Visual deprivation and the maturation of the retinotectal projection in *Xenopus laevis*

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SUMMARY

There has been a resurgence of interest, recently, in the possible role of neural activity in the ordering of synaptic connections in the lower vertebrate retinotectal system. Blockade of all neural activity, by chronic administration of tetrodotoxin (TTX), during the regeneration of the optic nerve in goldfish has been found to prevent the re-emergence of a fully ordered retinotectal projection. We sought to determine the effects of visual deprivation, a less radical perturbation of neural activity than that produced by TTX, on the initial development of the retinotectal projection. The contralateral visuotectal projection was studied in *Xenopus laevis* which had been reared in darkness from before the onset of visual function. The projection mapped electrophysiologically at metamorphic climax, or in postmetamorphic juveniles, showed a normal retinotopic topography. The topographic precision of the projection, as revealed by the multiunit receptive field sizes, was the same in light- and dark-reared animals. The laminar distribution, in the superficial neuropil of the optic tectum, of terminals from different classes of retinal ganglion cells was also normal. It is concluded that the specific retinotectal connections underlying these features of the projection are generated by intrinsic developmental processes which do not require visual experience. Among these intrinsic processes might be 'spontaneous' neural activity.

INTRODUCTION

In all vertebrate classes, optic fibres project to the contralateral optic tectum (superior colliculus). This structure plays a major role in the localization of stimuli in space and in the orientation of the organism to such stimuli (Trevarthen, 1968; Schneider, 1969; Wurtz & Albano, 1980). A prerequisite for this function is the orderly arrangement of optic fibre terminals in the tectum so as to produce a map of the retinal surface, and hence of the visual field, across this structure. The lower vertebrate retinotectal projection has been studied extensively with a view to understanding the developmental mechanisms responsible for the production of

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ordered neuronal connections. This system has long been viewed as an archetype of those systems in which ordered connections are produced by intrinsic developmental processes (Sperry, 1951, 1963; Gaze, 1970, 1984; Keating, 1976, 1981; Jacobson, 1978; Rager, 1980). It has been assumed that neural activity or visual experience had little role to play in the elaboration of the retinotectal projection. Harris (1980) furnished some support for this view. He demonstrated, in the newt, that an eye growing in an environment in which neural activity was suppressed by tetrodotoxin (TTX) was nevertheless able to elaborate a retinotectal projection. Within the limits imposed by neuroanatomical assessment of the projection, it was found to display at least general retinotopic order.

The search for the mechanisms ordering the retinotectal projection has involved both detailed studies of the normal developmental sequence through which the mature projection is established (Gaze, Keating & Chung, 1974; Gaze, Keating, Ostberg & Chung, 1979) and observations of the patterns of connections following a wide range of developmental perturbations (see Edds, Gaze, Schneider & Irwin, 1979). Both groups of investigations have revealed a considerable potential plasticity in retinotectal synaptic relationships. These results are difficult to accommodate within any simple variant of the theory of Neuronal Specificity which attributes selective synaptogenesis to selective recognition between appropriately labelled pre- and post-synaptic neuronal processes. The response to these difficulties has been the proposal of alternative mechanisms emphasizing the role of fibre ordering (Horder & Martin, 1978; Rager, 1980) or the elaboration of increasingly complex models, involving multifactorial contributions to the process of synaptogenesis (e.g. Fraser & Hunt, 1980; Meyer, 1982a).

This environment has led to a reconsideration of the possible role of neural activity in the detailed ordering of the retinotectal projection (Chung, 1974; Willshaw & von der Malsburg, 1976; Whitelaw & Cowan, 1981). These theoretical proposals have received recent experimental support. During regeneration of the optic nerve in goldfish, neural activity in the nerve was blocked by chronic intraocular administration of TTX (Meyer, 1983; Schmidt & Edwards, 1983). The resulting retinotectal projection was less well ordered than in untreated controls. Furthermore, in optic tecta caused to receive direct innervation from both eyes, the binocular input was found to segregate into 'stripes' (Levine & Jacobson, 1975; Constantine-Paton & Law, 1978; Law & Constantine-Paton, 1981; Springer & Cohen, 1981). This segregation did not occur if neural activity in the optic nerve was blocked by TTX (Meyer, 1982b; Constantine-Paton & Reh, 1983; Boss & Schmidt, 1984).

