The development of the retinotectal projection in *Xenopus* with one compound eye

By JOAN D. FELDMAN² AND R. M. GAZE¹

From the National Institute for Medical Research, Mill Hill

SUMMARY

Double-nasal and double-temporal compound eyes were constructed in *Xenopus* embryos at stages 32 and 37/38. A particular half was removed from the host eye anlage and replaced with the opposite half-eye from the contralateral side of a donor embryo. Control operations consisted of removing a half-eye and replacing it with a similar half from the ipsilateral side of the donor embryo. Whereas in control animals, each half-eye projected its fibres to the appropriate half-tectum, in operated animals each half of the compound eye spread its optic terminals across the entire rostrocaudal extent of the dorsal tectal surface.

The area of tectal surface covered by ganglion fibre terminals was similar in operated animals mapped at successive stages of development to that previously observed in normal animals at equivalent stages. Therefore the factors responsible for the extended distribution of fibre terminals from each half of a compound eye must exist at least from mid-tadpole life, and thereafter be continuously present throughout development.

INTRODUCTION

The retinotectal projections from surgically formed double-nasal (NN), double-temporal (TT) and double-ventral (VV) eyes in *Xenopus* have been previously described (Gaze, Jacobson & Székely, 1963, 1965; Gaze, Keating, Székely & Beazley, 1970; Straznicky, Gaze & Keating, 1971, 1974). To make an NN eye, the temporal half of a developing eye in a stage-32 (Nieuwkoop & Faber, 1967) *Xenopus* embryo is removed and replaced by the nasal half of an eye from the contralateral side of another embryo of the same stage. The compound eye thus formed has two poles of originally nasal origin, and the transplanted half-eye is in harmony with the original half in terms of dorsoventral polarity. In the case of the VV eyes the operation was done so as to preserve the original nasotemporal axis of the transplant.

In the above-mentioned experiments the animals were reared to beyond metamorphosis and were then used, some months to a year or so later, for electrophysiological analysis of the retinotectal projection. In each case, the usual result was that each half of the compound eyes mapped across the entire

¹ Author's address: National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA.
² Author's address: MRC Neuro-Immunology Project, Department of Zoology, University College London, Gower Street, London, WC1E 6BT.
dorsal surface of the tectum. The map from each half-eye tended to be properly
organized in that it was appropriately polarized with respect to the position
of the half-eye, and also internally consistent. Thus in the whole map each tectal
point received input from two places in the visual field, these two being mirror-
symmetrically arranged about the midline of the field (and thus the approximate
line of surgical union in the original operation – Gaze et al. 1963, 1965, 1970;
Straznicky et al. 1971, 1974).

Since the first publication of retinotectal maps from compound eyes (Gaze
et al. 1963) there has been extensive (and inconclusive) discussion of the meaning
to be assigned to the reduplicated projections found in these animals. Thus it
was argued (Gaze et al. 1963) that the ganglion cells of each half of a com-
pound eye might regulate their specificity characteristics at the time of operation
to follow the new high (or low) points of some hypothetical gradient across the
retina. Next, it was suggested (Sperry, 1965) that each half-retina might not
regulate, and that an input to the growing tectum such as two sets of nasally-
specified fibres from an NN eye, might lead to the overgrowth of the caudal half
of the tectum, which is the part specified to connect with nasal fibres. Since
fibres specified as temporal retinal fibres would be postulated to be absent in an
NN eye, a corresponding agenesis of the rostral tectum might be expected.

More recently an attempt was made to evaluate the idea of half-tectal over-
growth, by uncrossing the optic chiasma in animals with one compound eye and
allowing the fibres from the compound eye to innervate the normal tectum while
fibres from the normal eye innervated the tectum to which the compound eye
had previously projected (Straznicky et al. 1971). The results of these experi-
ments showed that both a compound and a normal eye could simultaneously
innervate one tectum. This makes the hypothesis of an overgrown half-tectum
less likely. However, as pointed out by the authors, the experiments do not allow
a distinction to be made between retinal regulation and retinal non-regulation
with spreading of connexions from a specified half-eye over the dorsal surface
of a specified whole tectum. This inability stems from the self-referential nature
of the experimental system where the retina is assessed in terms of the tectum
and vice-versa. These conclusions of Straznicky et al. (1971) are supported by
recent arguments put forward by Hunt & Jacobson (1973a).

