The relationship between retinal and tectal growth in larval Xenopus: implications for the development of the retino-tectal projection

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SUMMARY

The modes of retinal and tectal histogenesis, as well as the patterns of terminal distribution of optic axons in larval Xenopus were studied, using anatomical techniques. We show that the retina grows by adding strips of cells at its ciliary margin. The pattern of retinal growth is asymmetrical along the dorso-ventral axis of the retina. On the other hand, the tectum grows by adding newly formed cells caudo-medially. The most rapid tectal growth takes place between stages 50 and 53, and thereafter only a small proportion of cells are added near the midline of the caudal tectum. Despite such incongruent modes of retinal and tectal growth, retinotopic order is maintained throughout larval life.

We present here further evidence supporting the idea that connexions between the arrays of retinal and tectal cells shift progressively caudo-medially on the tectum during the period of growth. When the temporal pole of the retina is destroyed at various developmental stages, the density of degenerating synapses is always highest in the rostral pole of the tectum. Moreover, optic terminals stemming from the central retina spread caudally, invading newly maturing regions of the tectum. Quantitative analysis of the terminal distributions of optic axons suggest that orderly shifts in synaptic contacts between optic axons and tectal dendrites take place in the course of development. Ultrastructural observations on the maturing tectal neuropil are consistent with this view.

INTRODUCTION

Several recent studies on the peripheral and central nervous system have revealed that growing axons form transitory synapses, which are either eliminated or rendered functionally ineffective as development proceeds (Redfern, 1970; Gentschev & Sotelo, 1973; Hubel, Wiesel & LeVay, 1977; Rakic, 1977; Wall, 1977). These transitory synapses are believed to be the product of fail-safe developmental mechanisms which generate an initial excess of synaptic contacts. A further mechanism selects from these diffuse connexions a subset to be preserved as the adult pattern. In the amphibian tectum, however, the formation of...
neuronal circuitry appears to involve not only the elimination of excessive synapses, but also a gradual and ordered shift of connexions between retinal axons and tectal cells.

Evidence for this is based on several observations. Throughout larval life in *Xenopus*, the retina adds cells to its ciliary margin (Straznicky & Gaze, 1971) so that the retina grows by the peripheral accretion of rings of neurons. The tectum, on the other hand, grows in a curvilinear fashion by the addition of cells to its caudo-medial border (Straznicky & Gaze, 1972). Despite these incongruent modes of growth, the retina establishes synaptic connexions with the tectum early in the development of both structures (Chung, Keating & Bliss, 1974). Electrophysiological mapping revealed that, from stage 50 of larval life onwards, retinal terminals were arranged in an ordered fashion across the tectum (Gaze, Chung & Keating, 1972; Gaze, Keating & Chung, 1974). These facts led us to conclude that the early synaptic contacts are not permanent and that ordered adjustments of connexions take place throughout larval life.

Recently Jacobson (1976, 1977) has claimed that retinal histogenesis in *Xenopus* follows the same pattern as tectal histogenesis and that newly formed retinal ganglion cells connect to their newly formed counterparts in the tectum. If so, it is unnecessary to postulate a continual adjustment of synaptic relations in the growing visual system of *Xenopus*. We have therefore re-investigated the modes of retinal and tectal histogenesis and made further observations on the retino-tectal projection during development. This work has involved extensive use of the double-labelling technique devised in this laboratory by Scott & Lázár (1976). The results of the autoradiographic and electron microscopic studies reported here substantiate our earlier conclusions.

**METHODS**

(a) Materials

The data presented in this paper derive from studies on the visual system of 220 larval *Xenopus* between stages 50 and 66 (Nieuwkoop & Faber 1967). We also made use of some histological materials on larval *Xenopus* from stages 32 to 47 which were prepared for experimental series that have been reported previously (Straznicky & Gaze, 1971, 1972; Gaze et al. 1974).

(b) Autoradiography

Pulses of [³H]thymidine (10 µCi; specific activity: 15 Ci mm⁻¹) were injected intraperitoneally in 151 larval *Xenopus*. In 60 of these animals, a second, and sometimes a third, pulse of [³H]thymidine was administered at 10-day intervals after the first pulse. Sixty-two animals also received an intraocular injection of 2–6 µCi of [³H]proline (specific activity: 43 Ci mm⁻¹) 24 h before being killed. Autoradiographic sections were prepared according to standard procedure. The brains were embedded in paraffin wax and serial sections, cut at 15 µm in
the transverse, parasagittal or horizontal planes, were mounted on slides. The sections were coated with AR-10 stripping film (Kodak), developed after 9 days’ exposure and stained with neutral red.

(c) Retinal reconstruction

By approximating the shape of the retina as a hemispherical surface, we have represented the retinal surface on a plane, in the same way as the visual field is generally represented on a planar surface. In such a representation, the centre of the retina (and of the visual field) projects to the central pole of a circular disc and parallels of latitude of the hemisphere are transformed as circles around the central pole. Meridians of the hemisphere separated at a given angle are represented on the plane by diameters separated by the same angle. This representation is not an equal area representation although the areal distortions are not great.

Reconstruction of the retina from either horizontal or transverse sections of the eye, on this polar coordinate system, is relatively straightforward. Any such section is represented as an arc on the projection, its precise position and the degree of convexity or concavity of the arc depending upon the relative distance of the section from the horizontal or vertical meridian of the retina. Errors may arise if the plane of the section is not exactly horizontal or transverse. These errors become substantial only for those sections furthest away from the central section. For this reason, only about 50% of the retina was reconstructed for each eye, from those sections distributed about the central section. Complete retinal reconstructions were obtained by combining the results from eyes that had been horizontally sectioned with those that had been sectioned transversely.

Camera lucida drawings were made of every fifth section of the retina and the positions of cells which had incorporated [3H]thymidine were marked. From each drawing, the overall length of the section and the lengths of the retinal regions distal to the labelled cells were measured. The position of each section relative to the central meridian was determined and an arc representing the section, together with the positions on that arc of the labelled cells, was plotted on the polar projection. By repeating this process for each section examined, a conformal mapping of a hemispherical retina, and the position of the labelled cells, was constructed.

(d) Tectal reconstruction

The outline of the optic tectum viewed from its dorsal surface was traced from an enlarged photograph of the optic tectum of a stage-66 animal. The more rostral and lateral aspects of the tectum are not seen when viewed from a dorsal position. The dimensions of these areas were measured from histological reconstructions of the tectum. To represent the entire tectal surface on a plane, a ‘tear’ was introduced at the rostro-lateral border. This representation, although slightly distorted, preserves the linear dimensions of the tectum and will be
referred to as the ‘standard tectum’. Tecta from experimental animals were sectioned either horizontally or transversely. Camera lucida drawings were made of every fourth or fifth tectal section and the positions of cells labelled with $[^3]$H]thymidine were noted. The positions of thymidine-labelled cells were marked at corresponding positions on the standard tectum.

(e) Autoradiographic grain counting

Quantitative estimates of the tectal distribution of silver grains were made in 15 animals following intraocular injection of $[^3]$H]proline. Representative areas of the tectum were selected and sections of these areas were photographed with bright-field illumination. Enlargements to a magnification of 2000 were made and the number of silver grains contained in $10 \times 10 \mu m$ squares overlying the superficial tectal neuropil was counted. Usually the number of grains was counted in ten or more such squares for each position across the medio-lateral extent of the tectal section in transversely cut brains, or across the rostro-caudal extent of the section in parasagittally cut brains. By repeating this process for different tectal sections from the same brain, the relative distribution of label throughout the tectum was obtained.

