The development of the retino-tectal projection in *Xenopus laevis*: an autoradiographic and degeneration study

By T. M. SCOTT

From the Division of Developmental Biology, National Institute for Medical Research, Mill Hill

**SUMMARY**

The development of the retino-tectal projection in *Xenopus laevis* was studied using degeneration and autoradiographic techniques. At stage 47 only the rostral pole of the tectum receives optic fibres. By stage 50 half of the tectum is innervated and by metamorphosis all of the tectum except for narrow caudal and medial strips contains optic fibre terminals.

**INTRODUCTION**

Although the existence of sets of neurons which connect in a specific topographical fashion is well documented, the way in which these ordered connexions develop has received little attention. The lack of data on the development of these connexions can in part be attributed to the difficulty of studying these systems.

The retino-tectal system in amphibians is a convenient system in which to study the formation of connexions, in that the embryos develop over a period of weeks rather than days, allowing a more detailed study of development. In addition, the electrophysiology and morphology of the adult retino-tectal system in amphibians has been well described (see Gaze, 1970).

This study has set out to show how the outgrowing axons of retinal ganglion cells innervate the developing tectum in *Xenopus laevis*.

**MATERIALS AND METHODS**

**Degeneration**

In a previous study of amphibian optic terminals it was found that after eye removal, degenerating fibres and terminals could be seen in 1 μm plastic sections of optic tectum stained with toluidine blue (Scott, 1973). This technique has been applied to developing *Xenopus*.

1 *Author's address:* Department of Anatomy, London Hospital Medical College, Turner Street, London E1, U.K.
The left eye was removed from tadpoles at the following stages of development (Nieuwkoop & Faber, 1956): 45–53 inclusive, 55, 59, 62 and 64. The tadpoles were then kept alive for periods between 6 h and 10 days. The left eye was also removed from three adult *Xenopus* which were kept alive for 10 days. Whole brains were then removed from the tadpoles and adults and immersed for 20 min in a 4 % solution of formaldehyde in Millonig's buffer at 4 °C. Brains were transferred to a 1 % solution of osmium tetroxide in Millonig's buffer at 4 °C for a further hour. After fixation, dehydration was carried out through a graded series of alcohols followed by embedding in Araldite. Sections of 1 μm were cut at intervals of 25 μm through each brain in transverse, horizontal or parasaggital planes on an LKB ultrotome III. After mounting on glass slides, sections were stained in 1 % toluidine blue. Thin sections were also cut for electron microscopic observation.

**Autoradiography**

The technique used was that of intra-ocular injection of a labelled amino acid. Tritiated proline (New England Nuclear), 0.28 μl, specific activity 39.7 Ci/m mole, was injected by micropipette into the left eye of tadpoles at stages 48, 50, 52, 53 and 57; 2.8 μl of tritiated proline was injected into the left eye of three adult *Xenopus*. Previous studies using scintillation counting had established the rate of transport of labelled proline from the eye to the tectum in developing and adult tadpoles (G. Lazar & T. M. Scott, unpublished). Animals were killed about the peak of the slow phase of axonal transport, which was about 4 days in the case of tadpoles and adults.

Tadpole brains were fixed and cut as for degeneration. Adult brains were fixed in a similar way but embedded in paraffin wax and cut at 7 μm. Slides were dipped in Ilford L 4 emulsion and kept in the dark at 4 °C for 5 weeks in the case of tadpoles and 3 weeks in the case of adults. Slides were developed in Ilford ID 19 developer for 4 min, fixed in Hypam for 3 min and washed in tap-water for 20 min. One μm sections were stained in toluidine blue, 7 μm sections were stained in haemotoxylin and eosin.

**RESULTS**

**Degeneration in tadpoles**

Degeneration was found to begin by about 12 h, to reach a peak about 3 days and to decline from 6 to 10 days. At each stage, animals from different degeneration times were used to construct a degeneration map for that stage. The presence of degenerating terminals was confirmed at each stage by electron microscopy. 

**Stage 45–48**

At stage 45 both tecta and many other parts of the brain showed signs of degeneration, whether the eye had been removed or not. It was found to be impossible at this stage to say which degenerating terminals in the tectum contra-
lateral to eye removal were due to enucleation. By stage 47, however, the rate of natural degeneration in the tectum had decreased, and it was possible to distinguish degeneration due to enucleation. At this stage the tectum is about 350 μm in length. Degeneration can be found in the most rostral 100 μm of the tectum extending almost to the lateral edge and fairly close to the mid-line. The number of degenerating profiles observed was small compared with later stages. In animals estimated to be stage 48, terminal degeneration appeared to occupy the tectum to about the same extent as at stage 47. Degenerating terminals were few and found only in the most rostral part, forming a layer from about 30 μm deep to the surface to a depth of 70 μm.