Whatever the factors which are responsible for the projection of the ganglion
cells from each half-retina within the compound eye to the entire rostrocaudal
extent of the tectum, it is of interest to know how early they can be demonstrated,
and whether, once present, they persist continuously during development. Since
Gaze, Chung & Keating (1972) and Gaze, Keating & Chung (1974) have map-
ped retinotectal connexions in normal tadpoles it is now possible to study the
projections from tadpoles with compound eyes, and we have done this initially
in animals with one NN or one TT eye.

Since, in a TT compound eye, both temporal poles of the retina project (in
adult life) to the rostral pole of the tectum and the centre of the retina projects
to the caudal pole, it is of interest to ask how the projection develops. In normal *Xenopus* the different modes of growth of the retina and the tectum result in a progressive shift of the projection across the tectum with development. Thus the centre of the retina projects, in the young tadpole, to near the rostral margin of the tectum, whereas in the adult the same retinal cells project close to the geographical centre of the dorsal tectal surface (Gaze *et al.* 1974). And since the cells lying at the geographical centre of the adult tectum have not yet appeared when the axons from central retina first reach the tectum during development (Straznicky & Gaze, 1972), a change in intercellular retinotectal relationships must occur with growth; and the recent demonstration that optic axons form functional synapses early in larval life (Chung *et al.* 1974) indicates that the intercellular changes occurring in the developing retinotectal projection are probably synaptic changes. In this situation it becomes most relevant to know how the projection from the compound eye develops and this paper describes such an investigation.

**METHODS**

**Animals**

The animals used were *Xenopus laevis*. Embryos were produced in the laboratory by treatment of breeding pairs of adults with chorionic gonadotropin. Embryos and larvae were reared in 10% Stearns's solution (Stearns & Kostellow, 1958) and were staged with reference to Nieuwkoop & Faber (1967). When feeding started, the animals were fed on strained Heinz baby soup (beef and liver).

**Operations**

The techniques used to produce compound eyes were those devised by Székely (Gaze *et al.* 1963, 1965) and Straznicky (Straznicky *et al.* 1971). Embryos of the chosen stage were placed in depressions in the wax base of a Petri dish and were covered with 50% Holtfreter's solution (Rugh, 1962) to which 1/10000 MS 222 (tricaine methane sulphonate, Sandoz) had been added as anaesthetic. Operations were performed with sharpened tungsten needles and glass micro-pipettes.

In control operations, embryos at stage 32 were used. The temporal (or nasal) half of the eye was removed and was replaced by a temporal (or nasal) half-eye from the same side of another animal.

Double-nasal compound eyes were made at stage 32 and at stage 37/38. The temporal half of the eye was removed and replaced by a nasal half taken from the opposite side of another embryo. Thus normal dorsoventral polarity was preserved. Double-temporal compound eyes were made in a comparable fashion.

After operation the transplanted half-eye was kept in position by a small glass bridge. Twenty minutes after the operation the glass bridge was removed and
the animal transferred to full-strength Niu-Twitty solution (Rugh, 1962). The
operated eyes were examined 24 h later and failures were discarded.

Retinotectal mapping

The retinotectal projection from the operated eye was mapped electrophysiologically in later larval life, by the methods described by Gaze et al. (1974).

Histology

Tadpoles were fixed in Suza fixative at the end of the recording experiment and serial sections were stained with Holmes' silver method.

RESULTS

Controls: operated stage 32

Of the seven animals in this group, four had a nasal-half of the eye removed and replaced by a nasal-half in normal orientation and three had the temporal-half removed and replaced by a temporal-half in normal orientation.

Six of the seven animals gave normal maps. Fig. 1 shows the result from a nasal transplant and Fig. 2 shows the result from a temporal transplant. The remaining animal had a nasal transplant and the map was probably normal; in this case the eye was wrongly centred and the visual field coordinates were incorrect. No reduplicated field positions were found however, and that part of the field which could be mapped was normal.