In several animals which had also received a pulse of $[^3]$H]thymidine at a certain developmental stage, the distribution of grain counts of $[^3]$H]proline over the tectal neuropil was compared with the distribution of thymidine-labelled cell nuclei in the tectum. Sections through the retina of the eye into which the proline had been injected were also examined to compare the distribution of the proline and thymidine labels. In two of these animals, quantitative measures of this comparison were obtained. Low power photographs were taken of retinal sections and then a higher power montage of the inner plexiform layer was made. The number of grains in each $10 \times 10 \mu m$ square along the inner plexiform layer was then counted. The positions of the thymidine-labelled nuclei in the other retinal layers were marked on the low power photographs and direct comparison of the positions of thymidine and proline labels were made.

(f) Ultrastructural analysis of the distribution of optic terminals

Quantitative studies of the tectal distribution of degenerating synapses were made from electron microscopic observations of normal larval Xenopus, of animals in which one eye had been removed and of animals in which a lesion had been made at the temporal pole of the retina. The temporal pole of the left eye of 33 larval Xenopus of various developmental stages was destroyed with a heated tungsten needle. For comparison, the left eye was removed in 21 control animals. The operated animals were re-anaesthetized 24 or 48 h later and their brains prepared for ultrastructural analysis. Normal animals of various stages were treated in the same fashion.

The dorsal tectal surface was exposed and the animals were immersed in
aldehyde fixative for 18 h. Except for the addition of 1% glucose, the composition of this fixative was identical to that of Palay & Chan-Palay (1974). The optic tecta were then removed, post-fixed in osmium, dehydrated in ethanol and embedded in TAAB embedding resin. The embedded tecta were cut in the parasagittal plane and adjacent thin and ultra-thin sections were collected at every 50 μm interval lateral from the midline. Thin sections were stained with 1% methylene blue and 1% Azure II. The ultra-thin sections were collected onto an uncoated grid (Micron 400) and stained first with uranyl acetate and then with lead citrate (see Reynolds, 1963).

The ultra-thin sections were examined in a Philips 300 electron microscope and the tectal neuropil was scanned at a viewing magnification of approximately 35000. For the quantitative analysis of the distribution of degenerating synapses, five to eight squares (42 x 42 μm) distributed at equal intervals along the anterior-posterior axis of the parasagittal section were selected. The positions of the sampled regions were checked by making a camera lucida drawing of the adjacent thin section and matching this with the grid map of the ultra-thin section. For each selected grid square, the numbers of normal and degenerating synapses, as well as the numbers of vacant post-synaptic sites and of pieces of degenerating debris, were tabulated. The criteria used for identifying a synapse were thickened opposed membranes with clustered vesicles.

RESULTS

(a) The mode of retinal growth

Our observations on the mode of retinal growth in *Xenopus* agree, in general, with the previous findings of Straznicky & Gaze (1971); the retina grows in rings by the addition of cells to its ciliary margin. We also agree with Jacobson (1976) that after stage 53, relatively more growth occurs ventrally than dorsally. Figure 1A shows a stereo-reconstruction of the labelled right eye from a newly metamorphosed animal which had been given two injections of [3H]thymidine, one at stage 50 and one 10 days later. The asymmetry of the labelling is obvious. The orientation of the sections in relation to the head is shown in Fig. 1B.

Analysis of retinal growth in tadpoles labelled at various developmental stages and autoradiographed after varying survival times indicates, however, that in the earlier phases of retinal growth the asymmetry is the other way round and more cells are added dorsally than ventrally. This is illustrated (Fig. 2) by a series of transverse sections through the eye, at the level of the optic nerve head, arranged in order from younger to older developmental stages. As shown in Fig. 2d, which is a section from an animal labelled at stage 29 and autoradiographed at stage 54, the earliest formed retinal cells are those around the optic nerve head, arranged in order from younger to older developmental stages. Comparison of this section with that shown in Fig. 2c which comes from an animal labelled at stage 35 and autoradiographed at stage 48, shows that most of the retinal cells appearing between stages 29 and
Fig. 1. Stereo reconstructions of a transversely sectioned head of a *Xenopus laevis* at stage 66. The animal had received two pulses of \(^{3}H\)thymidine, one at stage 50 and the second one 10 days later. (A) A stereo pair of the right retina showing the positions of the thymidine label. Ventral retina is represented to the left in each diagram. (B) A stereo pair of the reconstruction of the whole head which permits the orientation of the eye within the head to be determined.

35 are added to retina dorsal to the optic nerve head. The rapid growth of the dorsal retina continues until late embryonic life as may be seen from the disparity in size between retina dorsal and ventral to the optic nerve head at these stages (Figs. 2a, b). This process of asymmetrical cellular accretion reverses about stage 53. Thus at stage 53 and earlier the optic nerve head is placed ventrally in the retina but after this the relatively greater growth of the ventral margin compensates for this asymmetry so that in later larval life the optic nerve head is approximately centrally placed at the back of the eye (Fig. 2d–h). Retinal growth in post-metamorphic life has recently been described by Beach & Jacobson (1979).

In contrast, the growth of the retina in the nasotemporal axis is symmetrical throughout larval life. Horizontal sections through the eye at the level of the optic nerve head show that the nasal and temporal extent of the retina is about equal at all stages (Fig. 3).
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Fig. 2. Camera lucida drawings of transverse sections through the eye at the level of the optic nerve head, at various developmental stages. The number accompanying the upper-left corner of each section indicates the stage at which the retina was prepared; the positions of [³H]thymidine labels, along with the stage at which [³H]thymidine had been injected, are indicated. The optic nerve is ventrally placed in the retina at stage 40 (a) and stage 45 (b). Relatively more cells are added to the dorsal retina during embryonic life (c and d), but this trend becomes reversed in midlarval life. At stage 57 (e) and stage 61 (f), the optic nerve is centrally placed, as a result of more ventral growth from about stage 55 (g). At metamorphic climax (h), the optic nerve head is situated somewhat dorsally. Calibrations: the bar on the left represents 250 μm and refers to a, b, and c. The bar on the right represents 500 μm and refers to d, e, f, g, and h. D, Dorsal; V, ventral.

Fig. 3. Camera lucida drawings of horizontal sections through the eye, at the level of the optic nerve head, at various developmental stages. The number accompanying the upper-left corner of each section indicates the stage at which the retina was prepared. Section (g) is from an animal which had received two injections of [³H]thymidine, one at stage 50 and the other at stage 55. Note that the optic nerve head is placed centrally throughout all developmental stages. Calibrations: The bar on the left represents 250 μm and refers to a, b and c. The bar on the right represents 500 μm and refers to d, e, f, g and h. N, Nasal; T, temporal.
Fig. 4. Reconstruction of retinal growth patterns. The retinae were reconstructed at stage 66 as described in the Methods and are represented on circular discs. Pulses of $[^3H]$thymidine were administered to animals at (a) stage 50, (b) stage 53 or (c) stage 56. Hatched areas indicate that portion of the retina present at stage 66 which had appeared since the time of injection. Solid lines between the hatched and open areas represent the position of thymidine-labelled cells in the retina at stage 66. (a) represents the mean of 20 retinae, (b) the mean of 13 retinae and (c) the mean of 10 retinae. D, Dorsal; V, ventral; N, nasal; T, temporal.

