The distribution of fibres in the optic tract after contralateral translocation of an eye in *Xenopus*

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**SUMMARY**

In *Xenopus* embryos of stage 30 the right eye was translocated, without rotation, to a left host orbit. Shortly after metamorphosis the visuotectal projection through the operated eye was mapped electrophysiologically and shown to be normal dorsoventrally but reversed nasotemporally. Labelling of small groups of retinal axons with HRP showed that the fibre trajectories from dorsal and ventral retina were normal, whereas fibres from nasally placed retina had diencephalic pathways and tectal terminations typical of temporal fibres, and fibres from temporally placed retina had diencephalic pathways and tectal terminations typical of nasal fibres. Thus from just beyond the chiasma the fibres had already achieved the major uniaxial rearrangement necessary to establish a normal tract distribution despite the eye translocation. The fibre rearrangement required to permit the formation of a nasotemporally inverted visuotectal projection appears, therefore, to occur not on the tectum or in the optic tract, but either within the nerve or at the chiasma.

**INTRODUCTION**

During normal development, optic fibres in *Xenopus* come to form a retinotopically ordered distribution of terminals across the surface of the optic tectum. The fibres of the optic tract are also ordered, although in a different manner from that found on the tectum.

The tectal distributions of optic fibres have been studied in a variety of experimental situations where the embryonic eye had previously been operatively disturbed. It has been found that after (1) rotation or contralateral translocation of the embryonic eye (Gaze, Feldman, Cooke & Chung, 1979), (2) the formation of double-nasal, double-temporal or double-ventral ‘compound’ eyes (Gaze, Jacobson & Szekely, 1963; Straznicky, Gaze & Keating, 1974) and (3) the construction of eyes with abnormally positioned ‘pie-slice’ grafts of retinal tissue (Conway, Feiock & Hunt, 1980; Willshaw, Fawcett & Gaze, 1983; Cooke & Gaze, 1983), the visuotectal maps later recordable electrophysiologically show abnormalities which in most cases reflect the nature of the operations done. Rotated eyes give rotated maps. Contralaterally translocated eyes give maps in which the dorsoventral organization is correct whereas the nasotemporal order is

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reversed. Compound eyes give projections in which the two halves of the operated retina map in orderly superimposition across the tectum to give a reduplicated map. In pie-slice eyes, the graft projects to the same region of the tectum as the host part of the retina of similar positional origin, to give a map with a localized reduplication.

All these observations point to one major conclusion, which is that whatever the nature of the retinal operation, the destination of the fibres from each group of retinal ganglion cells depends not on their final position in the eye but on their original embryonic position before operation. This means that fibres from cells which have been displaced with respect to their normal position in the orbit must have rearranged themselves somewhere in the pathway between the eye and the tectum. We would like to know whereabouts this fibre rearrangement occurs, since this could be relevant to our understanding of how the tectal distribution of optic axonal terminals is formed.

The fibres in the normal amphibian optic tract are also retinotopically ordered (Scalia & Fite, 1974, Rana; Fujisawa, Watanabe, Tani & Ibato, 1981a, Rana; and 1981b, Cynops; Steedman, 1981, Xenopus; Fawcett & Gaze, 1982, Xenopus). In experimentally modified visual systems, it is found that fibres from compound eyes in Xenopus run in the region of the optic tract which is appropriate to fibres from the type of retina forming the compound eye. Thus fibres from a double-ventral eye all run in the medial branch of the optic tract (Straznicky, Gaze & Horder, 1979), fibres from a double-nasal eye spread out widely across the tract and enter the tectum through both medial and lateral branches, and fibres from a double-temporal eye approach the tectum as a narrow group running up the middle of the tract (Steedman, 1981; Fawcett & Gaze, 1982). In this paper we discuss the distribution of fibres in the optic tract after contralateral translocation of the eye. We show that, after such an operation, the required reorganization of the optic fibres has already occurred by shortly beyond the optic chiasma.