NN eyes: operated stage 32

In this group, eight animals were recorded. Seven gave maps with NN type reduplication and one gave no responses. Figure 3 illustrates the result from an animal mapped at stage 53. The other reduplicated maps were of various degrees of completeness and were recorded between stages 52 and 54.

TT eyes: operated stage 32

In this group 18 animals were recorded. Six gave TT type reduplications, eight gave uninterpretable results (in most cases because of too few recording points) and four showed no responses.

A reduplicated map of the TT pattern, recorded at stage 52, is shown in Fig. 4. The other reduplicated maps of this group were recorded between stage 51 and stage 62.

NN eyes: operated stage 37/38

All five animals in this group gave NN maps. Once such result, recorded at stage 57/58, is shown in Fig. 5. Three other animals of this group were mapped at stages 54–55 and in the remaining animal the larval stage was not recorded.
Fig. 1. The retinotectal projection from a newly metamorphosed *Xenopus* in which the nasal half-eye was removed at stage 32 and replaced with a similar half-eye from the same side of a sibling tadpole. The upper diagram represents the optic tectum, seen from above; the numbers on the tectal diagram are electrode positions. Filled circles represent positions from which no response was recorded. The arrow points rostrally along the tectal midline. The lower diagram is the left visual field showing the stimulus positions corresponding to the electrode positions on the right (contralateral) tectum. The perimeter chart extends for 100° outwards from the fixation point. The animal is to be considered as sitting behind the chart, looking at the observer through the centre of the chart (fixation point). N, nasal; T, temporal; I, inferior (ventral); S, superior (dorsal). The map is similar to that seen in a normal animal of a corresponding age. The conventions used in this figure are also used in the other illustrations.
Fig. 2. Projection from a stage-56 *Xenopus* tadpole in which the temporal half-eye was removed at stage 32 and replaced with a similar half-eye from the same side of sibling tadpole. Number '20' represents a field position obtained with the electrode at position 20 but advanced further into the tectum and a similar convention applies to the other map illustrations.
Retinotectal projection in Xenopus

Fig. 3. Projection from a double-nasal compound eye, operated at stage 32 and recorded at stage 53.

TT eyes: operated stage 37/38

Four out of nine animals in this group gave TT maps. Three maps were uninterpretable and two animals gave no responses. A projection from an animal at stage 62, and showing TT reduplication, is illustrated in Fig. 6. The other three reduplicated projections were mapped between stages 58 and 62.

In those experiments where multi-unit receptive field sizes were estimated, we noticed that these tended to be larger rostrally on the tectum than caudally, both in the case of NN and TT eyes. We did not find any consistent histological
Fig. 4. Projection from a double-temporal compound eye, operated at stage 32 and recorded at stage 54.

differences in the ganglion cell distribution at the retinal margin, between NN eyes and TT eyes. The fact that multi-unit receptive fields from caudal tectum are smaller than those found rostrally in both NN and TT eyes (as well as in normal animals) seems therefore to be related more to the nature of the projection at the tectum than to the position of the relevant ganglion cells in the eye.
Fig. 5. Projection from a double-nasal compound eye, operated at stage 37/38 and recorded at stage 57/58.
Fig. 6. Projection from a double-temporal compound eye, operated at stage 37/38 and recorded at stage 62.

DISCUSSION

The first point that we may note is that the control operation where an extirpated half-eye is replaced by a similar half-eye in normal orientation, does not give a reduplicated projection. Thus the trauma associated with the operation to form a compound eye is not sufficient, in itself, to give the reduplication effect. Also, the fact that control operations consistently produce normal maps, whereas compound eyes give rise to reduplicated maps, argues against the possibility that in compound eyes, an unknown proportion of reduplicated
maps are produced by a degeneration of the transplanted half-eye, followed by twinning regeneration from the remaining half-eye. Furthermore, we have recently shown (Feldman & Gaze, 1975) as have Berman & Hunt (1975) that most surgically formed half-eyes go on to reconstitute a normal projection; few give rise to twinning regeneration.