Quantitative studies on the mode of retinal growth between stage 50 and 66 were made by reconstructing 43 retinas from stage-66 animals which had been labelled with $[^3H]$thymidine at stage 50 ($n = 20$), 53 ($n = 13$) or 56 ($n = 10$). The results are illustrated in Fig. 4 in the form of the mean of each group. The unshaded area represents the region of retina that was present at the time of labelling, and the shaded area represents the region of retina added between the time of labelling and metamorphosis. Labelled cells occupied the junction between the two areas.

These diagrams illustrate the rate of retinal growth between these developmental stages and show clearly both the symmetry of retinal growth in the naso-temporal retinal axis and the asymmetry of retinal growth in the dorso-ventral axis. For example, it may be calculated from the data of Fig. 4a that, of the surface area of the retina present at stage 66, 68% is occupied by retinal cells that appeared after stage 50. From these reconstructions, we have also deduced a growth curve for the retina, as shown in Fig. 5.
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Fig. 5. The increase of surface area of retina with development. This is expressed as the proportion of the retina present at stage 66 which was present at the developmental stage indicated. The proportion was obtained from data presented in Fig. 4, which represents the means of animals labelled at a particular developmental stage. Within a particular developmental stage there was, however, a wide variation in the degree of retinal maturation; this variation is probably due to the fact that staging was done by limb-bud criteria and brain maturation is not well correlated with limb development. On the basis of the positions of thymidine labels in the tectum, we have divided some of the developmental stages into sub-groups, as shown in the figure. The calculation of surface area at a given stage was based on the solid angle subtended by the retinal area contained within the thymidine label.

(b) The mode of tectal growth

Quantitative studies on the mode of tectal growth between stages 50 and 66 were made by histological reconstructions of 38 tecta from stage-66 animals which had received $[^3]H$thymidine at stage 50, 53 or 56. The results are in agreement with the previous conclusions of Straznicky & Gaze (1972). If a single pulse of $[^3]H$thymidine is given at stage 50, horizontal sections of the tectum from a stage-66 animal reveal a diagonal band of labelled cells running from near the midline at the rostral end of the tectum to a more lateral position caudally. Our present results and the previous work of Straznicky & Gaze (1972) indicate that the tectum rostrolateral to this diagonal band is formed prior to the time of label administration, whereas tectum caudo-medial to the diagonal band formed after this time. The direction of tectal growth is thus from rostrolateral to caudo-medial.

Figure 6 shows the overall pattern of tectal growth. The portions of the tecta present at stage 66 which had been added after the administration of $[^3]H$-thymidine at stage 50, 53 or 56 are shaded. Note that the few cells which are
The tectal outline of a stage-66 animal was constructed according to procedures described in Methods. Pulses of \[^3\text{H}\]thymidine were administered to animals at (a) stage 50, (b) stage 53, or (c) stage 56. Hatched areas represent the area of tectum that appeared between the administration of the thymidine and stage 66. Solid lines between the hatched and open areas represent the position of thymidine-labelled cells in the tectum at stage 66. (a) represents the mean of 15 tecta (b) the mean of 13 tecta, and (c) the mean of 10 tecta. M, Medial tectal border; R, rostral.

added to the tectum after stage 56 are located near the midline of caudal tectal areas (Fig. 6c).

The direction of tectal histogenesis is clearly reflected in the morphology of the neuropil. This can be illustrated by reference to ultrastructural features found in the caudal, middle and rostral tectum of a stage-53 tadpole (Figs. 7–10). The neuropil of the most caudal tectum consists of an open network of intersecting axons, glial processes and immature dendrites with abundant extracellular spaces (Fig. 8a). The axons and dendrites in this area are small (0.1–0.2 \(\mu\)m diameter) and contain only a few microtubules (Fig. 8b, c). Along the preterminal lengths and at the apices of these neuronal processes are small growth cones filled with an amorphous floccular cytoplasm (Fig. 8b) and containing either a dilated network of smooth reticular membranes (Figs. 8c and 9a) or larger irregular vacuoles (cf. Del Cerro & Snider, 1968; Tennyson, 1970; Westrum, 1975). There are very few specialized contacts between neuronal processes and profiles filled with synaptic vesicles are very rarely seen.

In the middle region of the tectum, the neuropil proliferates and the extracellular spaces are less wide. Here are found dilated axonal profiles filled with
vesicles. Most of the synaptic or specialized neuronal contacts are very immature initial contacts. These usually occur between growing axons and dendrites and appear as the apposition of plasma membranes which are slightly denser than normal with the intervening gap filled with electron-opaque cleft material (Fig. 9b). Slightly more mature contacts have a few synaptic vesicles clustered at the presynaptic membrane (Fig. 9c). Pronounced post-synaptic membrane thickenings that are either unapposed or contacted by morphologically unspecialized profiles (Fig. 9d) are here defined as vacant post-synaptic sites, and their significance will be discussed later.

In the rostral tectum, the extracellular spaces are greatly reduced and the neuropil is densely packed with large synapses and other neural and glial processes. There are numerous axonal profiles filled with synaptic vesicles as well as mature synapses which are formed immediately adjacent to the actively growing regions of both axons and dendrites (Fig. 10a, b). These mature
Fig. 8. For legend see opposite.
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synapses have larger presynaptic profiles filled with synaptic vesicles and a longer and more pronounced post-synaptic thickening (Fig. 10c).

As the animal matures, the depth and density of the tectal neuropil organization increases further (see Fig. 18) until the extracellular spaces virtually disappear. In this context it is relevant to note that the volume of the tectum continues to increase after metamorphosis. In the 2 years following metamorphic climax, the rostro-caudal and medio-lateral tectal linear dimensions increase from about 1 to 2-3 mm. Similarly, the thickness of the tectum, especially that of the superficial neuropil (stratum opticum), increases gradually as the animal matures. This enormous increase in the tectal volume is probably due mainly to the elaboration of axonal, dendritic and glial processes. Some slight histogenesis does continue into early adult life but these new cells are added only to the periventricular layer. The changes in the morphology of neurones and the concomitant adjustments of synaptic relations that this must entail during adult life deserve further investigation.

(c) The incongruent modes of retinal and tectal growth

In the preceding sections, the modes of retinal and tectal histogenesis were summarized. If synaptic relationships during this period of continual growth were to remain invariant, then the modes of retinal and tectal growth should be congruent. Thus, the oldest retina should project to the oldest part of the tectum and connexions from newly grown retina should project to newly grown tectum.

Our data show no such congruence. This is demonstrated by comparison of the actual modes of the retinal and tectal growth with those modes which would be required for congruence. The baseline for these calculations is the normal visuo-topic projection to the tectum at stage 66 (Fig. 11). This electrophysiological mapped projection of the visual field of one eye upon the contralateral optic tectum may be used to deduce the nature of the retinal projection

Electron micrographs of the superficial caudal tectum from a stage-53 animal. Photomicrographs were obtained from square 5 in Fig. 7a.