Given this resurgence of interest it seemed appropriate to examine the effects of visual deprivation on the development of the retinotectal projection. Visual deprivation will affect the pattern of neural activity in the optic nerve but will not produce the total block of neural activity associated with TTX treatment. Xenopus laevis larvae were placed in darkness at stage 35/36 (Nieuwkoop & Faber, 1967) which is before the onset of visually driven electrical activity in the retina (Witkovsky et al. 1976). They were reared in darkness until a terminal experiment
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in which the retinotectal projection was mapped electrophysiologically. Three aspects of the retinotectal projection were assessed:

(i) detailed order in the projection as it is normally mapped;

(ii) the size of the multiunit receptive fields (MURFs) recordable at one tectal locus – this is a quantitative index of the precision with which optic fibres terminating at a given tectal site arise only from ganglion cells at a restricted, retinal locus; and

(iii) the differential depth distribution of terminals from different classes of retinal ganglion cells (Maturana, Lettvin, McCulloch & Pitts, 1959; Keating & Gaze, 1970). A brief account of some of these results has appeared (Dawes, Grant, Keating & Nanchahal, 1984).

METHODS

*Xenopus laevis* embryos were produced in the laboratory by mating adults following injection of human chorionic gonadotrophin. Such embryos were divided into two groups. The control group was raised under normal diurnal lighting conditions. The experimental group was raised, from hatching at stage 35/36 (Nieuwkoop & Faber, 1967), in darkness. Both groups were maintained in tanks containing oxygenated Stearns (+ iodide) solution. Until metamorphosis they were fed three times/week on strained baby food (Heinz). The water was changed once/month. After metamorphosis *Tubifex* worms were supplied once/week, at which time also the water was changed.

Dark-rearing

The animals were reared in tanks in a light-tight cabinet in a light-tight room. This latter was fitted with a light-tight double-entry lock. As a check on the upper limit of ambient illumination within the light-tight cabinets, on several occasions photographic film was exposed within the cabinet for a period of one week. On development, this film (Kodak Plus X Pan Professional Sheet Film 4147) showed no evidence of light exposure. The sensitivity of this film is such that this evidence indicates an upper limit light energy level of $10^{-15}$ J cm$^{-2}$ s$^{-1}$. The actual light level was probably considerably less than this. No visually driven activity is detectable electrophysiologically from an animal under comparable lighting conditions. Moreover, this regime was sufficient to produce marked effects on another system of visual neuronal connections in *Xenopus*, the intertectal system, the maturation of which does seem to require normal visual experience (Keating & Feldman, 1975; Grant & Keating, 1981).

The visually deprived animals do experience a minimal degree of visual exposure during feeding and water changing. Food and water were changed under conditions of very dim red light and occupied about 30 s per week. The cumulative time of visual experience under such limited conditions would, therefore, be about 5 min for a stage-66 animal and 30 min for a one-year-old animal. Precautions to minimize visual experience were also taken during the preparation for terminal electrophysiological experiments. The animals were transported from the animal accommodation to the laboratory in light-tight tanks, in which they were anaesthetized. They were immediately fitted with thick light-occluding eye covers prior to dissection. One eye remained covered during the acquisition of data on the retinotectal projection through the other eye. No differences were noted between response properties early in the experiment and those late in the experiment, that is after the eye had received several hours of visual experience. Similarly, initial recordings through the eye that had been covered throughout the experiment, showed response properties identical to those obtained through the eye that had been exposed for several hours.
Electrophysiological recordings

(i) Mapping the contralateral visuotectal projection

Contralateral visuotectal projections were mapped by standard electrophysiological techniques. In metamorphosing or three-month postmetamorphic juveniles the visuotectal projections were mapped with the animals immersed in Niu–Twitty solution in a transparent perspex dome (Gaze et al. 1974). One-year postmetamorphic juveniles were mapped in air (Gaze, Keating, Szekely & Beazley, 1970).

(ii) The depth distribution of unit types

Units recorded from optic fibre terminals in the superficial neuropil of the optic tectum were classified according to their responses to moving black stimuli, to spots of light and to changes in background illumination. 96% of units were easily classifiable into one of three categories (Keating & Gaze, 1970; Chung, Gaze & Stirling, 1973). 'Sustained' units show little response to changes of background illumination, respond better to small stimuli than to larger ones, and their characteristic feature is a sustained response to an object moved into their receptive field and held stationary there. 'Event' units respond to both 'on' and 'off' of background illumination or light spots within the excitatory receptive field. They respond briskly to stimuli moving across their receptive fields but give no sustained response to stationary stimuli. 'Dimmer' units respond preferentially to large rather than small stimuli and respond to the switching 'off' of a light spot situated within their receptive field. The characteristic feature of these units is their response to background illumination which consists of a burst of activity at each decrement in background illumination, the response becoming more vigorous as darkness is approached. Unclassifiable units were omitted from further analysis.