The fact that control operations produced normal maps whereas retinotectal projections from compound eyes showed reduplication, means also that in the presence of temporal fibres, the nasal fibres within a retinal population project to the rostral half-tectum; whereas in the presence of another nasal half-retina, the original nasal fibres of the compound-eye population spread across the entire tectum. Thus if we are to consider the projection from each half of a compound eye as the result of a regulatory phenomenon in each half-eye, the regulation only occurs in these experiments when the remaining half-eye is confronted with a similar half. Such retinal regulation would require the transfer of information mutually between the two half-eyes and the spread of information throughout the whole of each half-eye up to as late as stage 38. Gap junctions such as might credibly be involved in the transfer of this sort of morphogenetic information, have been found to be present between cells throughout the retina only up to stage 31 (Dixon & Cronly-Dillon, 1972). Up to the present time such intercellular junctions have not been demonstrated in central retina as late as stage 38.

An alternative explanation for the mode of projection of each half of a compound eye could involve competition between fibres from a population which was graded in some fashion from, for instance, most-nasal to least-nasal. With such a scheme, in a normal eye, least-nasal fibres would represent the fibres from the temporal pole of the retina (see Gaze et al. 1963); in the case of a TT eye, least-nasal fibres would come from the vertical midline of the eye.

The main result shown here is that when NN or TT eyes are formed at stage 32 or 37/38, the typical compound eye reduplications of the visual projection may be present throughout larval life. Thus it is very likely that the reduplicated projections are to be found from as early as an ordered projection can be mapped. The earliest projection recorded in the present experiments was at stage 51/52.

In larval life the projection from an NN or TT eye resembles that in a normal animal in that in young larvae the projection is restricted to the rostral part of the tectum (Figs. 3 and 4). The experiments show no difference between NN and TT eyes, in the rate at which caudal tectum becomes innervated.

Another property of the normal tadpole map which is shown by the compound NN and TT maps in larvae, is the non-uniformity (Gaze et al. 1974) of the projection. Thus in a normal Xenopus larva the visual projection is distorted in that there is an expanded representation over the dorsal tectum of the temporal pole of the field. In the larval NN projection this distortion tends to be seen at both nasal and temporal poles of the field (Figs. 3 and 5). In the larval TT projection the expanded representation is found particularly at the centre...
of the visual field (Figs. 4 and 6). Thus in both NN and TT larval projections, the expanded representation occurs over that part of the tectum which is growing – dorsocaudal tectum (Straznicky & Gaze, 1972).

The mechanisms underlying the expanded representation of part of the visual field on dorsocaudal tectum are not understood, either in the compound projections of the present paper or in the normal animal (Gaze et al. 1974). The fact that multi-unit receptive fields tend to be smaller in caudal tectum than in rostral tectum is presumably related partly to the histological structure of the developing tectum, where the number of fibres apparently arborizing in rostral tectum is much greater than those doing so more caudally (Gaze et al. 1974).

In all the operated animals presented here, the polarity of host and donor segments of the compound eye remained as they were at the time of the operation. This finding is in marked contrast to those of Hunt & Jacobson (1973b) and Hunt (1974) where the polarity of both segments of a compound eye were seen to change in cases where eyes were compounded from non-identical fragments, e.g. a nasal and ventral combination.

The compound NN retina appears to grow in a similar manner to the normal retina; that is, at the ciliary margin (Feldman & Gaze, 1972). If the TT retina grows also in this fashion, and if the tectum connected to the compound eye grows in a normal manner (Straznicky & Gaze, 1972), then the shift of the visual projection with growth of the visual system will be very much more extensive in these animals than in the normal. In a TT projection, for example, both temporal poles of the retina project, in the adult, to rostral tectum and the centre of the retina projects to caudal tectum. In the larva, as we show in this paper, the entire visual projection from a TT eye projects to rostral tectum. Thus with growth through the larval stages to beyond metamorphosis, the projection of the central retinal ganglion cells moves from rostral tectum to the caudal pole. The retinotectal projection in *Xenopus* therefore retains extensive plasticity up to at least stage 38, when optic fibres are about to reach the tectum.

**REFERENCES**


Retinotectal projection in Xenopus


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