(a) At low magnification, the large extracellular space (e) is evident with, a few ascending dendrites (D) and scattered bundles of small axons (A). Calibration: 2 μm.

(b) Protruding from the side of a small axon is a growth cone (C) with both floccular cytoplasm and some basal tubules of smooth reticulum (sr).

(c) Two growth cones (C) found just under the pial surface (PS) are shown. One contains floccular cytoplasm and has filopodia (f), whereas the other contains the basal smooth reticulum (sr) and a few neurotubules (t). Calibration: 0.5 μm for (b) and (c).

A, axons; C, growth cone; D, dendrite; DB, degenerating debris; e, extracellular space; f, filopodia; G, glial process; m, mitochondrion; mf, myelin figure; N, small neurone; PS, pial surface; sr, smooth reticulum; SV, synaptic vesicule; t, neurotubule.
Fig. 9. For legend see opposite.
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to the tectum. We assume that the optics of the Xenopus eye at stage 66 are not significantly distorted so that a light ray from a particular visual field position falls by simple camera inversion upon a corresponding retinal position (Land & Stirling, 1975). Then, knowing the mode of retinal growth, we are able to specify the tectal region onto which retinal ganglion cells that are generated between two given stages will subsequently project. From the data on retinal and tectal growth (Figs. 4 and 6), together with the electrophysiological map (Fig. 11), we have constructed the diagrams illustrated in Fig. 12.

Thus, in Fig. 12a, growth between stages 50 and 66 is considered. The retinal ganglion cells generated between stages 50 and 66 occupy a wide peripheral annulus. At stage 66, the axons of these retinal ganglion cells project to a corresponding annulus on the tectum. If retina and tectum were to grow in congruent fashion, tectal growth after stage 50 should be represented by this tectal annulus. In fact, tectal growth from stage 50 to 66 occurs not in an annulus but in the caudomedial sector of the tectum; therefore, actual tectal growth is quite different from that predicted on the basis of a congruence with retinal growth.

The same point may be made in the converse way by considering the mode of retinal growth that would be required for it to be congruent with the actual tectal growth that occurred between stages 50 and 66. At stage 66, the portion of retina that projects to the area of the tectum which was added between stages 50 and 66 occupied the naso-ventral sector of the retina. Thus, for retinal growth to be congruent with tectal growth from stages 50 to 66, it should have occurred only in this naso-ventral sector. Since retinal growth between these two stages occurs in the form of a somewhat asymmetrical annulus (see Fig. 4), it is clear that actual retinal growth is quite different from that predicted on the basis of a congruence with tectal growth.

Similar considerations for retinal and tectal growth between stages 53–66 and stages 56–66 result in the diagrams illustrated in Fig. 12b and Fig. 12c, respectively. Note that between these stages also the modes of retinal and tectal growth are quite disparate.

**Figure 9**
The mid-tectal superficial neuropil of a stage-53 tadpole. Photomicrographs were obtained from square 3 in Fig. 7a for (a) and (b), and from square 4 in Fig. 7a for (c) and (d).

(a) A growth cone (C) appears at the tip of a relatively large axon (A).

(b) A small axon profile (A) filled with synaptic vesicles (sv) forms an initial contact, as indicated by *, with a dendritic spine (D). The membranes are slightly denser than usual and the cleft is filled with electron-opaque material.

(c) An immature synapse with a few synaptic vesicles (sv) lies against a pronounced post-synaptic thickening (*) on a dendrite (D) with a growth cone (C).

(d) A pronounced membrane thickening (*) at the tip of filopodium (f) is apposed by the unspecialized membrane of a presumptive axon (A). The cleft is filled with electron-opaque material. Calibration: 1 μm for (a) and 0.5 μm for (b), (c) and (d).
Fig. 10. For legend see opposite.
(d) Autoradiographic evidence for shifting connexions

The above analysis of the modes of retinal and tectal growth, together with the results of previous electrophysiological studies (Gaze et al. 1972, 1974; Chung et al. 1974), leads us to postulate that connexions between retina and tectum shift gradually in the course of development. For example, the retinal fibres, which in later life will project onto the middle of the tectal surface, initially arborize at the rostral pole of the tectum and form synapses with nearby dendrites. We now present autoradiographic evidence for such a shift of connexions. A pulse of $[^3H]$proline was administered to the eye at stage 50, and the distribution of silver grains was examined either at stage 50 (24 h after injection) or at stage 66.

For this comparison to be valid, it was necessary to show that the $[^3H]$proline injected into the eye at stage 50 labelled only those retinal cells present at that time and did not label those retinal ganglion cells that appeared later. Retinal sections from two stage-66 animals, which had received $[^3H]$proline and $[^3H]$-thymidine at stage 50 were examined. The entire extent of the inner plexiform layer of the retina was divided into contiguous $10 \times 10 \mu m$ squares and silver grains in each square were counted. The positions of thymidine-labelled nuclei were marked on low power photographs of the retinal sections, so that direct comparison of the position of the thymidine and proline labels could be made. Figure 13 shows the distribution of silver grains across the retina in one such animal; the positions of thymidine-labelled cells are indicated by arrows. It may be seen that the retinal cells which have incorporated $[^3H]$proline are those which were generated before or very close to the time of proline injection.

If the eye and optic axons are labelled with $[^3H]$proline at stage 50 and the animal killed 24 h later, then the distribution of silver grains in the tectum is most dense at the rostral tectal pole, and the density declines in the rostro-caudal direction. Two transverse sections of one such animal, with the densities of silver grains indicated, are shown in Fig. 14. The average number of silver

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**Figure 10**
The rostral superficial tectal neuropil of a stage-53 tadpole. Photomicrographs displayed in (a), (b) and (c) were obtained, respectively, from squares 1, 3 and 2 of Fig. 7a.

(a) A large presynaptic terminal (A1) filled with synaptic vesicles (sv) and mitochondria (m) synapses just proximal to a growth cone (C), as indicated by an asterisk. Other synapses are also indicated by asterisks.

(b) An axon (A) forms three synaptic contacts (*) just proximal to its own growth cone (C).

(c) Relatively mature synaptic contacts (*) are characterised by the large presynaptic profile (A) filled with synaptic vesicles (sv), some of which are clustered near pronounced post-synaptic thickenings. Note the reduced extracellular space (e) in all three micrographs. Calibration: 0.5 $\mu m$. 
Fig. 11. Contralateral visuo-tectal projection in a normal stage-66 Xenopus. The projection of the visual field of the right eye to the left optic tectum is shown, mapped with the optic axis of the right eye centred on the perimeter. The tectal diagram represents a dorsal view, with the rostral aspect of the tectum directed as shown (R). From each numbered electrode position on the tectum, stimulation of correspondingly numbered visual field position elicited electrical responses. The dashed line on the tectal diagram is obtained by unfolding the rostral and lateral tectum, which cannot be viewed from the dorsal aspect. N, Nasal; D, dorsal; T, temporal; V, ventral; M, midline.

grains per 10 × 10 μm square in the rostral tectum is (129 ± 8) and in a section 200 μm more caudal was 97 (± 20).

In contrast, in an animal which received an intraocular injection of [3H]-proline at stage 66 and was killed 24 h later, the tectal distribution of optic axons, as indicated by grain counts, is uniform. Counts from a parasagittal section from one such animal are illustrated in Fig. 15.