In normal anurans these different unit types assume a laminar distribution in the superficial neuropil (layers 8 and 9) of the tectum (Maturana et al. 1959; Keating & Gaze, 1970; Chung et al. 1973; Chung, Bliss & Keating, 1974). One of our primary concerns in this paper was whether visual experience was necessary to effect this laminar distribution. To determine this we would ideally have liked to make absolute measures of the depths from the surface of the tectum at which the different unit types are recorded. While this is feasible in Rana it is not in Xenopus. The superficial neuropil of a one-year postmetamorphic Xenopus is only some 200–250 μm thick, which is approximately half that of adult Rana. Moreover the pia mater is thicker in Xenopus than in Rana, so problems of dimpling and drag during an electrode penetration are correspondingly greater in Xenopus. For these reasons the laminar distribution of unit types in normal and in dark-reared Xenopus was assessed by noting the relative positions, within any penetration, of units of different type.

(iii) The plotting of multiunit receptive fields

A recording microelectrode at one locus in the superficial neuropil of the optic tectum may record from only one or from several optic fibre terminals. In the latter case the recording is referred to as a multiunit recording and the receptive field, stimulation of which produces activity in the recording microelectrode, is a multiunit receptive field (MURF). The size of the MURF is an index of the precision of spatial organization in the retinotectal projection. It indicates the size of the retinal locus containing the ganglion cells whose fibre terminals project to the vicinity of the microelectrode position.

The measured size of the MURF may be influenced by the selectivity and sensitivity of the recording microelectrode, and by the level and signal-to-noise ratio of the amplification. We attempted to minimize the possible effects of such variables. Microelectrodes were selected for their capacity to record multiunits. When possible, and this was usually the case, the same recording microelectrode was used for experiments on both light-reared and dark-reared animals. The amplification levels of the signal were constant throughout the experimental series. During multiunit recordings, data from the recording microelectrode were fed through a window discriminator. The level of the window was set 100% above the noise level. For a recording to be characterized as multiunit, we required that it be constituted of at least three different spikes, above the window level, as revealed by differences in spike height and shape.
Analysis in this paper is limited to multiunits of the ‘event’ type (or Class III of Maturana, Lettvwin, McCulloch & Pitts, 1960; Grùsser, Grùsser-Cornehls & Bullock, 1964). Our main purpose was to compare the size of MURFs in normal and in dark-reared animals. The MURFs of different unit types are themselves different in normal animals. Inclusion of different unit types would, thus, have introduced an additional variable. ‘Event’ units are the most commonly encountered.

The sizes of ‘event’ MURFs were plotted quantitatively using computer control of the visual stimulus and analysis of the acquired spike data. Visual stimuli consisted of square dark stimuli moved systematically, under computer control, across a large television screen. The approximate position of the MURF was first determined on an Aimark perimeter as in the procedure by which the contralateral visuotectal projection is normally mapped. The perimeter was then removed and the television screen positioned so that the MURF was approximately centred on the screen. The screen was situated 38 cm from the animal’s eye. At this distance the area of the visual field occupied by the effective stimulus area of the screen was 64.5° in the nasotemporal and 34.5° in the superior–inferior axis of the field. The stimulus routinely used was a dark square of side 6° moved at a velocity of 44° s⁻¹. The background luminance of the screen was 170 cd m⁻² and the contrast between the visual stimulus and this background was 0.98.

The screen was traversed systematically by the visual stimulus. Starting at one corner of the screen the stimulus moved across the screen either vertically or horizontally. It was then stepped 1.5° on a direction orthogonal to the sweep, and the vertical or horizontal sweep repeated. This process was repeated until the entire screen had been traversed by the moving stimulus. Spikes occurring during the traverse were timed and recorded by the computer in a manner similar to that described by Hodos, Dawes & Keating (1982). The computer produced a two-dimensional matrix representation of the screen. This field was divided into 1.5°×1.5° areas and the number of spikes resulting from stimulation of that area was presented as an element in the 43×23 matrix. The measurement of a MURF size involved the pooling of matrices from traverses involving stimulus movement in both horizontal and both vertical directions. The diameter of the MURF was measured from this matrix output – the horizontal diameter from responses to vertical stimulus movement and the vertical diameter from responses to horizontal movement. The diameter recorded was the mean of these two diameters. To eliminate errors of spurious readings due to occasional spontaneous or artefactual spikes, the excitatory receptive field size of the MURF was considered to be that in which individual matrix elements were 10% or more of the maximum element size. It was the diameter of this field that was measured. The computer, also, produced a graphical representation of this information. This involved a pseudo-three-dimensional plot in which the x axis represented the nasotemporal dimension of the field, the y axis the superoinferior dimension of the field and the z axis the number of spikes evoked in response to stimulation of the corresponding 1.5°×1.5° of the visual field.