METHODS

Embryonic operations

Adult Xenopus were induced to mate by injection of chorionic gonadotrophin. Viable embryos were sorted and raised in 10% Niu-Twitty solution at room temperature. For operations the embryos were dejellied and staged according to the normal table of Nieuwkoop & Faber (1967). Selected embryos were anaesthetized in 1:4500 MS222 (tricane methane sulphonate, Sandoz) in 66% Niu-Twitty solution, and positioned for operating on a wax base. The left eyes of stage-30 host embryos were excised with tungsten needles and replaced by right eyes grafted from donor embryos at the same stage, particular care being taken to ensure that the dorsoventral orientation of the graft was normal (Fig. 1). Small glass bridges were used to hold the graft in place whilst healing occurred. The operated embryos were then allowed to recover in oxygenated 50% Niu-Twitty solution. All animals were reared to metamorphosis in aerated 10% Stearns solution at 22°C, and fed on Beef and Vegetable dinner (Boots) and subsequently on fresh Tubifex worms.

Visuotectal mapping

Under anaesthesia with MS222 (1:3000) the optic tecta of the newly metamorphosed animals were exposed and photographed. The animal was then positioned on a Plasticine plinth inside a
Perspex sphere containing a solution of 1:9000 MS222 in oxygenated Niu-Twitty solution. The sphere was then placed at the centre of an Aimark perimeter with the eye under investigation centred and the other eye covered with a Plasticine shield. A hole at the top of the sphere permitted the insertion of both an indifferent electrode and a recording microelectrode into the saline. The recording electrode was glass-insulated tungsten, tipped with platinum, and had a tip diameter of 10 \( \mu m \).

All recording was performed in a darkened room. Visual stimulation was by means of a black disc which subtended 10 degrees at the eye and which was moved against an illuminated background on the perimeter arc. The microelectrode was placed serially at various positions on the optic tectum and lowered into the tissue until a maximal extracellular multi-unit response was picked up on visual stimulation. The response was passed into a conventional AC amplifier of high input resistance and bandwidth 100 Hz–10 kHz, and displayed on an oscilloscope as well as being channelled through an audioamplifier to a loudspeaker. In successfully recorded animals the skin overlying the tecta was sutured back into position and localized application of horse radish peroxidase (HRP) was made to the operated eye.

**HRP labelling**

The anaesthetized animals were positioned so that the area of retina to be labelled was uppermost. The sclera was pierced and a fine needle inserted into the vitreous. The retina was lesioned by being crushed between the needle and a pair of forceps held against the outer surface of the sclera. Small pieces of recrystallized HRP (Boehringer grade 1) were inserted through the sclera and allowed to dissolve in the region of the lesion. The animals were then allowed to recover in oxygenated 50% Niu-Twitty solution.

**HRP processing**

48 h after the application of the HRP the animals were deeply anaesthetized in 1:1500 MS222 and perfused through the heart with 0.25 M-sucrose followed by 2.5% gluteraldehyde in 0.1 M-phosphate buffer at pH 7.4. The brains were dissected free, demembranated, and placed in fresh fixative at 4°C for 1 h. After washing for 30 minutes in phosphate buffer the brains were reacted using the cobalt DAB method of Adams (1977), at 4°C. Reacted brains were dehydrated in ethanol and cleared in methyl salicylate for viewing as whole mounts.

![Diagram of eye translocation](image)  

**Fig. 1.** The nature of the operation. A right eye is translocated, without rotation, in place of a left eye. N, nasal; T, temporal.
RESULTS

The contralateral translocation of an eye, without rotation, reverses the nasotemporal axis of the eye but leaves the dorsoventral axis unaltered. In this paper we concentrate on the organization in the tract of fibres representing the nasotemporal axis of the eye.

In normal *Xenopus*, fibres from temporal retina run from the chiasma to the tectum in a tightly organized group which passes up the middle of the optic tract, and enters directly onto rostral tectum, where the fibres terminate (Fig. 2A,B). Fibres from nasal retina, on the other hand, pass up the diencephalon widely dispersed across the entire width of the optic tract. These fibres then mostly travel through either the medial or the lateral branch of the tract, to reach their region of termination in caudal tectum (Fig. 2C,D). Dorsal fibres are confined to the posterior edge of the diencephalic tract, turn caudally at the tectodiencephalic junction into the lateral branch of the tract and innervate lateral tectum. Fibres originating in ventral retina are found in the anterior part of the tract, course round the medial edge of the tectum in the medial branch of the tract and terminate medially on the tectum.

