeiss Particle Size Analyser TGZ 3 from enlargements made from × 1000 pictures of the cells. The preceding data were derived from direct measurement at × 1000 from Polaroid photographs.



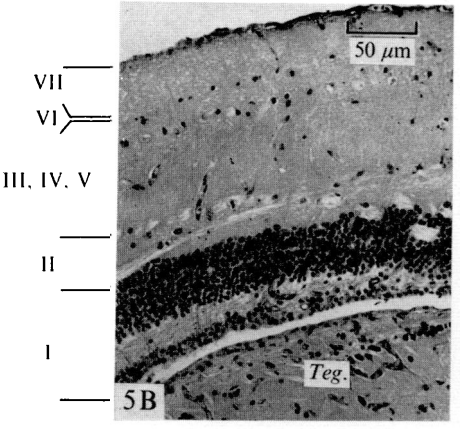




Fig. 4. (A) *Astyanax mexicanus* (river fish) adult. Cross-section through midtectum. The optic lobes (*OL*) are full and meet in the midline over the torus longitudinalis (*TL*); *Teg.*, tegmentum. Bodian silver. (B) *Astyanax hubbsi* (cave fish) adult. Cross-section through midtectum. The optic lobes (*OL*) are poorly developed, and leave a midline gap over the torus longitudinalis (*TL*); *Teg.*, tegmentum. Bodian silver.

Fig. 5. (A) River fish tectum enlarged to show subdivisions of layers. Layer I: stratum griseum periventriculare. Layer II: stratum fibrium album profundum. The three central layers (III, IV, V) are not distinctly delineated. Layer VI: stratum plexiforme and fibrium album externum. Layer VII: stratum marginale. Cresyl violet. (B) Cave fish tectum showing reduction in thickness, especially in layers III to VI. The cells in the stratum griseum periventriculare are smaller and more tightly packed. Cresyl violet.