RESULTS

The contralateral visuotectal projection was mapped in 39 dark-reared animals. 9 of these animals were mapped at metamorphic climax, 7 at three months after metamorphosis, 18 at one year postmetamorphosis, and 5 at three–five years postmetamorphosis. The result from an animal mapped at metamorphic climax is shown in Fig. 1, and that from a one-year-old juvenile in Fig. 2. Both projections show detailed retinotopic ordering comparable to that seen in normal animals. No disturbance of order in either the dorsoventral or nasotemporal retinal axis was observed.

A more quantitative measure of the precision of connections in the contralateral visuotectal projection is given by the size of the multiunit receptive field (MURF) recordable from a single tectal locus. MURF sizes were measured for 29 event multiunits from eight normal animals and for 35 event multiunits from six dark-reared animals. All animals were aged one year postmetamorphosis. The mean
Fig. 1. Contralateral visuotectal projection through the right eye to the left tectum in a stage-66 Xenopus raised in total darkness from stage 35/36. The outline of the dorsal surface of the left tectum is shown above with the midline arrow pointing rostrally. Below is shown the visual field of the animal's right eye. The animal may be imagined as positioned behind the chart representation with the optic axis of its right eye centred on the origin of the chart, looking out at the reader. The disc is the outline of a standard perimetric chart representing the visual field extending out for 100° of visual angle in all directions from the centre. The numbers on the tectal diagram represent microelectrode positions. The corresponding numbers in the visual field representation indicate the localized visual field positions, stimulation of which evoked activity at the corresponding microelectrode position. N, S, T, I - Nasal, superior, temporal, inferior poles of the right visual field. Bar equals 200 µm.
Fig. 2. Contralateral visuotectal projection through the right eye to the left tectum in a one-year postmetamorphic juvenile *Xenopus* raised in darkness from stage 35/36. Conventions as in Fig. 1. Bar equals 200 μm.
Fig. 3. Pseudo-three-dimensional plots of an 'event' multiunit receptive field in (A) a normal one-year postmetamorphic *Xenopus* and in (B) a one-year postmetamorphic *Xenopus* reared in darkness from stage 35/36. (Details in Materials and Methods.) The horizontal axis represents 64·5° of the nasotemporal field axis and the vertical axis represents 34·5° of the superoinferior field axis.

MURF diameters (±s.d.) for the two groups were normal animals 20·5° ± 5·8°, dark-reared animals 21·1° ± 6·9°. The difference was not significant. These values are different from those given in our preliminary communication (Dawes et al. 1984). For that communication we measured receptive field diameter using a criterion of matrix elements containing 50% or more of the maximum element size, whereas here we present data using a 10% criterion (see Materials and Methods). Illustrative plots of MURFs from a light-reared and a dark-reared animal are shown in Fig. 3.

To study the relative depth distribution at which different unit types were recorded, units were classified by standard criteria (see Materials and Methods) into one of three classes – sustained, event or dimmer. The unit’s depth position, relative to that of other unit classes recorded in the same electrode penetration, was noted. For this purpose a total of 89 penetrations were made in fifteen normal
animals and 69 penetrations in eight dark-reared animals. The animals were aged one year postmetamorphosis. In 50 of these penetrations (28 in normal animals and 22 in dark-reared animals) only one unit type was recorded during the penetration. These were thus discarded from the analysis. The remaining 108 penetrations were categorized according to the number of unit types recorded and the relative depths of those unit types. The data for normal and for dark-reared animals are shown in Fig. 4. In 42 penetrations all three unit types were recorded. 30 of these penetrations were in normal and 12 in dark-reared animals. In all 42 penetrations the sustained units were always the most superficial, the dimmer units the deepest, and event units were found at intermediate depths. In 66 penetrations (31 in normal and 35 in dark-reared animals) only two of the three unit types were recorded. In no case did the relative position, in depth, of these unit types deviate from the normal. We conclude from these data that the normal laminar distribution of unit types is present in dark-reared animals.