Thirty-three animals with translocated eyes on the left side were mapped after metamorphosis. Thirteen gave visuotectal maps which showed an inversion of the nasotemporal axis. One of the others gave an uninterpretable map with several reduplicated positions and the remainder either failed to connect or gave too few tectal responses to permit a map to be drawn. These are not considered further.

In several cases the final position of the operated eye showed a partial rotation in the orbit. This varied in different animals from 30° clockwise to 50° anticlockwise as judged by the position of the ventral fissure. In nine of the thirteen recorded animals there was a discrepancy between the orientation of the eye and the orientation of the map. In each case the map showed an anticlockwise rotation relative to the eye, and this varied in different animals from 30° to 110°. The mismatch between the position of the eye and the orientation of the map, and the fact that it was consistently in one direction, are intriguing observations, for which we can at present offer no explanation.

Since the destination of each ganglion cell fibre depends on the embryonic origin of its parent cell, rather than the final position of the cell in the eye, we have used the information derived from the visuotectal map to identify retinal position. In

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Fig. 2. (A) Photograph of a normal postmetamorphic *Xenopus* brain in which fibres from temporal retina had been labelled with HRP. The fibres form a compact band and terminate rostrally on the tectum. In this and the following photographs and drawings (except Fig. 6) the brain is seen from the dorso-lateral aspect, caudal is upwards and dorsal to the right. (B) *Camera lucida* drawing of the same preparation. Bar equals 1 mm. (C) Photograph of a normal brain in which fibres from nasal retina had been labelled with HRP. The fibres are widely distributed across the tract and pass via both branches to terminate caudally on the tectum. (D) *Camera lucida* drawing of the same preparation. Bar equals 1 mm.
Optic fibre distribution in translocated Xenopus eyes

Fig. 2.
this way we have been able to label quadrantic positions in the eye despite the varying degrees of rotation of the eye and of the map.

Of the thirteen successfully recorded animals, four had nasal (i.e. originally temporal) retina labelled, four had temporal (i.e. originally nasal) retina labelled, two ventral retina and three dorsal retina. In nine cases the labelled optic fibres reached the tectum via the optic tract. In three cases the fibres entered the brain by an abnormal route and in one case the fill was unsuccessful.

**Nasal retinal fills**

In all four cases where the nasal pole of the retina was lesioned and filled, the labelled fibres, from ganglion cells of originally temporal character, behaved as do normal temporal fibres. The distribution of the filled fibres in the diencephalic region of the optic tract was typical of fibres of anatomically temporal origin. The fibres coursed dorsally as a tight group in the central region of the optic tract. At the tectodiencephalic junction, they passed directly onto rostral tectum where they terminated (Fig. 3).

**Temporal retinal fills**

In three of the four animals in this category the labelled fibres from the temporal pole of the retina, coming from ganglion cells of originally nasal character,
Fig. 3. (A) Photograph of a brain in which fibres from nasally positioned retina in the transplanted eye had been labelled with HRP. The narrow band of fibres and their rostral tectal terminations are typical of temporal fibres. (B) Camera lucida drawing of the same preparation. The neuropil halfway up the tract is the neuropil of Bellonci and that near the ventral surface of the brain is the basal optic neuropil. Bar equals 1 mm. (C) Visuotectal map through this translocated eye. In this and the following maps the drawing on the right is a dorsal view of the right optic tectum, seen from above. The numbers represent electrode recording positions and the arrow points rostrally, along the midline. The diagram on the left represents the visual field of the translocated eye. N, S, T, I = nasal, superior, temporal, inferior. The eye is to be considered as being on the far side of the chart, looking out at the observer through its centre. The chart extends for 100° outwards from the centre. The numbers on the chart represent optimal positions for the visual stimulus for each of the correspondingly numbered tectal positions. Field positions corresponding to lateromedial rows of tectal positions have been joined with lines so as to make the orientation of the map more obvious. It may be seen that in comparison with a map from a normal left eye (shown in Fig. 3D) this map is reversed nasotemporally and rotated by some 60°–70° anticlockwise. (D) Visuotectal map from a normal left eye.
behaved as do normal nasal fibres. They were found spread across the width of the
diencephalic optic tract and showed no evidence of any coherent grouping. At the
tectum they entered both the lateral and medial branches of the tract and coursed
around the tectum to terminate in caudal neuropil (Fig. 4). In the fourth case the
fibres followed an abnormal pathway to the tectum, although the map could not be
distinguished from the other three.