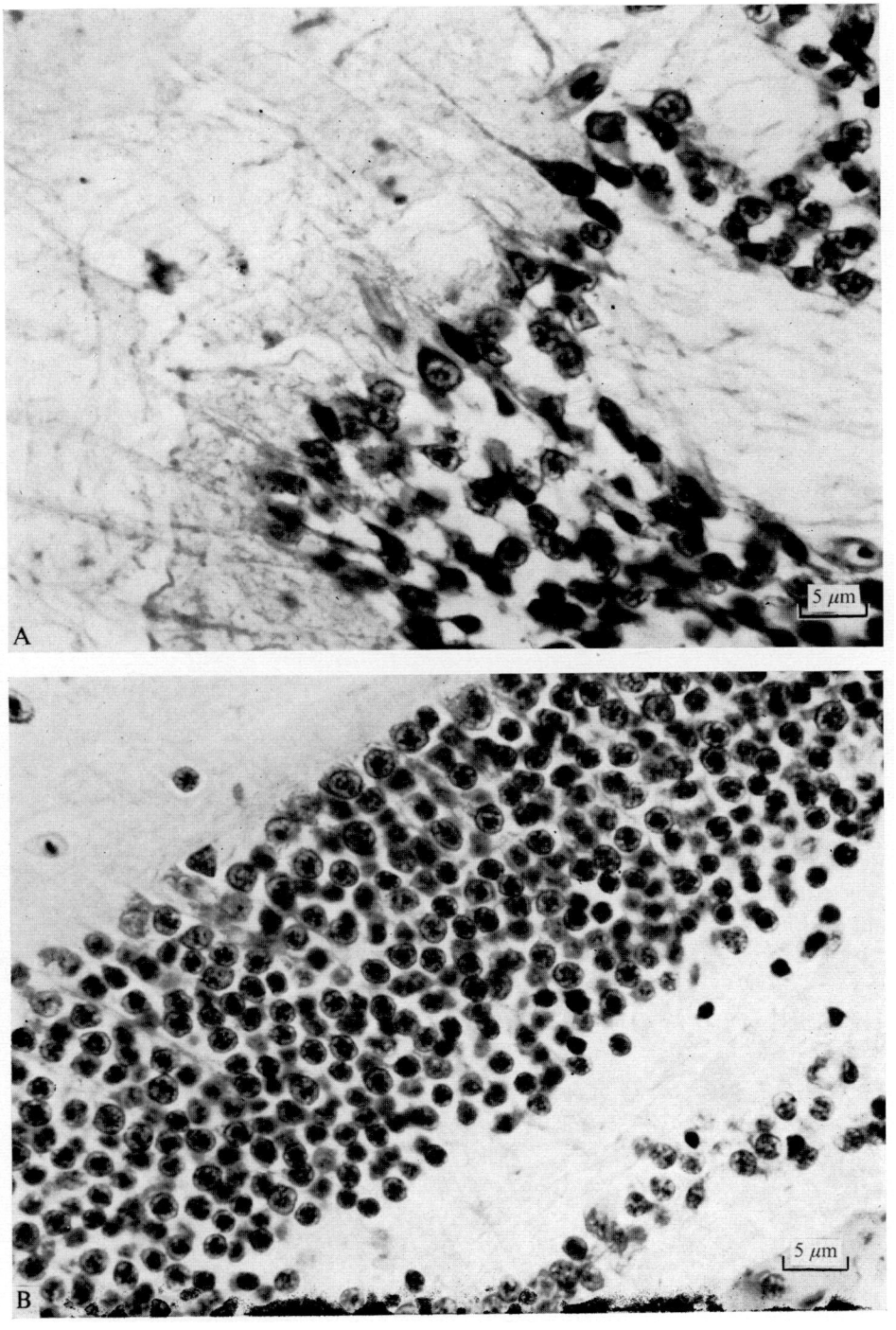



Fig. 6. (A) River fish adult, stratum periventriculare. The neurons are large, generally pyriform in shape, giving rise to many distinct fibres coursing to the more superficial tectal region (to the left). Cresyl violet. (B) Cave fish adult, stratum periventriculare. The nuclei are smaller, little neuronal differentiation is to be seen. The cells are compacted and uniform in appearance. Cresyl violet.

The stratum periventriculare (I) in the river fish (Fig. 6A) is densely populated with relatively small pyriform neurons polarized so that their tapering ends send long fibres perpendicularly to, in some cases, as high as layer VI; the vast majority of these fibres are lost in layers IV and V. Observation with phase-contrast microscopy of cresyl violet stained material shows that almost all of these neurons produce these long fibres. Layer II is richly endowed with bundles of fibres, probably the tectal efferents largely from the periventricular cells, running laterally and then downward through layer I. The neurons in the central region (III, IV, V) are larger than those in the periventricular layer, but are sparsely distributed. These are often bipolar with well-developed fibres; they generally tend to be polarized perpendicularly. Layer VI is well-developed, and large bundles of optic fibres can be seen coursing through it.

The stratum periventriculare of the cave fish also contains the greatest concentration of tectal neurons, but three distinct differences are noted in this layer: (1) the neurons are more tightly packed; (2) their nuclei are obviously smaller; and (3) the vast majority are small, round cells, only very few having a pyriform shape and fibre process (Fig. 6B). The overall thickness of this layer is, however, about equal to that of the river fish. This does not mean that this layer contains more cells, since the optic lobes of the cave fish are considerably smaller in circumference (compare Fig. 4A and B). Layer II is less distinct since there are smaller bundles of efferent fibres. Layers III, IV and V are reduced in thickness due to lack of fibres (apical dendrites) from the periventricular layer, absence of optic fibres, and less fibre production from smaller, less well-differentiated neurons in the central layers. Layer VI lacks the optic afferents, hence it is greatly decreased in thickness. There are a few fibres running in this layer, probably afferents from other sensory sources.

*One-eyed catfish versus normal* I. nebulosus (*Fig. 7*) – *differentiation of stratum periventriculare.* Cross-section through the optic lobes of the one-eyed catfish shows a deficiency in tectal development on the non-innervated side; its stratum periventriculare contains a large percentage of small round cells with deeply staining nuclei, with very few cells having well-developed fibres (Fig. 8A). In the innervated side these cells are distinctly larger, with many more pyriform shaped neurons having better developed perpendicularly oriented fibres ascending into the tectum; however, there are also a significant number of small round cells present, but much less than on the non-innervated side (Fig. 8B). In the stratum periventriculare of the normal catfish tectum, innervated by a much thicker optic nerve, the neurons are considerably larger, pyriform shaped with prominent fibres, and only an occasional small round cell present (Fig. 8C).

*Experimental results*

A. *Effect of light versus darkness on embryonic optic tectal development.* Two groups of five zebrafish embryos raised at 28 °C were analysed; one group was allowed to develop during normal light conditions in the laboratory, the other