DISCUSSION

The experiments reported in this paper sought to examine the hypothesis that visually driven neural activity participates in the ordering of connections in the

![Diagram](image_url)
developing contralateral retinotectal projection of *Xenopus laevis*. This is, we believe, the first study involving mapping of the retinotectal projection in a lower vertebrate visually deprived from before the onset of visual function. Our results indicate that visually driven neural activity is not required for the maturational elaboration of a highly ordered projection.

The effects of visual environment on the maturation of visual structures have been investigated in some teleost fish (Grun, 1979; Jeserich & Rahmann, 1979; Rahmann, Jeserich & Zeutsius, 1979; Zeutsius & Rahmann, 1984; Zeutsius, Probst & Rahmann, 1984). Retinal development apparently proceeds normally under conditions of visual deprivation but can be accelerated slightly by visual experience (Grun, 1979). The initial development of optokinetic responses and of tectal structure was not affected by visual deprivation. Prolonged dark rearing did produce deficits in optokinetic behaviour and in tectal lamination and changes in synaptic ultrastructure in the tectum. The topography of the retinotectal projection was not examined in these studies. Moreover, prolonged dark rearing produced marked general effects on motility and growth. The fish did not mature to adulthood and only a few survived 100 days of dark rearing. Given these profound general effects, it is possible that the deficits in visuomotor behaviour and tectal structure were not primary responses to visual deprivation. They may have reflected secondary effects of the disruption of general mechanisms necessary for growth and survival.

The effects of various forms of visual deprivation on the development of the mammalian visual system have been the focus of many investigations (reviewed in Wiesel, 1982; Sherman & Spear, 1982; Fregnac & Imbert, 1984; Boothe, Dobson & Teller, 1985). Dark rearing or binocular lid suture produces some abnormalities in the dorsal lateral geniculate nucleus and visual cortical areas. The topography of the projections is not affected but receptive field properties of cortical cells are less specific and less responsive than normal (Spear, Tong & Sawyer, 1983). Responses in the superior colliculus, following developmental binocular deprivation, display a marked reduction in directional selectivity and a reduced response to the ipsilateral eye (Flandrin & Jeannerod, 1975; Hoffman & Sherman, 1975). It appears, however, that these effects are secondary to defects in the visual cortex or corticotectal pathway since similar effects are produced in normal cats following visual cortical lesions (Sterling & Wickelgren, 1970; Mize & Murphy, 1976). The actual retinotectal input itself does not appear to be affected by binocular visual deprivation.

The maturation of the vertebrate retinotectal system thus seems to be less dependent upon visual experience than does the mammalian geniculocortical system. Visual deprivation alters, but does not eliminate, neural activity in visual pathways. Total blockade of propagated sodium action potentials may be achieved by administration of TTX, a sodium channel blocker. If neural activity has a role to play in the maturation of visual pathways then such a role may be revealed by observing the effects of TTX.
Harris (1980, 1984) addressed this question in an ingenious series of experiments on the developing amphibian retinotectal system. He transplanted an embryonic eye cup from *Ambystoma trigrinum* to an embryonic newt of the species *Taricha torosa*. This latter species manufactures TTX in sufficient quantities to suppress action potentials in a TTX-sensitive species such as *Ambystoma*. The transplanted eye from *Ambystoma* thus grew in an environment in which it was incapable of generating action potentials. The transplant grew, apparently normally, and elaborated a retinotectal projection. Of necessity the topography of this projection had to be assessed neuroanatomically. Evidence for a reasonable degree of retinotopicity was presented. The methodology forced by the paradigm would not have revealed small differences in the organization of the projection.

On the other hand, in the mammalian visual system, Archer, Dubin & Stark (1982) reported that the development of retinogeniculate connections was affected much more profoundly by TTX blockade than by visual deprivation. The ipsilateral retinotectal projection in mammals during development displays an initial coverage of the greater part of the superior colliculus, but then in early postnatal life undergoes restriction to rostral colliculus (Land & Lund, 1979). This restriction is associated with selective retinal ganglion cell death (Insausti, Blakemore & Cowan, 1984). Fawcett, O'Leary & Cowan (1984) showed that TTX blockade of neural activity prevented this cell death and the associated restriction of the ipsilateral retinotectal projection.