The distribution of silver grains in the tectum of stage-66 animals, which had received an intraocular injection of [3H]proline (as well as an intraperitoneal injection of [3H]thymidine) at stage 50, was analysed in 15 animals. The density of silver grains was highest in central tectal areas; in many cases, tectal areas
Fig. 12. Diagrams illustrating the non-congruence of the modes of retinal and tectal growth. In (a) is shown the growth from stage 50 to stage 66, in (b) that from stage 53 to stage 66 and in (c) that from stage 56 to stage 66. For each developmental period, a representation of the retina and tectum at stage 66 is shown. Within each representation is shown the actual growth that occurred during the period (vertical hatching). Also shown is the pattern of growth that should have occurred in that structure for its growth mode to be congruent with the actual growth observed in the other structure (horizontal hatching). This latter pattern was deduced from the normal visuo-tectal projection illustrated in Fig. 11. Thus, the pattern of retinal growth required for congruence with the actual mode of tectal growth is calculated by determining, from the map, that area of retina which, at stage 66, projects to the area of tectum which appeared in the developmental period being considered. Conversely, the pattern of tectal growth required for congruence with the actual mode of retinal growth is calculated by determining, from the map, the tectal projection of the area of retina that actually did grow during the period.
Fig. 13. Grain counts from inner plexiform layer of a stage-66 animal which received an intraocular injection of \([\text{H}]\)proline and intraperitoneal injection of \([\text{H}]\)thymidine at stage 50. A transverse retinal section was photographed at high magnification, and the inner plexiform layer was divided into 156 adjacent 10 × 10 \(\mu\)m squares. The number of grains in each square was counted. The number of grains per square is plotted against the dorso-ventral axis of the retina. Arrows indicate the positions of cells labelled with \([\text{H}]\)thymidine. Note that the high density of proline labels are seen only in those retinal areas that existed at the time of injection.

that appeared after stage 50 contained proline-labelled optic terminals. The mean grain counts for different tectal positions from one such animal are shown in Fig. 16a. The dotted line marks the site of tectal cells that incorporated most labelled thymidine. Figure 16b represents the predicted distribution of retinal axons from ganglion cells born before stage 50 onto the tectum at stage 66 in the particular animal from which the grain counts of Fig. 16a were obtained. This prediction was obtained by the methods used in constructing the diagrams of Fig. 12. The predicted distribution accords reasonably well with the data obtained.

Figure 17 shows the density of silver grains in two transverse sections from animals labelled at stage 50 and killed at stage 66. The sections through the tectum show higher grain counts in central tectal areas with lower counts laterally. The highest counts occur medial to the most lateral thymidine-labelled tectal cells, indicating that optic fibres from retinal ganglion cells generated before stage 50 project at metamorphic climax onto tectal cells generated after stage 50.
Fig. 14. Proline grain counts from transverse sections of the tectum of an animal which had received an intracocular injection of \(^{[3]H}\)proline at stage 50 and was killed 24 h later. The upper section is rostral tectum, and the lower section is from a mid-tectal area. At each site indicated, five 10 × 10 \(\mu\)m squares were counted, except in one case where only two squares were counted (in this case no standard deviation is given), and the mean \(±\) standard deviations are shown. The grain density is highest rostrally and uniformly distributed medio-laterally, apart from a reduction at the medial margin.

(e) **Ultrastructural analysis of degenerating synapses**

We have also investigated the distribution of degenerating synapses in the optic tectum after destroying the temporal pole of the retina at various stages. Since the temporal pole of the retina is growing, while rostral tectum is not, the fibres from these new temporal pole retinal ganglion cells should project to the rostral tectum, displacing the previous innervation from this pole more caudally. If such a process does take place, at all developmental stages the degenerating synapses should be concentrated at the rostral pole of the tectum. This we have
confirmed by tabulating the number of degenerating synapses along the anterior-posterior axis of the tectal neuropil following a lesion of the temporal pole of the retina. As controls, we also observed the distribution of degenerating synapses in normal developing optic tectum and in the tectum following enucleation of the contralateral eye.

(i) Degenerating synapses following eye enucleation

The ultrastructural synaptic configuration of optic terminals in *Xenopus* is similar to that described for *Rana* (Székely, 1973). The terminals of optic axons form large (0.7–1.5 μm) presynaptic profiles filled with scattered, round agranular vesicles and an occasional mitochondrion (Fig. 18a). These profiles usually contact spine-like dendritic protuberances with pronounced asymmetrical post-synaptic membrane thickenings. The presynaptic profile often contacts more than one profile in the plane of section. In non-optimum planes of section the optic profiles may appear smaller and under these circumstances are not so easily distinguished from other synaptic elements.

Following removal of the eye, a sequence of degenerative changes in the optic terminals takes place, and the time course of this process depends on the age of the animal. For example, in animals at stage 50, degenerating synapses
Fig. 16. Proline grain counts from the tectum of an animal which had received an intraocular injection of [3H]proline at stage 50 and which was killed at stage 66. The animal also received a pulse of [3H]thymidine at stage 50. Grain counts were made from representative sites over the whole tectum. In (a) these counts are displayed on a standard tectum as used in the previous figures. The broken line represents the position of cells containing most thymidine label. The area containing the densest proline is outlined. (b) Predicted distribution of proline label over the tectum in the same animal on the basis of assumptions detailed in the text and illustrated in Fig. 12. There is reasonable accord between the predicted distribution of label and that observed in (a).

are observed within 12 h after operation, and all the degenerating debris is removed by the fourth post-operative day. In contrast, the onset of degeneration in metamorphic animals is slower, and debris lingers on until 15 days after operation. Despite the difference in time course, the sequence of degeneration appears the same in animals at all stages. The process starts with the appearance in the presynaptic terminals of abnormal organelles, such as myelin figures, multivesicular bodies and cytolyosomes (Fig. 18b). Subsequently, synaptic vesicles are clumped together as the cytoplasmic density increases (Fig. 18c). The presynaptic profiles then collapse and become electron-dense, the mitochondria swell and disintegrate. The entire degenerating synaptic complex is
Fig. 17. Representative transverse sections of the tectum together with proline grain counts taken from two animals which received an intraocular injection of $[^3H]$proline at stage 50 and which were killed at stage 66. The animals were given, in addition, two pulses of intraperitoneal $[^3H]$thymidine, the first at stage 50 and the second at stage 53. At each site indicated the number of proline grains in 11–20 $10 \times 10 \mu m$ squares was counted and the mean ± s.d. are shown. The dashed lines represent the boundaries of thymidine-labelled cells. It may be seen that the highest proline counts are found medial to the most lateral thymidine-labelled cells which are those cells which incorporated thymidine at stage 50. (a) represents a section from the rostral third of the tectum, and (b) a section from mid-tectum.
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frequently seen engulfed by glial cytoplasm (Fig. 18d). The final step in the
degeneration process is when the axonal and synaptic debris coalesce within
the glial cytoplasm (Fig. 18f) to become the characteristic dark, osmiophilic
dots seen under the light microscope. Although the number of pieces of glial
debris was counted at the time, we have not included it with the degenerating
synapse counts, since such debris can appear anywhere along the axonal
trajectory (Scott, 1974; Longley, 1978).