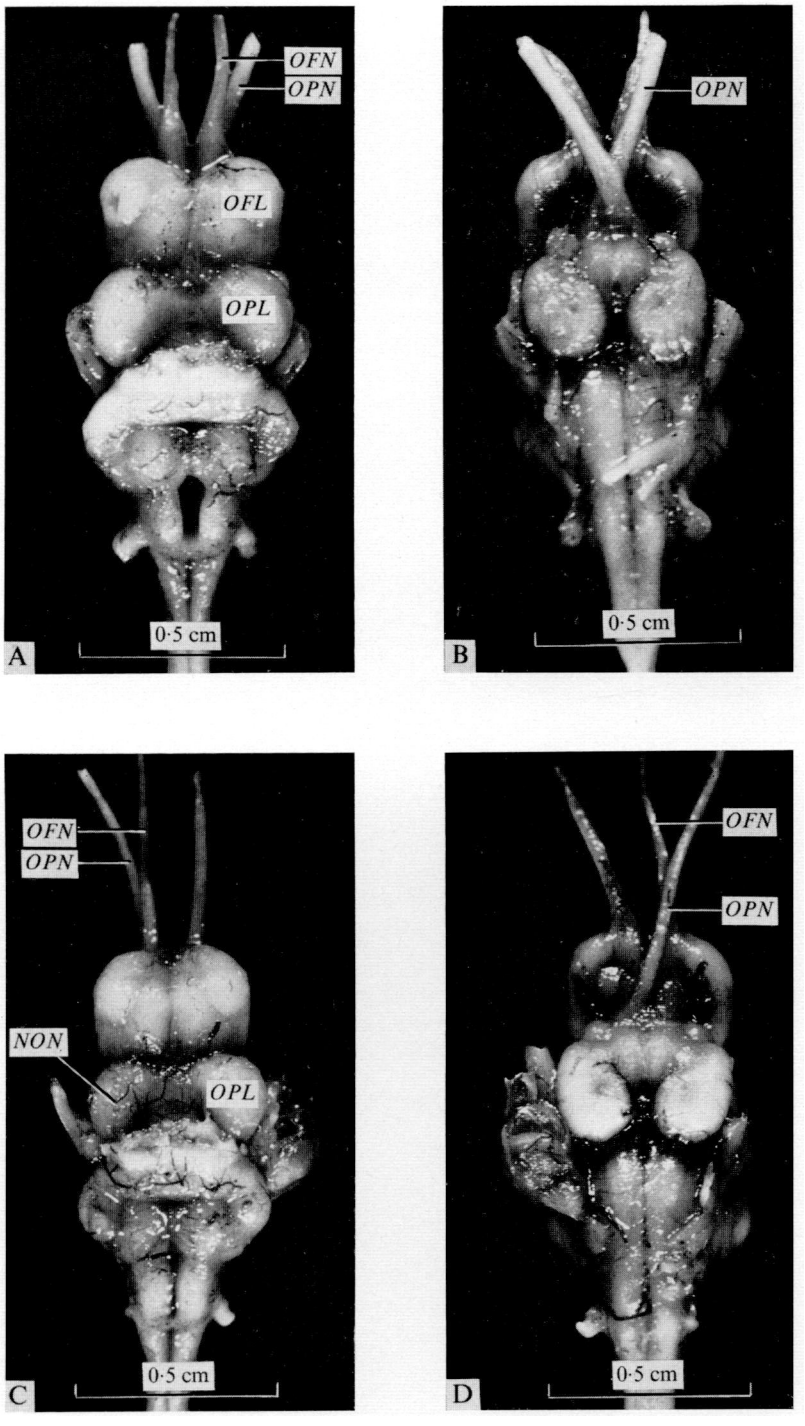
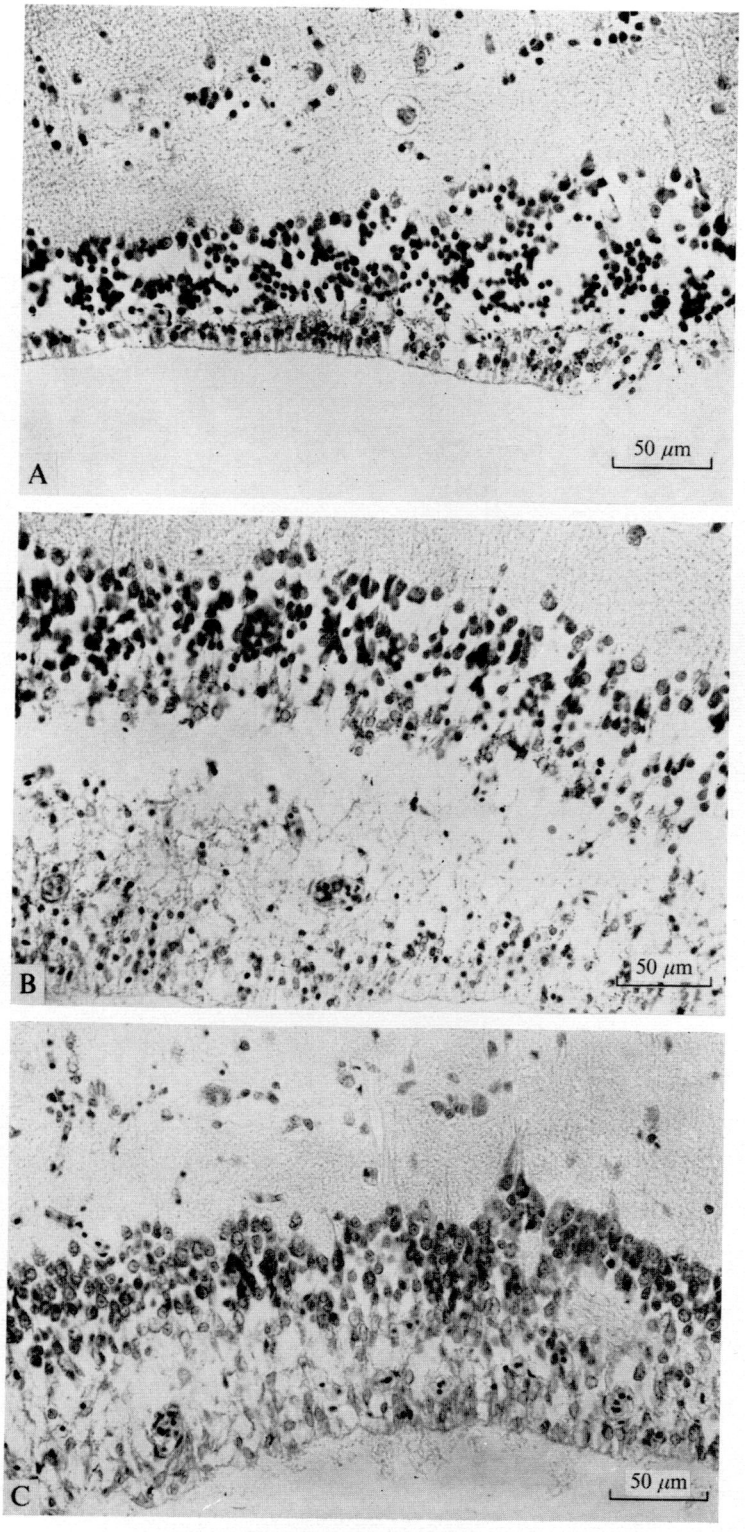