Fig. 4. (A) Photograph of a brain in which fibres from temporally positioned retina in
the translocated eye had been labelled with HRP. The wide distribution of the fibres in
the tract, and their caudal termination on the tectum, are typical of nasal fibres. (B)
Camera lucida drawing of the same preparation. The Bellonci, basal optic and pretectal
neuropils are shown. Bar equals 1 mm. (C) Visuotectal map through this translocated
eye. The map is reversed nasotemporally and rotated by some 60°-70° anticlockwise
(compare with normal map in Fig. 3D).
Ventral and dorsal fills

In the three animals of this category in which fibres from the translocated eye followed the optic tract, fibres arising from dorsal and ventral poles of the retina behaved as do fibres in unoperated control animals. Ventral fibres, spread over the anterior part of the diencephalic tract, swept dorsally around the front of the tectum to enter the medial branch of the tract and terminated medially on the tectum (Fig. 5). Dorsal fibres formed a narrow band in the posterior part of the

Fig. 5. (A) Photograph of a brain in which fibres from ventrally positioned retina in the translocated eye had been labelled with HRP. As in a normal projection, the fibres all turn medially in the tract and end medially on the tectum. (B) Camera lucida drawing of this preparation. Bar equals 1 mm. (C) Visuotectal projection through this translocated eye. In comparison with a normal projection (Fig. 3D) the map is reversed nasotemporally and rotated some 50° anticlockwise.
diencephalic tract, turned abruptly at the tectodiencephalic junction to enter the lateral branch of the tract, and terminated laterally on the tectum (Fig. 6). In two animals fibres from the retina reached the tectum via grossly abnormal pathways.

Fig. 6. (A) Photograph of a brain in which fibres from dorsally positioned retina in the translocated eye had been labelled with HRP. As in a normal projection, the fibres turn laterally and end laterally on the tectum. This preparation is seen from the ventrolateral aspect. (B) Camera lucida drawing of this brain. Bar equals 1 mm. (C) Visuotectal projection through this translocated eye. In comparison with a normal projection (Fig. 3D) the map is reversed nasotemporally and rotated by some 40° anticlockwise.
Abnormal pathways

In three animals the paths taken by the optic fibres were abnormal. In two cases fibres entered the brain via the oculomotor root and both tecta were innervated. In the third animal the filled optic fibres entered the brain at the ipsilateral tectodiencephalic junction, forming a dramatic striped projection over the rostrotemporal tectum. In all three cases the visuotectal maps and the distribution of the tectal terminations were indistinguishable from the cases where normal pathways were taken.

DISCUSSION

The retinotectal pathway in Xenopus comprises nerve fibres arising from the ganglion cells of the retina and projecting to the main visual centre, the optic tectum. In recent years considerable progress has been made towards understanding the nature of the ordering of fibres in the normal amphibian optic pathway (Scalia & Fite, 1974; Gaze & Grant, 1978; Bunt, Horder & Martin, 1979; Fawcett, 1981; Fujisawa et al. 1981a; Steedman, 1981; Cima & Grant, 1982; Fawcett & Gaze, 1982; Holt & Harris, 1983; Reh, Pitts & Constantine-Paton, 1983; Scalia & Arango, 1983; Holt, 1984).

The arrangement of fibres in the Xenopus optic tract must reflect the various developmental influences that have helped to form it. Prominent among these are: 1) Time. The approximately concentric growth of the retina, which occurs throughout larval life, is reflected by the fact that the radial, age related, dimension of the retina is represented by an inner–outer distribution of optic fibres in the nerve and tract (Gaze & Grant, 1978; Cima & Grant, 1982, Xenopus; Dawnay, 1979, goldfish, abstract; Reh et al. 1983, Rana; Fawcett et al. 1984, Xenopus). 2) Fibre–pathway interactions. Optic fibres are able to choose the correct branch of the tract as they approach the tectum (Attardi & Sperry, 1963, goldfish; Straznicky et al. 1979; Steedman, 1981; Fawcett & Gaze, 1982, Xenopus; Fujisawa et al. 1983a, Rana). 3) Fibre–fibre interactions. It has been found that in instances where fibres of similar retinal origin from two eyes, or from two halves of a compound eye, project to the same tectum, they terminate in the same general region of the tectum yet show segregation into discrete stripes or patches (Levine & Jacobson, 1975; Constantine-Paton & Law, 1978; Fawcett & Willshaw, 1982). In two other cases it also seems likely that fibre–fibre interactions are involved: in goldfish, regenerating fibres are able to recognize tectal ‘markers’ provided by a previous, abnormal, fibre population, and terminate accordingly (Schmidt, 1978); and when regenerating fibres from a compound eye are made to innervate a normal tectum, they form a restricted distribution of terminals, alongside fibres of similar retinal origin from the normal eye (Gaze & Straznicky, 1980).