Figure 19 shows the rostro-caudal distribution of degenerating synapses in
the tectum after contralateral eye enucleation at stages 50, 53, 58 and 66. Each
square marked on the tectal neuropil was scanned systematically under a
viewing magnification of 35000 and the number of intact and degenerating
synapses, as well as vacated post-synaptic sites, was tabulated. The patterns of
degenerating synaptic distributions following removal of the eye are essentially
similar to the tectal distribution of silver grains in our autoradiographic pre-
parations, following intraocular injection with [3H]proline at the same de-
velopmental stage (Fig. 19). In the tectum of a stage-50 tadpole, the degenerating
synapses are essentially confined to its rostral half. The extent of the tectum
covered by degenerating synapses expands further caudally in the later stages
of tadpole life; by stage 66, degenerating synapses are uniformly distributed
along the rostro-caudal axis, apart from a reduced concentration at the most
caudal pole.

(ii) Degenerating synapses following retinal temporal pole lesion

Using the same method of analysis, we have examined the tecta of 16 tad-
poles in which the temporal pole of the contralateral retina had been destroyed
24 or 48 h earlier, at stage 50, 53, 58 or 66. At all stages of development analysed,
fibres originating from the temporal retina formed synaptic contacts with
dendrites of tectal cells in the most rostral tectum. To ascertain the extent of
the retinal lesions, we have reconstructed the retinai, according to the pro-
cedures detailed previously. The shaded areas of the disc in Fig. 20 represent
the damaged parts of the retina. Synapses of all categories in each grid square
on the tectal neuropil indicated in Fig. 21 were tabulated and the number of
degenerating synapses was always highest in the rostral 200 μm of the tectal
neuropil. We therefore conclude that retinal fibres from larval Xenopus form
synaptic contacts with available tectal cells, and those originating from the
temporal retina occupy synaptic sites in the most rostral region of the tectum.

(iii) Spontaneous synaptic degeneration in the tectum

If our hypothesis is correct, then throughout larval life synaptic reorganiza-
tion is taking place in the tectal neuropil. One way in which this could occur is
by the degeneration of those synaptic connexions which are no longer appropri-
ate, and the establishment of new synaptic connexions by the presynaptic fibre.
On this view one might expect, therefore, to find significant levels of synaptic
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degeneration occurring in the larval tectum. Our ultrastructural observations on the optic tectum of 15 normal tadpoles provide results which are consistent with this view. The neuropil of the normal tadpole, unlike that of the adult animal, contains large numbers of spontaneously degenerating synapses.

The number of normal and degenerating synapses was counted in grid squares (42 × 42 μm) covering the rostro-caudal extent of the tectum in parasagittal sections. In the larval stages the density of tectal synapses shows a rostro-caudal gradient, whereas in the tectal neuropil of a metamorphic animal it is about uniform. Thus, in tadpoles of larval stages 50 and 53, there was an average of 83 synapses per grid square in the rostral tectum (n = 14, S.E.M. = 9.9, n = no. of grid squares counted), 62 synapses per grid square on central tectum (n = 13, S.E.M. = 6.5), and 26 synapses per grid square on caudal tectum (n = 12, S.E.M. = 5.3). In stage-66 animals, there was no difference between the synaptic densities from rostral, central or caudal tectum, and the overall synaptic density was increased to 160 (n = 51, S.E.M. = 5.8).

The proportion of degenerating synapses to intact synapses was highest in the youngest tadpoles and diminished with age. Thus, degenerating synapses comprise 9.2% (±0.9, n = 7, n = No. of animals) in tadpoles of stages 50, 53 and 58 but only 3.9% (±1.0, n = 6) in stage-66 animals.

Although all the phases of synaptic degeneration are found in normal tecta at all stages of larval life (Fig. 22), the initial phases with abnormal presynaptic inclusions predominate. Myelin figures are the most frequent form of electron-dense debris found, not only in these spontaneously degenerating synapses (Fig. 22a), but also in other axonal and dendritic processes (Fig. 22c, d). Examination of Fig. 23 reveals that myelin figures can be formed by collapsing or retracting membranes, such as the collapsing membranes of dendritic and axonal growth cones. By analogy, we believe that the myelin figures seen in the

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**FIGURE 18**

A sequence of degenerative changes seen in the tectal neuropil of a stage-66 *Xenopus* 1–4 days after the removal of the contralateral eye.

(a) A normal presynaptic axon profile (A) with pleomorphic synaptic vesicles (sv) contacts two post-synaptic profiles (*).  
(b) A presynaptic profile (A) contains a myelin figure (mf). Such abnormal organelles are one of the first signs of degeneration.  
(c) In an early dark degenerating synapse, the presynaptic profile (A) has collapsed, the cytoplasm is darker and the vesicles are clumped.  
(d) A late dark degenerating synapse (A) is partially engulfed by a glial process (G): the presynaptic cytoplasm is very dense.  
(e) An early dark degenerating synapse (A) and two small post-synapse profiles (*) are totally engulfed by a glial process (G).  
(f) Large aggregations of degeneration debris (DB) are engulfed by glial processes (G). Calibration: 0.5 μm for (a)–(e), and 2 μm for (f).
Fig. 19. Distribution of degenerating synapses in the tectum after removal of the contralateral eye. The stage at which the eye was enucleated is indicated. The graphs are from individual animals and the curves are fitted by eye. For each animal, the number of degenerating synapses in the marked squares, on the corresponding camera lucida drawings of the tectum, is plotted against the distance in $\mu$m of the square from the rostral pole of the tectum. The dashed line is the average number of degenerating synapses per square seen in normal tecta from matched control animals. The tecta are cut in parasagittal section and the lateral distance from the midline is indicated for each. Survival times are 1 day for stage 50, stage 58 and stage 66, and 2 days for stage 53. Calibration: 0·5 mm. C, Cerebellum; TV, tectal ventricle.
spontaneously degenerating synapses are also formed by the collapsing or retracting membranes of adjacent growth cones. Unlike those in enucleated animals, degenerating profiles in the normal tectum are seldom engulfed by glial processes (but see Fig. 22d).

The immature tectum also contains a number of post-synaptic membrane thickenings which are unopposed by presynaptic profiles (Fig. 24d). On average, 1.1 (±0.2, n = 13 animals) of these post-synaptic sites per grid square were seen in normal animals of all developmental stages examined compared with an average of 9.0 (±1.6, n = 9) vacant post-synaptic sites per grid square seen 1–5 days after enucleation of one eye.

A consistent feature of the developing tectal neuropil at all stages of development is the large number of growth cones and of immature synapses (Fig. 24). This is particularly marked in the most superficial neuropil within about 20 μm of the pial surface where the extracellular space is considerable. In this area there are abundant small axons, initial contacts, immature synapses, growth cones and vacant post-synaptic sites as well as more mature synapses. Thus in the immature tectal neuropil, axons and dendrites with growth cones and immature and mature synaptic configurations are interspersed with degenerating synapses and with vacant post-synaptic sites. These ultrastructural
Fig. 21. Distribution of degenerating synapses in the tectum after a lesion in the temporal pole of the retina. The stage at which the lesion was made is indicated. The graphs are from those animals, whose retinal lesions were reconstructed in Fig. 20. For each animal, the number of degenerating synapses in the marked squares on the corresponding tectal drawing is plotted against the distance of the square in μm from the rostral pole of the tectum. The dashed line is the average number of degenerating synapses seen in normal tecta from matched control animals. The tecta are cut in parasagittal sections and the lateral distance from the midline is indicated for each. Survival times are 1 day for stages 50, 53 and 66, and 2 days for stage 58. Calibration: 0.5 mm. C, Cerebellum; TV, tectal ventricle.
features could well be a manifestation of developmental processes involved in the remodelling of synaptic relationships between retinal fibres and tectal neurons.