Fig. 7. (A) Normal catfish, *Ictalurus nebulosus*, dorsal view; tongue of cerebellum overlying optic lobes (*OPL*) is cut away. The optic nerves (*OPN*) are broader than the olfactory nerves (*OFN*); *OFL*, olfactory lobe. (B) Normal catfish, ventral view showing optic chiasma. (C) One-eyed Oregon catfish, *I. nebulosus*, dorsal view; the right optic nerve is missing, the left optic nerve (*OPN*) is no larger than the olfactory nerve (*OFN*). The left, non-innervated, optic lobe (*NON*) is slightly, but significantly, smaller than the right, innervated, optic lobe (*OPL*). (D) One-eyed Oregon catfish, ventral view, showing only one optic nerve at chiasma.


A
50 μm
B
50 μm
C
50 μm

kept in total darkness from minutes after fertilization until sacrifice at 7 days of age. In both groups each half tectum was measured as to weight of paper tracings of the combined white and grey matter, and for the white and grey matter separately. The mean values show insignificant differences between the two groups (Table 1 A).

Tectal nuclear size in the grey matter (subventricular zone) was also compared in the two groups. The mean diameters of the tectal nuclei were the same in both groups (Table 1 B).

B. *Optic cup extirpation in river* Astyanax. Optic cups were removed unilaterally in six embryos, before optic nerve development, at 18 h of age, and the embryos were sacrificed at 1 week of development (Fig. 9). The mean group weight of the innervated tectal sides was 1·35; the non-innervated sides weighed 0·96 (Fig. 2), a reduction of 27·9 % in the weight of the non-innervated side. The mean reduction in white matter was 41·1 %, and of grey matter was 10·2 %.

Tectal nuclei on the innervated side measured $10{\cdot}07 \pm 0{\cdot}71$ (range of $9{\cdot}25 \pm 0{\cdot}45$ to $10{\cdot}47 \pm 0{\cdot}84$), and in the non-innervated side measured $9{\cdot}11 \pm 0{\cdot}71$ (range of $8{\cdot}54 \pm 0{\cdot}72$ to $9{\cdot}55 \pm 0{\cdot}74$), representing a mean reduction of nuclear size of 9·5 % in the non-innervated side (Fig. 3, column 3). The spreads of the data show little overlap between the two groups with highly significant $P$ values.

C. *Optic cup extirpation in zebrafish.* In 72 h-old embryos, following unilateral extirpation of eye *Anlage* at 30 h of age, the non-innervated tectum is already smaller than the innervated. At this stage the normal tectum has been innervated by the optic nerve for about 28 h. With phase-contrast microscopy, fibres are seen arising from the grey matter of the innervated side to penetrate the superficial layer of the tectum; these fibres are absent on the non-innervated side. The origin of these fibres was difficult to demonstrate in the very compact mass of subventricular zone cells. Separating the cells by shrinkage allowed for their visualization. This was done by prolonged exposure (72 h) of the sections to 100 % alcohol during dehydration. With this method, and stained with cresyl violet or basal fuchsin, many of the cells on the innervated side had a pyriform shape, were polarized so that their long axes were perpendicular to the white tectal border, and formed distinct fibres heading toward it. On the non-innervated side there was no polarization to be seen; the cells were round, oval or irregularly shrunken, and did not have distinct fibre processes (Fig. 10 A, B, C).

In eight zebrafish embryos that were similarly operated upon and examined

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Fig. 8. (A) One-eyed adult catfish, *Ictalurus nebulosus*, stratum periventriculare of non-innervated side. Many small, round, dark staining undifferentiated cells are present. Cresyl violet. (B) One-eyed adult catfish, stratum periventriculare of innervated side. The neurons are larger and more differentiated with fewer small, round, dark staining cells present. Cresyl violet. (C) Normal two-eyed catfish, stratum periventriculare. The neurons are considerably larger with well developed fibre processes to be seen. There are no small, dark, undifferentiated cells present. Cresyl violet.