A role for neural activity in the ‘fine tuning’ of connections in the lower vertebrate retinotectal projection has been suggested by observations on the effect of TTX blockade during the regeneration of optic fibres, following nerve section, in adult goldfish. Meyer (1983) and Schmidt & Edwards (1983) reported that, while the resulting retinotectal projection showed normal polarity and general retinotopic order, the precision of retinotopicity was considerably reduced compared to that of normal or control regeneration maps. These findings suggest that neural activity does play a role in the refinement of topographic order in the retinotectal projection.

If one were to extrapolate these findings on the regenerating goldfish retinotectal projection to the initial development of the system, and then compare them with our findings that dark rearing does not affect the fine tuning in the developing retinotectal projection, then it might be concluded that it is ‘spontaneous’, i.e. non-visually driven neural activity, that plays this refining role. Willshaw & von der Malsburg (1976) presented a model of the organization of specific connections between two neuronal arrays such as the retina and tectum. This model assumed some initial polarity information in the arrays, but beyond that the major organizing mechanism lay in a postulated coherence of spontaneous activity in neighbouring ganglion cells. Such statistical coherence has been described in retinal ganglion cells of goldfish (Arnett, 1978), rabbit (Arnett & Spraker, 1981) and cat (Mastronarde, 1983).

A test of this hypothesis might be provided by rearing under conditions of stroboscopic illumination. Stroboscopic illumination would be expected to entrain
the 'spontaneous' activity and render the firing of all ganglion cells coherent, thus removing the neighbourhood connotation of such coherent firing. Schmidt & Eisele (1985) report that, in goldfish, the retinotectal projection resulting from regeneration of the optic nerve fails to show refined topography if the fish are exposed only to stroboscopic illumination. Experiments are in progress to determine whether *Xenopus*, reared under stroboscopic conditions, also show disruption of their retinotectal projections.

A somewhat discordant note to the line of reasoning being developed here has, however, been sounded by Schmidt & Eisele (1985). Three goldfish were maintained in darkness during regeneration of the optic nerve. Schmidt & Eisele found that the resulting retinotectal projection showed reduced topographic precision in that the MURF size was larger than normal. Schmidt (1985) takes the view that the coherence of 'spontaneous' activity in neighbouring ganglion cells is alone insufficient to produce topographic precision in the projection. Refinement of topographic order requires additional coherence provided by local visual stimulation.

Further work will be required to determine whether the difference between our results and those of Schmidt & Eisele (1985) reflects a species difference, or a difference between development of the projection and regeneration. It is interesting that the visual discrimination behaviour of goldfish, following optic nerve regeneration in darkness, is not different from that of fish maintained during regeneration under normal diurnal lighting conditions (Leitner, Francis & Gazzaniga, 1982). Moreover, Yoon (1975) reported that the retinotopically ordered compression of the entire visual field onto rostral tectum, that follows caudal tectal removal in goldfish, occurred even if the fish were maintained in darkness.

Little analytical attention has been given to the mechanisms responsible for what might be called a third dimension of the retinotectal projection. Different retinal ganglion cell classes, revealed by different receptive field properties, send their axons to different laminae in the superficial neuropil of the anuran optic tectum (Maturana et al. 1959). This laminar distribution is restored following regeneration of the optic nerve (Keating & Gaze, 1970). Chung et al. (1973) made the intriguing observation that in adult *Xenopus*, maintained under prolonged stroboscopic lighting conditions, this laminar distribution was disrupted. We find no such disruption under conditions of visual deprivation. The juxtaposition of these two findings may indicate that 'spontaneous' activity plays a part in generating the laminar distribution. Tucker & Hollyfield (1977) describe quite marked changes in the inner plexiform layer of dark-reared *Xenopus*. One might have expected this to produce significant changes in the receptive field properties of ganglion cells in dark-reared animals. In terms of the qualitative criteria used to classify receptive field types we did not observe any changes but quantitative studies may reveal functional correlates of the morphological changes described by Tucker & Hollyfield.

The present study is intended as a contribution to a dissection of the mechanisms generating ordered neuronal connections. Such mechanisms may be broadly
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classified into those independent of neural activity and those utilizing neural activity. The latter class may be further subdivided into intrinsic mechanisms utilizing 'spontaneous' neural activity and those requiring environmentally driven neural activity. Our data seems to indicate that in the anuran retinotectal system the normal highly ordered connections are generated without the participation of visually driven neural activity.

REFERENCES


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