**DISCUSSION**

Several conclusions can be drawn from present and previous studies about the developmental processes associated with the formation of the mature retino-tectal map. First, the modes of retinal and tectal histogenesis are incongruent. Second, optic axons, soon after their arrival at the tectum, form synaptic contacts with tectal cells, while maintaining throughout development the correct polarity and order of the retinal map onto the tectum. Finally, our findings suggest that, as a consequence of the above facts, synaptic connexions between the retinal and tectal cells shift gradually as the animal matures.

(a) Incongruent modes of retinal and tectal growth

The growth of the retina by accretion of cells to its peripheral margin has been demonstrated in a number of vertebrate species; in fish by Hollyfield (1972), Johns (1977), Meyer (1978); in *Rana* by Currie (1974); in *Xenopus* by Straznicky & Gaze (1971), Hollyfield (1971); in chick by Fujita & Horij (1963) and in mouse by Sidman (1961). Similarly, there is little disagreement about the direction of cellular proliferation in the optic tectum. Straznicky & Gaze (1972) found that the rostro-lateral portion of the tectum appears first in *Xenopus* and that tectal histogenesis proceeds by the serial addition of bands of cells to the caudo-medial margin of the optic tectum. A similar pattern of histogenesis was described for the chick optic tectum (LaVail & Cowan, 1971) and in adult goldfish (Meyer, 1978).

Recently, Jacobson (1976, 1977) claimed that retinal growth during later larval stages of *Xenopus* can be viewed essentially as the addition of crescents of cells to the ventral retina and as such is congruent with tectal histogenesis occurring during this period. Given this congruence of retinal and tectal histogenesis, he maintains, there is no necessity to postulate continuously re-adjusting retino-tectal connexions.

Our results, however, are at variance with Jacobson's interpretation. Although more growth occurs in ventral retina than in dorsal retina during part of this developmental period, the annular pattern of growth is maintained throughout. To emphasize the disparate patterns of retinal and tectal growth, we have illustrated how the retina should grow, given the observed pattern of tectal growth, for the two patterns of growth to be congruent (Fig. 12). Conversely, we can show how the tectum should grow, given the observed pattern of retinal growth, if the retinal and tectal growth are to be congruent (Fig. 12). It is clear, from these illustrations, that the patterns of retinal and tectal histogenesis, for any given developmental period between stages 50 and 66, are not congruent. The postulate of continuously adjusting retino-tectal connexions
Fig. 22. For legend see opposite.
cannot, therefore, be dismissed on the grounds that its underlying premise of incongruent retinal and tectal growth was incorrect.

(b) Morphological evidence for shifting connexions

An ideal support for the hypothesis of re-adjustment of retino-tectal connexions during growth would be the histological demonstration of the postulated shift. Thus, a localized lesion of central retina of tadpoles of various developmental stages should be followed by degenerating optic terminals initially distributed to rostral tectum, but at later developmental stages the degenerating terminals should be found not at most rostral tectum but further caudally in more central tectal areas. Since this approach is not feasible, we studied the tectal projection from central retinal areas at various developmental stages, using autoradiographic methods.

There are at least two methodological difficulties with the method we have used. First, traces of \(^{3}\text{H}\)proline injected intraocularly may remain there for some time, and become incorporated by those retinal cells that are generated after the time of injection. We are able to control for this possibility by examining the distribution of proline label in the retina of a stage-66 animals that had received \(^{3}\text{H}\)proline and \(^{3}\text{H}\)thymidine at stage 50. The retinal ganglion cells labelled with proline were largely those within the retinal area bounded by thymidine-labelled retinal cells, thus indicating that there had been no significant uptake of proline by those retinal ganglion cells generated after stage 50 (Fig. 13). Second, when the survival time is long, \(^{3}\text{H}\)proline injected intraocularly becomes distributed throughout the entire extent of the optic axon. Thus, grains will appear not only in those tectal areas where the fibres terminate but also those areas through which they pass on their way to their termination. One might expect, however, that the concentration of label at the site of the profuse pre-terminal arborization would be higher than at the sites of axonal passage. We hoped, therefore, that quantitative grain counting might indicate the site of optic terminals.

**Figure 22**

Spontaneously degenerating synapses in the tectum of normal *Xenopus.*

(a) A presynaptic profile (A) from a stage-66 animal in the initial phase of degeneration contains a myelin figure (mf).

(b) The presynaptic profile (A) of an early dark degenerating synapse from a stage-53 tadpole has electron-dense cytoplasm. In (a) and (b), mitochondria (m) and membrane thickenings (*) are indicated.

(c) A presumed axon (A) from a stage-66 animal contains a large lump of debris (d).

(d) A late dark degenerating synapse (A) is engulfed by a glial process (G) in a stage-66 animal. Both the presynaptic profile (A) and post-synaptic profile, as well as membrane thickening (*) are engulfed. The positions from which the photomicrographs were obtained are shown in Fig. 7: (a) is from 7b, square 2; (c) is from 7a, square 4; (c) and (d) are from 7b, square 3.
Fig. 23. For legend see opposite.
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Our findings provide clear support for the view that the terminals of central retinal fibres move in a caudo-medial direction in the tectum as the animal matures. The tectum of an animal labelled with [³H]proline at stage 50 and killed within 24 h shows high counts of silver grains distributed throughout the rostro-lateral tectum (Fig. 14). If, on the other hand, following labelling at stage 50, the tectum is examined at stage 66, the density of silver grains is highest not in the rostro-lateral tectal areas but nearer the middle of the tectum (Figs. 16, 17). There is a relative reduction of silver grains in the most rostral and lateral tectal areas despite the fact that axons terminating more centrally will be coursing through these areas.

In addition, it may be seen that the area of high grain counts spreads caudo-medially beyond those tectal cells which had incorporated thymidine following labelling at stage 50 (Fig. 17). Given the modes of retinal and tectal growth (Figs. 4, 6) it is easy to demonstrate that the extent of this caudo-medial spread relative to the thymidine-labelled cells will depend on the developmental stage at which [³H]thymidine and [³H]proline are given (Fig. 25), and the region of tectum examined. If, for example, labels are given at stage 53 or later, there will be only a very slight disparity between the boundary of proline label and the band of thymidine-labelled tectal cells. This disparity is such that in these regions it might well not be detectable within the limits of experimental observation. This may explain why Jacobson (1976, 1977) detected no significant disparity.

The distribution of degenerating synapses in the tectum following temporal pole lesions in the retina provides additional evidence for the hypothesis of shifting connexions. Optic axons from the temporal retinal pole of a stage-50 tadpole synapse with tectal cells in the rostral pole, as do those from the temporal pole at stages 53, 58 and 66. Unless the older synaptic connexions shift progressively in a caudal direction as newly arriving temporal fibres terminate at the rostral tectum, an ordered retino-tectal map of this retinal region, which exists in the mature animal, could not be formed. Longley (1978) also observed

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**Figure 23**
The formation of myelin figures in normal Xenopus. (a), (b) and (c) show a hypothetical sequence for the collapsing of membranes of growth cones (C) to form myelin figures (mf) in the tectal neuropil.