In the various forms of compound and pie-slice eyes, where the integrity of the eye has been destroyed at operation, the ganglion cells are abnormally ordered
relative to each other. Interactions, involving some form of fibre–fibre recognition between these abnormally arranged retinal fibres, could play a part in the establishment of the fibre order that is seen. The results of earlier experiments led to the suggestion that the retinal ganglion cells are labelled according to their embryonic position within the eye anlagen (Sperry, 1943, 1944, 1945) and recently molecular gradients in the chick retina, which might be used as positional markers, have been described (Trisler, Schneider & Nirenberg, 1981). If the growing nerve fibres could recognize and use such labels, restoration of the embryonically specified neighbour relations could occur by relative reordering amongst the fibres themselves.

There are two kinds of operation where the integrity of the eye is preserved: rotation of the eye within the orbit, and translocation to the contralateral orbit.

Unfortunately, early eye rotation grossly disturbs and misaligns the two ends of the optic stalk along which the nerve fibres grow from the ventral part of the eye to the ventral surface of the forebrain. Even if the distal optic stalk does connect with the brain, the paths taken by the ingrowing optic nerve fibres are often very abnormal. The fibres are in many cases not confined to the optic tract and therefore cannot be usefully analysed in the manner used in these experiments.

When the eye anlage is translocated from one side of a donor head to the opposite side of a host head, without rotation, the nasotemporal axis of the retina of the translocated eye will be reversed with reference to the rest of the animal, yet the dorsoventral orientation of the eye will be normal. Such operations facilitate connection between the two opposed ends of the optic stalk. In visuotectal maps made from translocated eyes the nasotemporal axis is reversed while the dorsoventral axis is not.

To achieve this type of projection the fibres arising from the nasal and temporal poles of the retina must rearrange their relative positions in the projection, leaving the dorsoventral relationship between the fibres normal. Such a uniaxial rearrangement cannot be achieved by any simple rotation of the array of fibres as a whole. To give the projection found, there must be an extensive reorganization of the fibres. For example the populations of fibres from nasal and temporal margins of the eye could pass through each other to invert the nasotemporal axis while preserving the orientation of the dorsoventral axis. Since the diencephalon is growing as successive generations of optic fibres enter the tract, fibres already in the tract become enmeshed among other fibre networks and glial structures. This makes any rearrangement of relative fibre order within the tracts impossible.

By moving the eye as a whole the positioning of the ganglion cells relative to each other is undisturbed, so there is no basis for reordering based upon fibre–fibre recognition to take place within the eye or the nerve. However, once the fibres from the grafted eye reach the host tissue they are incorrectly positioned with respect to the brain. Any rearrangement of the fibres in the pathway should therefore be due to interactions with the surrounding tissues rather than between the fibres themselves.
In our experiments we have used HRP labelling of selected populations of fibres in translocated eyes to look for such fibre rearrangements in the optic tract and tectum. The results show that throughout the diencephalic optic tract and on the tectum, fibres from nasal parts of the eye behave as would normal temporal fibres; and temporally positioned retinal fibres behave as would normal nasal fibres. Fibres arising from the dorsal and ventral poles of the eye behave as in normal control animals. This means that the relative reordering of the fibres is achieved before they reach the main part of the diencephalic optic tract; that is, the rearrangement must occur between the optic nerve head and just beyond the contralateral side of the chiasma. This rearrangement appears to involve a fibre–pathway interaction, in which the nerve fibres are responding to some aspect of the environment through which they are growing.

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REFERENCES


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