Table 1 A. *Comparison of effects of light versus darkness on relative amounts of white and grey matter in embryonic zebrafish tecta**

| Development in light | | | | | | | Development in dark | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spec. no. | Weight† each tectum | | Weight grey matter each tectum | | Weight white matter each tectum | | Spec. no. | Weight each tectum | | Weight grey matter each tectum | | Weight white matter each tectum | |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) |
| 304 | 0·60 | 0·48 | 0·38 | 0·26 | 0·22 | 0·22 | 297 | 0·43 | 0·51 | 0·14 | 0·20 | 0·29 | 0·31 |
| 300 | 0·68 | 0·58 | 0·33 | 0·27 | 0·35 | 0·31 | 294 | 0·56 | 0·51 | 0·25 | 0·27 | 0·31 | 0·24 |
| 303 | 0·37 | 0·46 | 0·19 | 0·25 | 0·18 | 0·21 | 296 | 0·49 | 0·52 | 0·27 | 0·30 | 0·22 | 0·22 |
| 299 | 0·50 | 0·50 | 0·30 | 0·29 | 0·20 | 0·21 | 298 | 0·61 | 0·52 | 0·40 | 0·31 | 0·21 | 0·21 |
| 302 | 0·63 | 0·65 | 0·37 | 0·40 | 0·26 | 0·25 | 295 | 0·72 | 0·70 | 0·46 | 0·46 | 0·26 | 0·24 |
| Mean | 0·55 | 0·53 | 0·31 | 0·29 | 0·24 | 0·24 | Mean | 0·56 | 0·55 | 0·30 | 0·31 | 0·26 | 0·24 |
| | Weight both tecta (15) | | Weight grey matter (16) | | Weight white matter (17) | | | Weight both tecta (18) | | Weight grey matter (19) | | Weight white matter (20) | |
| | 1·08 | | 0·60 (55 %) | | 0·48 (45 %) | | | 1·11 | | 0·61 (55 %) | | 0·50 (45 %) | |

* All larvae killed at 7 days of development. † Weight in grams of paper tracing.

Table 1 B. *Effects of light versus darkness on nuclear diameter of subventricular neurons*

| Mean diameter of 18 tectal nuclei | | | |
|---|---|---|---|
| Spec. no. | Raised to 7 days in light | Spec. no. | Raised to 7 days in dark |
| 299 | 9·20 | 295 | 8·78 |
| 304 | 8·87 | 297 | 9·76 |
| 300 | 9·72 | 296 | 9·66 |
| 303 | 9·40 | 294 | 9·51 |
| 302 | 8·93 | 298 | 8·78 |
| Mean | 9·22 (90 cells) | Mean | 9·22 (90 cells) |

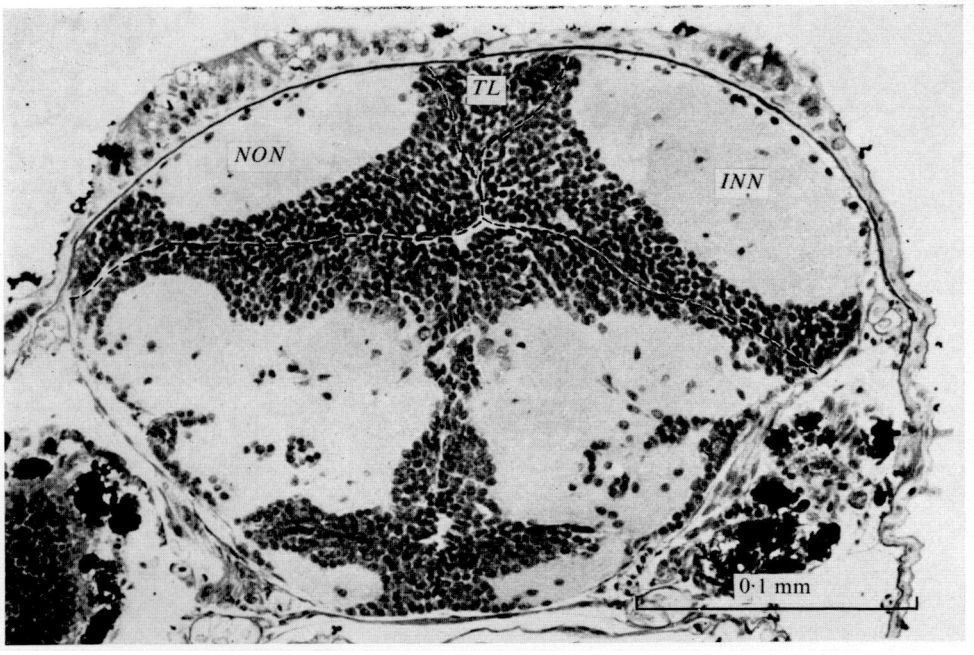
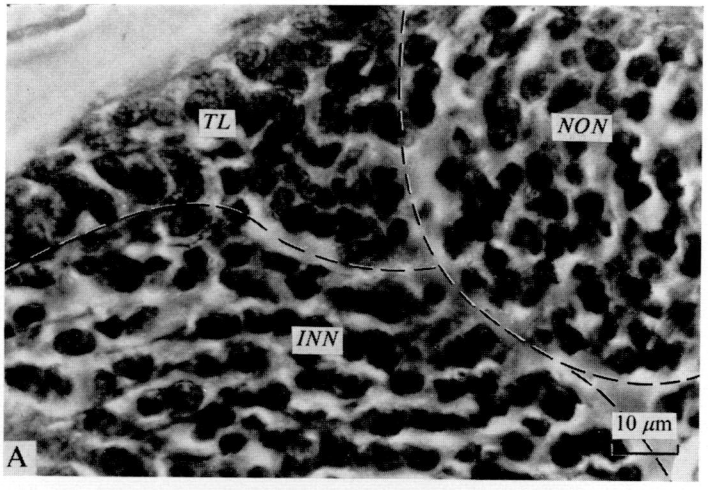