(d) A small protuberance from an axon (A) contains a myelin figure (mf).

(e) A myelin figure (mf) is seen in a presynaptic profile. Post-synaptic profiles are indicated by asterisks in (e), (f) and (g).

(f) and (g) Presynaptic profiles (A) are associated with growth cones (C), which are identified by the presence of the tubules of smooth reticulum (sr).

All the photomicrographs illustrated here are from stage-66 animals, except (b) and (f) which were obtained from a stage-53 animal. Calibration: 0.25 μm. See Fig. 8 for other abbreviations.
Fig. 24. For legend see opposite.
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degenerating terminals in the rostral pole of the tectum following localized lesions of the temporal retinal pole in *Xenopus* tadpoles of stage 51.

These findings, taken together, lead us to conclude that the oldest retinal ganglion cells make synapses initially with the earliest-formed tectal cells and subsequently shift their connexions to more caudo-medially situated tectal cells.

*(e) Ultrastructural evidence of synaptic remodelling*

The synaptic remodelling that we propose could take one or more of several forms. The initial synaptic connexions that are formed may, when they become inappropriate, remain present morphologically but be functionally dormant (Mark, 1974; Wall, 1977). Alternatively, the inappropriate connexion may degenerate and vacate its post-synaptic site while the preterminal axon sends out a new collateral to form appropriate connexions at a new site. If the latter process were to occur, hints of this synaptic remodelling may exist in the ultrastructural configurations of the tectal neuropil.

The presence of abundant degenerating synapses and vacant post-synaptic sites in the larval optic tectum (Fig. 9) suggests that formed synapses are not necessarily permanent but can be eliminated by degeneration and re-absorption. We have no unequivocal evidence that these spontaneously degenerating presynaptic profiles and vacant post-synaptic sites pertain to optic fibre synapses, but we have observed that such spontaneously degenerating synapses are absent if the tectum is examined a month after removal of the eye. If they are indeed optic synapses, then the process of synaptic elimination and remodelling that occurs in larval life differs in several respects from the elimination and re-creation of synaptic sites that occurs after section and regeneration of the optic nerve in adult frogs.

In the adult animal following section of the optic tracts, entire synaptic complexes are removed by glial phagocytosis, sparing only a small proportion of vacated post-synaptic sites (Ostberg & Norden, 1979). Regenerating optic

**FIGURE 24**

Immature superficial tectal neuropil from a metamorphic *Xenopus*.

*(a)* Superficial caudal neuropil is characterized by open extracellular spaces (e) and an abundance of small axon profiles (A), some of which are dilated and filled with synaptic vesicles (sv) and mitochondria (m). Glial process (G), small neuron (N) and pial surface (PS) are indicated. *(b)* A small axon (A) forms an initial contact (*) with dendritic spine. The opposed membranes are slightly thickened and the gap is filled with electron-opaque material. *(c)* An immature synapse; the dilated axon terminal (A) is filled with scattered synaptic vesicles (SV) some of which are clustered at the presynaptic membrane opposite the post-synaptic thickening (*). *(d)* A vacant post-synaptic membrane thickening (*) opposed by a non-synaptic profile. A nearby axon (A) has a lateral growth cone (C). *(e)* A more mature synapse with a presynaptic axon (A) completely filled with synaptic vesicles (SV). Note the relatively open extracellular space (e) in all the illustrations. Calibration bars are 0.5 µm.
Fig. 25. Predicted relative positions of proline label and thymidine label in the tectum of a stage-66 animal which had received an intraocular injection of $[^3H]$-proline and an intraperitoneal injection of $[^3H]$thymidine at the developmental stage indicated. The procedures used to derive this figure are the same as those used for Fig. 16b. The dashed line represents the position of thymidine-labelled cells and the vertical hatching represents the predicted tectal position of high concentration of proline. The various developmental stages were subdivided by the same criteria as indicated in Fig. 5. The predictions were based on the assumption that the label was administered at (a) early stage 50, (b) mid stage 50, (c) late stage 50, (d) stage 53, (e) early stage 56, and (f) late stage 56. Note that the relative positions of thymidine and proline labels are critically dependent on the stage at which labels are administered.

terminals form synapses by inducing new post-synaptic thickenings. The synaptic remodelling that is occurring in the normal larval optic tectum appears to be different. In the developing neuropil, most of the degenerating debris is seen as membranous and lysosomal inclusions in axonal and dendritic processes as the presynaptic terminals simply withdraw without glial intervention, leaving the post-synaptic thickening intact. We are unable to determine whether the vacated sites are reoccupied by a new presynaptic profile or whether they disappear later. In the latter case, presumably new post-synaptic sites would be induced by pre-synaptic processes.

A further ultrastructural characteristic of the tectal neuropil in tadpoles is the presence, throughout the entire neuropil, of growth cones and immature synaptic contacts. Together with the presence of degenerating synaptic profiles, and vacated post-synaptic sites, these observations suggest strongly that synapses are formed, broken-down and re-formed during tectal maturation.
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(d) Implications for selective nerve connexions

The findings reported here substantiate our previous conclusion derived from analysis of the retino-tectal projection (Gaze et al. 1972, 1974) and of current source-sink distribution (Chung et al. 1974) in larval Xenopus. This conclusion is inconsistent with the view that unique and persisting affinities between appropriately labelled pre- and post-synaptic cells stipulate neuronal connexions. Instead, specific synaptic relationships during maturation are undergoing a continuous modification, while preserving the overall polarity of the projection. Thus, selective neuronal connexions, in this system at least, are the product not of discrete selections by a given retinal axon of a specific and stable optimal tectal target but the available population of presynaptic fibres is matched, within the constraints of an overall mechanism governing the polarity of the projection, to the available post-synaptic sites in the target area. Implied in such a mechanism is the concept of some form of competition between fibres of the presynaptic array for the available post-synaptic space.

Similar views had been reached earlier (Straznicky, Gaze & Keating, 1971; Gaze & Keating, 1972) on the basis of the apparent plasticity of retino-tectal synaptic connexions following partial destruction of the retina or the tectum. It is possible that the plasticity of connexions observed in the earlier experimental situations was merely the expression of the same controlling mechanisms which are operating in the normal course of development.

Such a mechanism seems to be forced on a system in which two neuronal arrays are functionally interconnected during a period in which the two arrays grow in a non-congruent fashion. The advantage of such disparate modes of retinal and tectal growth for neurogenesis is unknown, as are the factors governing spatial patterns of histogenesis. It may be relevant that the tectum is a neural structure which receives input from other sensory modalities in addition to visual inputs. Perhaps a pattern of tectal histogenesis congruent with that of retinal histogenesis is inappropriate to the construction of spatially ordered maps of these other sensory modalities.

Whatever the reasons for the disparate mode of retinal and tectal histogenesis in the amphibian visual system, a consequence appears to be the controlled continuous adjustment of synaptic relations between retina and tectum. An adequate understanding of the mechanisms governing selective neuronal connexions must accommodate this naturally occurring neural plasticity.

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