**Stage 49–52**

Between these stages an increase in the number of degenerating terminals takes place with an associated increase in tectal coverage. Tectal coverage begins to catch up with tectal growth and by stage 50 half of the tectum receives optic innervation (Fig. 1a). The terminals appear to be more dense and in clusters. They are spread about in a layer about 45 μm thick. Degeneration is almost never found deeper than this layer. Degenerating particles in a stage-52 tadpole are shown in Fig. 2A. By stage 52 the tectal coverage has increased to two-thirds (Fig. 1b).
Fig. 2. (A) Part of a transverse section of a stage-52 tectum, 2 days after eye removal. Degenerating particles can be seen as dense dots.

(B) A transverse section through the caudal pole of a stage-53 tectum. No degenerating particles are visible.

(C) Part of a transverse section through an adult tectum, 10 days after eye removal. Degenerating particles can be seen as small black dots.

(D) An electronmicrograph of a degenerating optic terminal (T) in the tectum of a stage-52 tadpole, 16 h after eye removal. The terminal contains vesicles and dense material and is still attached to its post-synaptic element.

(E) An electronmicrograph of a degenerating terminal (T) being engulfed by a glial cell process together with its post-synaptic element. Stage-53 tadpole, 1 day after eye removal.

(F) Part of a transverse section through the tectum of a stage-53 tadpole. The left eye had been injected with tritiated proline. Exposed grains can be seen as a band just in from the surface of the tectum.
Stage 53–55

Between these stages there is a marked increase in tectal growth and in tectal coverage (Fig. 1c). The tectum increases to a length of about 1300 μm, with degenerating terminals being found in almost all areas except for narrow caudal and medial strips. The caudal part of the tectum is shown in Fig. 2B. The outer layer has many cells but no optic innervation.

Stage 56–59

The tectum continues to grow, with optic terminals being present in all areas except for narrow caudal and medial strips. Between stage 59 and metamorphosis, tectal growth continues but at a much reduced rate. Tectal coverage at stage 59 is shown diagrammatically in Fig. 1d.

Degeneration in adults

Tectal coverage in the adult *Xenopus* is similar to that at metamorphosis in that there are narrow caudal and medial strips without optic terminals. Degeneration of optic terminals is shown in Fig. 2C as dense dots in layers 9 and 8.

Endpoint degeneration

The presence of degeneration terminals after eye removal was checked at each tadpole stage by electron microscopy. For the purpose of this paper it was sufficient to establish whether synapses were being formed by optic axons or not. Further observations on optic axon synaptogenesis are being prepared for publication. Degenerating optic synapses could be distinguished from about stage 47 onwards. A degenerating optic terminal is shown in Fig. 2D at 16 h after eye enucleation. A degenerating terminal is shown being engulfed by glia in Fig. 2E.

Autoradiography

Unlike degeneration, autoradiography could not be used to demonstrate terminals alone but to show which part of the tectum received optic axons. The results of autoradiography closely resembled those of degeneration. A typical result is shown in Fig. 2F, where grains can be seen in a band just in from the surface in a situation similar to the dots in a degenerated tectum.

Discussion

The way in which the tectum becomes innervated by optic fibres in developing *Xenopus* resembles other waves of development. Tectal cell division occurs during development in antero-posterior and latero-medial directions (Straznicky & Gaze, 1971). Differentiation of tectal cells also takes place in this fashion (Lázár, 1973).

It has not been possible from this study to say how optic innervation of the
tectum influences the differentiation of tectal cells, but it was noted that optic terminals were absent from areas which showed only the beginnings of cell differentiation.

The results of this study follow closely the results of a physiological study by Gaze, Chung & Keating (1972). Areas which were electrophysiologically silent were found to have no optic innervation. Areas which may have been expected to contain larger numbers of optic terminals than others could not be distinguished due to the variation in section thickness.

I would like to thank Dr R. M. Gaze and Dr Gy. Lázár for their assistance with this work.

REFERENCES


(Received 4 September 1973)