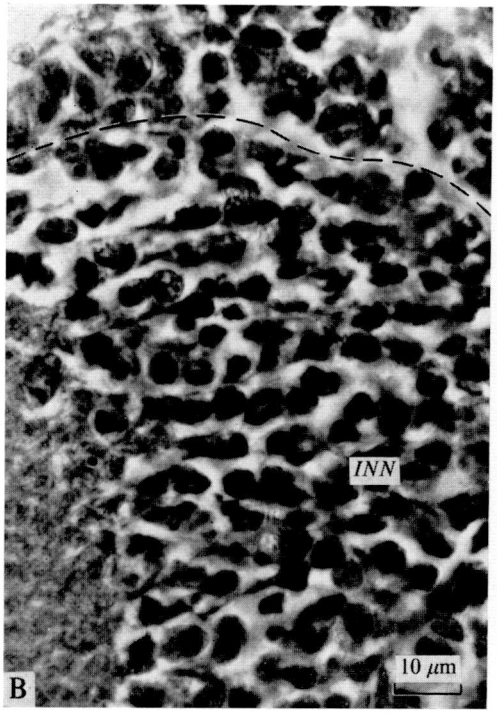

Fig. 9. Seven-day-old *Astyanax mexicanus* embryo, cross-section through midbrain. Right eye *Anlage* extirpated at 18 h of age. The non-innervated left tectum (*NON*) is significantly smaller than the innervated right tectum (*INN*). *TL*, torus longitudinalis. Haidenhain's haematoxylin.

at 1 week, the mean tectal weight was 0·96 on the innervated side, and 0·72 on the non-innervated side (Fig. 2); a reduction of 23·8 % in non-innervated tectal size. The white matter of the non-innervated tectum was reduced by 39·2 %, and the grey matter by 6·8 %.

The mean nuclear diameter of the subventricular cells in the innervated tectal side was $9{\cdot}20 \pm 0{\cdot}83$ ($8{\cdot}76 \pm 0{\cdot}77$ to $9{\cdot}76 \pm 0{\cdot}77$), and in the non-innervated side it was $8{\cdot}34 \pm 0{\cdot}74$ ($8{\cdot}07 \pm 0{\cdot}59$ to $8{\cdot}72 \pm 0{\cdot}75$); a decrease of 9·3 % in the size of the non-innervated tectal nuclei — there was no overlap in the means of the two groups, with highly significant $P$ values (Fig. 3, column 4).

Seven 4- to 6-week-old one-eyed larvae (eye *Anlage* removed at 30 h) were studied for later effects. At this age adult type layer stratification becomes apparent. The non-innervated side is smaller than the innervated side. Layers II through VI are decreased in thickness and fibre content, and dendrite outgrowth from the periventricular layer is reduced. Nuclear size differences persisted at this age. In a 4-week-old larva, the mean nuclear diameter in the stratum periventriculare was $13{\cdot}23 \pm 1{\cdot}42$ on the innervated side, and on the non-innervated side there was a decrease in size of 9·7 % to $11{\cdot}95 \pm 1{\cdot}27$. In a 6-week-old larva, nuclei in the stratum periventriculare on the innervated side measured $13{\cdot}31 \pm 1{\cdot}24$, and on the non-innervated side measured $12{\cdot}34 \pm 1{\cdot}18$, a decrease of 7·3 % (Fig. 3).



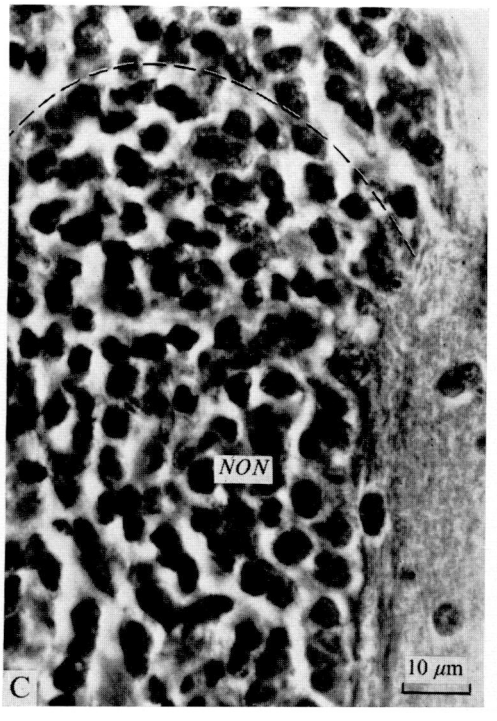




Fig. 10. (A) Zebrafish embryonic tectum, 72 h of development. One eye *Anlage* removed at 30 h of age. *INN*, innervated tectal side; *NON*, non-innervated side, corresponding to the absent optic nerve; *TL*, torus longitudinalis. The neurons in the innervated side have distinct fibre processes, and are polarized in the direction of the tectal white matter. The cells in the non-innervated side do not show the development of fibres, and are not polarized. Basal fuchsin, Heine phase-contrast. (B) Innervated side as above. Tectal white matter to the left. (C) Non-innervated side as above. Tectal white matter to the right.

