Retino-tectal projections from half-ventral and half-dorsal eye rudiments in Xenopus

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SUMMARY

When the ventral half of a developing eye in Xenopus larvae was removed at stage 32, the remaining fragment rounded up and developed into an eye which looked macroscopically normal by mid-larval stages. Eyes from half-dorsal rudiments were usually small, had more than one ventral fissure, and showed abnormal pupils. The contralateral retinotectal projection was always found to be normally ordered when mapped in later tadpole stages, or, just after metamorphosis. No mirror-image duplicated maps were seen, as was found previously in eyes deriving from half-nasal and half-temporal rudiments.

It is concluded that the ‘rule of distal transformation’ does not apply to eyes which are generated from embryonic rudiments.

INTRODUCTION

In a previous paper (Feldman & Gaze, 1975) the nasal or temporal half of the developing eye in Xenopus larvae was removed, and in later tadpole life the contralateral retino-tectal projection was mapped. It was found to be normal in two-thirds of the animals, and a mirror reduplicated map was observed in the remaining animals. An analogy between the regenerating limb stump and the development of an eye from a partial rudiment was considered. It was proposed (McDonald, 1976) that an eye with the centre removed might reduplicate its pattern of retinal projection. If this were true then the generation of eyes from half-ventral and half-dorsal eye fragments should produce retinae which project normally, or retinae which project in a mirror reduplicated fashion about the horizontal axis. The proportion of these two results should be similar to that seen from eyes derived from half-nasal or half-temporal fragments. Normal maps would result from those half-eyes in which the centre had not been removed, i.e. where the residual eye fragment was slightly more than half; reduplicated maps would result from those eye fragments in which an exact half or fragment slightly more than half had been removed. Although it was possible to choose to make one-third and two-third eye fragments in order to test the hypothesis more directly, it was decided to make approximately

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half-ventral and half-dorsal eyes as a first step, for two reasons: first, to allow a direct comparison to be made with the results observed (Feldman & Gaze, 1975) when half-nasal or half-temporal eyes were made; second, to test that it was in fact possible to obtain optic outgrowth from half-dorsal eyes, since at stage 32 when these eye fragment operations are done, the embryonic fissure in *Xenopus* closes; and if this is not closed in time, eye formation will be defective with blindness likely to result (Lopashov & Stroeva, 1964). Removal of the ventral half eye is most likely to interfere with the tissues in the region of the embryonic fissure, and the latter reason was held to account for the failure of double-dorsal ‘compound’ eyes to develop optic nerves (Straznicky, personal communication).
METHODS

The operations to produce half eyes, and the method of mapping the retino-tectal projections were similar to those previously described (Feldman & Gaze, 1975). However, a different dome was developed to facilitate mapping of the inferior visual fields of experimental tadpoles. The dome is illustrated in Fig. 1, which shows that in place of the half-sphere described in Feldman & Gaze (1975), a complete sphere, made by gluing two 'Perspex' half-spheres together, was used. Circular holes were then cut at the top and bottom of the globe, and the tadpole fixed on a strip of Plasticine mounted on a rubber bung which fitted the inferior circular hole.

Half eyes were made at stage 32 (Nieuwkoop & Faber, 1967). Animals were mapped either during tadpole life or after metamorphosis.

RESULTS

The eyes which developed from originally ventral fragments were indistinguishable from normal at the time of mapping, and only one ventral fissure was present (Fig. 2A). The half-dorsal rudiments resulted in eyes which were often two-thirds the size of normal (Fig. 2B). Sometimes the eye was misplaced forwards as compared with the normal eye, the pupil was most often half the normal size, and two or three fissures were often present (Fig. 2B).

Optic nerves were seen in all animals examined with Holmes' silver stain; the optic nerves from dorsal rudiments (Fig. 2C) were not as well developed as they were in eyes from half-ventral fragments, where they appeared normal.

Control normal animals were mapped in the new type of dome, and the inferior visual field was seen to be recorded easily (Fig. 3).