In a 6-month-old fish whose eye *Anlage* was removed, the non-innervated tectum was underdeveloped, paralleling the findings in the cave fish and non-innervated catfish tectum. The hypoplastic side was considerably shrunken back from the midline leaving the torus longitudinalis uncovered.

## DISCUSSION

The findings in this study demonstrate that, in the absence of optic innervation, incomplete tectal development results largely from a deficiency in differentiation of the neurons of the stratum griseum periventriculare. In the cave fish embryos, and in the non-innervated tecta of river fish and zebrafish embryos, the subventricular neurons manifest failure in differentiation by a decrease in nuclear size and fibre production, with an associated reduction in the amount of tectal white matter. In the larval and adult fish the consequences of the failure of differentiation of the periventricular neurons are more obvious. The failure of apical dendrite and efferent fibre development of the periventricular neurons can be seen to result in a decrease in size and complexity of layers II through V. Lack of optic innervation also contributes to a deficiency in layers IV, V and VI due to the absence of optic fibres in these layers.

Non-innervated tecta of embryonic river fish and embryonic tecta of cave fish were compared to tecta of normal river fish embryos. There was 41·1 % less white matter in the non-innervated tecta of river fish, and 49·5 % less white matter in the cave fish tecta. The nuclei of the subventricular cells in the non-innervated river fish tecta were 9·5 % smaller than in the normal river fish embryo. In the cave fish embryo the subventricular cell nuclei were 12·2 % smaller. While the findings in the non-innervated river fish and cave fish tecta are roughly comparable, the 'natural denervate' is somewhat more hypoplastic. This may represent the beginning of mutation taking place in the genome of the cave fish governing tectal cell differentiation.

The findings in the non-innervated catfish tectum closely parallel those in the cave fish. The lack of development of the cells of the periventricular layer is obvious, and the hypoplasia of the superficial layers is similar to that of the cave fish. Observations in the catfish are of further interest in that they add another parameter for the evaluation of the dependence of differentiation on optic afferent ingrowth, i.e. 'dosage dependence'. The one optic nerve of the Oregon catfish had fewer optic neurons than normal, since its retina was considerably underdeveloped with fewer than normal ganglion cells (N. Peters, Hamburg, personal communication). The tectum innervated by this nerve was more differentiated than the non-innervated side, but not as well differentiated as the normal catfish tectum innervated by a large optic nerve from a well-developed retina – hence the more optic afferent fibres, the better the differentiation of the periventricular layer.