The distribution of experimental results was as follows. Sixteen animals with eyes derived from half-ventral fragments were recorded; these all showed normal retino-tectal projections (Fig. 4). No mirror-image reduplicated maps were seen, either, in animals with eyes developed from half-dorsal fragments. In the latter series of experiments, out of a total of 16 animals four normal maps were recorded (Fig. 5). Incomplete maps but with sufficient points to indicate a normal ordering of points and no reduplication, were observed in seven animals. Unlocalizable responses were recorded from three animals, and responses could not be elicited at all in two tadpoles.

DISCUSSION

It can be seen from Figs. 4 and 5 that practically the whole visual field could be very easily mapped in these animals. Figure 4, for example, shows a very far ventral field corresponding to the rectal surface explored, which probably represents almost the entire surface of the tadpole tectum.
Fig. 2. (A) Left half-ventral eye from *Xenopus* ½V/6, photographed 2 months post-metamorphosis. The eye looks normal. The arrow points to the ventral fissure. (B) Left half-dorsal eye from *Xenopus* ½D/2, photographed 2 months post-metamorphosis. The eye is out of shape, and two ventral fissures can be seen (arrows). (C) Left-half-dorsal eye from *Xenopus* ½D/11. ON, Optic nerve. Calibration 100 μm.
Fig. 3. Projection of left visual field upon right optic tectum in normal *Xenopus* tadpole at stage 61, using spherical globe. The upper diagram represents the right optic tectum seen from above; the numbers in this diagram are electrode positions. The lower diagram is the left visual field showing the stimulus positions corresponding to the electrode positions. The superscript ‘a’ represents a field position obtained with the electrode at the same position as that at the number marked without an ‘a’; however, the electrode is advanced deeper into the tectum. The perimeter chart extends for 100° outwards from the centre of the visual field. 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 pole; T, temporal pole; I, inferior (ventral) pole; S, superior (dorsal) pole.
Fig. 4. Projection of left visual field from a half ventral fragment in *Xenopus* ½V3 upon right optic tectum, recorded at stage 56.
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Fig. 5. Projection of left visual field upon right optic tectum from a half-dorsal fragment in Xenopus ½D/2, recorded 2 months post-metamorphosis.
Mapping of retino-tectal projections from eyes which developed from dorsal fragments proved to be more difficult than in the case of eyes derived from ventral fragments. Responses were usually smaller in amplitude, more easily fatiguable, and often unlocalizable. Why this occurred is not known.

In the animals of this series, both retino-tectal projections from eyes which had developed from half-ventral, and those from half-dorsal fragments, were normal. In no cases was mirror-reduplication present. These results differ from those of Berman & Hunt (1975), who reported that in 30% of eyes from ventral rudiments, mirror reduplication was observed. Half-dorsal rudiments were not made by these authors. The present results also contrast with our previous observations (Feldman & Gaze, 1975), where in projections from half-nasal or half-temporal eyes, 30% of cases showed mirror-reduplication.

The reasons for the discrepancies in the results from our laboratories and those of Berman & Hunt are not known. In the same way, the difference between two further sets of results remains unexplained. McDonald (1976) found normal maps when mid-line lesions were made in embryonic eyes of stage 32 in Xenopus, whereas Hunt & Jacobson (1974) reported reduplicated maps when mid-line lesions were made across embryonic Xenopus eyes. It could prove that the resolution of such differences would throw light on the factors which determine polarity. Our results for example, might predispose towards the idea that retinal cells arranged along dorso-ventral and nasotemporal axes have different properties, as has been suggested by the cell adhesion experiments of Gottlieb, Rock & Glaser (1976). Alternatively, differences in the concentrations of operating solutions employed by different laboratories might be held to account for variance in results. In the present experiments, for example, half eyes were removed with the embryo in a medium comprising 50 vol. of Holtfreter's solution, 5 vol. of Steinberg's solution and 45 vol. of distilled water.

These results do not provide any support for the notion that the 'rule of distal regeneration' may apply to eyes which develop from partial eye rudiments. Neither do the results of a more direct testing of the hypothesis by McDonald (1976). This worker found that peripheral one-third fragments made along several eye axes regenerated normal maps in 50% of cases.

REFERENCES


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