Hamburger (1955) questions how the inflow of optic fibres at the superficial

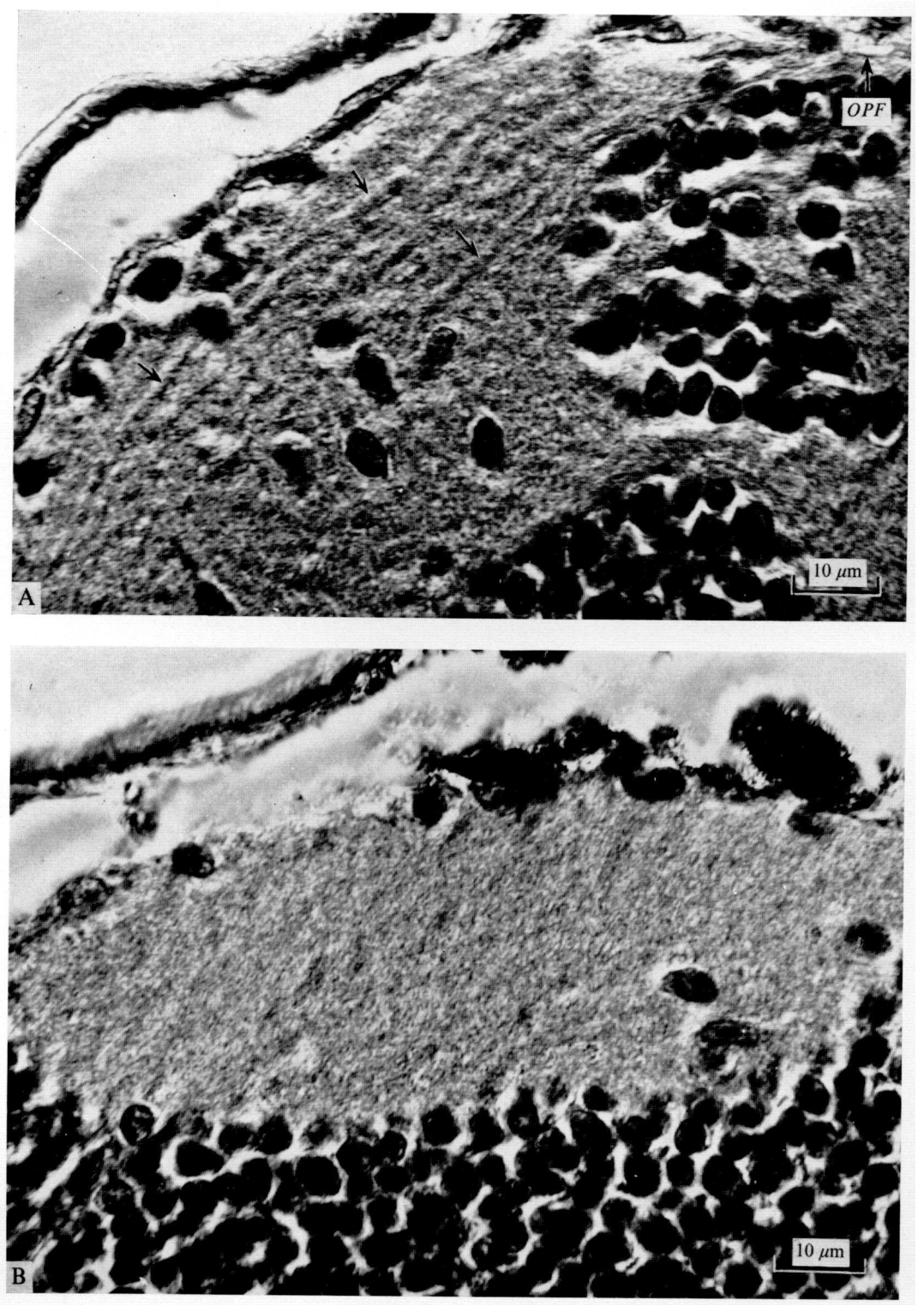



Fig. 11. (A) 72 h old zebrafish embryonic tectum, innervated side, optic fibres (*OPF*) entering at lateral tectal corner, and seen coursing as bundles (at arrows) in the outer half of the white matter. Haidenhain's haematoxylin, Heine phase-contrast. (B) 72 h old zebrafish embryonic tectum, non-innervated side. Optic *Anlage* removed from contralateral side at 30 h of age. The optic bundles seen in (A) are absent.

layer of the optic tectum controls development in the deepest layer. This point is answerable by looking to the early embryo. Differentiation of subventricular cells, 28 h after optic nerve ingrowth, as compared to the lack of differentiation on the non-innervated side, demonstrates very early developmental optic afferent interaction with these cells. In the 3-day-old embryo, the afferent optic fibres course through the marginal tectal zone in close proximity to the subventricular zone (Fig. 11). Contact between these fibres and the underlying cells is easily comprehensible. Then, as growth occurs, dendrite prolongation with attached optic axon synapses shifts the synaptic zone to more superficial tectal layers. Later ingrowing optic fibres, following the pioneering fibres, can synapse with the elongated dendrites in the outer zones.

Angevine (1970), noting that differentiation of neurons in the diencephalon correlates with afferent axon ingrowth, speculates that ingrowth of axons might induce differentiation. The finding in this study that axonal ingrowth is a prerequisite for further differentiation of the cells of the subventricular zone lends credence to these speculations.

Szentágothai & Hámori (1969) indicate that the dendritic growth and differentiation processes of a post-synaptic neuron are more dependent upon orderly connexions than the primary afferent neuron whose axons appear to be driven by intrinsic goal-directed programmes. This is consistent with the observations reported in this study. The optic afferent axons appear goal-directed, and the subventricular cells dependent for differentiation upon their connexions.

In the zebrafish raised in the dark grossly normal tecta (under the light microscope) developed in the absence of light stimulation (see also Bondy & Margolis, 1969), indicating that functioning connexions are not necessary to stimulate differentiation. However, bioelectric activity may be present in the retino-tectal fibres despite the absence of light stimulation. Crain, Peterson & Bornstein (1968) blocked bioelectric activity with Xyolcaine in foetal rat spinal cord and neocortex tissue cultures, and showed that synapse formation and neuron differentiation occurred nevertheless. Goodman (1932), Gyllensten, Malmfors & Norrlin (1965), Valverde (1967, 1968), Szentágothai & Hámori (1969), Fifková & Hassler (1969), Fifková (1970), Shapiro & Vukovich (1970) and others have shown that function does produce an increment of maturation by demonstrating that visual deprivation results in a lack of development of dendritic spines in the neurons of the visual centres. However, at least in centres dependent upon peripheral innervation, it appears that function is unnecessary for the gross differentiation of the central neuroblast to synapse formation and the production of a mature neuron.

The mechanism by which the optic afferent fibre stimulates differentiation of the subventricular neuron remains unknown. Recent work offers some leads for attacking this problem. Grobstein (1967) suggests that surface associated macromolecules (glycoproteins) may be functioning as inducing substances. There may be actual transport of substances across the interneuronal membranes

(Payton, Bennett & Pappas, 1969) to produce and maintain differentiation. Axoplasmic transport (Barondes, 1970) may have a developmental function in the embryonic nervous system in making specific macromolecules produced by the pericaryon available at the membrane of the growth cone.

Interaction between nerve cells appears of primary importance in neurogenesis as shown by the dependence of central development on peripheral innervation. In the optic tectum interaction begins with the establishment of a relationship between an ingrowing growth cone of an afferent optic neuron and a (specific?) tectal neuroblast, thought to occur by contact affinity. After the initial contact is made, there is seemingly a further interaction stimulating differentiation. Synaptic transmission may be necessary for further maturation to occur. And lastly, continued interaction may be concerned with the maintenance of the differentiated state.

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