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Evidence for Plasticity of Intertectal Neuronal Connections in Adult Xenopus [and Discussion]

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Evidence for plasticity of intertectal neuronal connections in adult *Xenopus*

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Each optic tectum in *Xenopus* receives two visuotectal projections of the binocular portion of the visual world, one from each eye. These two visuotectal projections at each optic tectum are superimposed and in register, an arrangement made possible by the functional organization of a topographically ordered system of intertectal neuronal connections. Following surgical rotation of a larval eye in *Xenopus* a rearrangement of intertectal connections may take place during metamorphosis. The nature of the intertectal reorganization is such that the two visuotectal projections at each optic tectum remain in register. This synaptic reorganization requires visual experience.

The capacity of the intertectal system to respond to such changes in interocular geometry reduces with age but even in adult life a residual plasticity of intertectal connections exists. In the adult animal an acute rotation of one eye by 90° evokes no reorganization of intertectal connections. An equivalent cumulative change in interocular relationship in adult life which is achieved very gradually rather than acutely can, however, lead to an appropriate reorganization of intertectal connections. It is suggested that the normal role of this residual intertectal plasticity is to compensate for the normal changes in interocular geometry that occur with growth in the post-metamorphic animal.

In the South African clawed frog, *Xenopus laevis*, experimental alteration of the normal relation between the two eyes by, for example, the surgical rotation of one eye in larval life, can result in a rearrangement of the pattern of neuronal connections linking the rostral regions of the two optic tecta (Keating 1968; Gaze, Keating, Székely & Beazley 1970; Keating 1975*a*; Keating, Straznicky & Gaze, in preparation). Two conditions necessary for such a rearrangement to occur are firstly, that the surgery be performed before a certain 'critical period' which covers the later stages of metamorphic climax and early post-metamorphic life (Keating, Beazley, Feldman & Gaze 1975; Keating & Feldman, in preparation) and secondly that the animal be permitted binocular visual experience during that period (Keating & Feldman 1975; Keating 1975*a*; Keating, in preparation).

A clue to the normal developmental role for this experimentally demonstrable plasticity may be provided by considering the normal physiological function of the intertectal system. The organization of the anuran visual system involves a projection of retinal fibres from the eye to the contralateral optic tectum. These fibres are distributed across the optic tectum in a retinotopic fashion, and serve to produce a map of the visual field of one eye on the surface of the contralateral tectum. This contralateral visuotectal projection may be mapped electrophysiologically. A polysynaptic intertectal pathway links the rostral regions of the right and left tectum, and provides the second stage of a pathway from an eye to the ipsilateral optic tectum. This ipsilateral visuotectal projection may also be mapped electrophysiologically (Gaze 1958; Gaze & Jacobson 1962; Keating & Gaze 1970). The topography of the intertectal connections is revealed by observing the transformation of the visual field projection as it is transmitted from one tectum to another. In the normal animal the intertectal system is organized so as to link the loci in the two tecta which are activated from a single locus in the binocular portion of the visual field. Thus at either tectum the maps of binocular visual space through the two eyes are in register, and this congruence is achieved by the intertectal system.

During normal development, however, the geometrical relationship between the two eyes changes. The eyes migrate from a lateral to a fronto-superior position on the head and the interocular distance increases as the skull grows. Just before metamorphic climax the two eyes are 5 mm apart and the angle between the two ocular axes is 130° while by late adult life the eyes are 20 mm apart and the interocular angle is 50°. If one defines as 'corresponding pairs' those pairs of retinal loci, one in each retina, looking at a common locus of binocular visual space then the change of interocular geometry with development means that the pairs of retinal loci which are actually 'corresponding' will change continuously during growth. Since a 'corresponding pair' of retinal loci projects directly to a pair of tectal loci, then as the retinal corresponding pairs change with growth so the 'tectal corresponding pairs' must also change. To preserve a detailed congruence of the ipsilateral and contralateral visuotectal connections from metamorphosis to late adult life, the intertectal connections must always link 'corresponding' tectal pairs. If such tectal pairs change with changing interocular relations, then the intertectal connections themselves must undergo a continuous adjustment. This remodelling of intertectal connections could be achieved by a mechanism, involving binocular visual experience, such as that generating the plasticity of intertectal connections in response to rotation of one eye. We have suggested that this represents the normal developmental role of intertectal plasticity (Keating 1974; Keating *et al.* 1975).

If this suggestion is correct, then fairly large scale remodelling of intertectal connections is required during metamorphosis when relative interocular movement is great, smaller scale remodelling is required during early juvenile life when relative interocular movement is small and only very slight adjustments are involved in post-juvenile life since the interocular changes occur only very gradually at this stage. Our previous eye rotation experiments have demonstrated that the potential plasticity in the intertectal system is large during the early phases of metamorphosis (Keating *et al.* 1975) and is progressively reduced during later metamorphosis and in the first two months of post-metamorphic life (Keating & Feldman, in preparation). This work indicated that the capacity for change of the intertectal system, as shown in the experimental situation, parallels the capacity that would be required by the system were it to play the developmental role that we have suggested.

What then required demonstration was that at later stages of post-metamorphic life, the intertectal system was still capable of a small scale plasticity. It must be able to respond to changes in binocular geometry which, although they may be cumulatively considerable, involve at any one time only very small changes. The problem was that our original experimental approach is not capable of detecting very small changes, because very small eye rotations in adult life are very difficult to maintain, the eye promptly de-rotates. In addition, our mapping technique while capable of revealing intertectal changes corresponding to rotations of 25° and more, does not possess the resolution necesssary to detect the much smaller changes that would correspond to those occurring in normal post-metamorphic development.

To study the reaction of adult intertectal connections to continuous small changes in binocular geometry advantage was taken of a process that had been viewed previously as contributing to the failure of an experiment, namely, the de-rotation of experimentally rotated eyes. This phenomenon of eye de-rotation occurs in some 25–40 % of our animals. The majority of these de-rotations occur in the first few days after the operation and the greater part of the remainder de-rotate during metamorphic climax. In a small number of animals (some 5 % of the total number operated) the eye remained rotated until after metamorphosis but then slowly de-rotated in adult life.

Since the de-rotation in these latter animals was very gradual, an opportunity was provided to examine the reaction of adult intertectal connections to gradual change and to compare it with their response to rapid changes. In this paper the resultant pattern of intertectal connections in animals in which one eye de-rotated spontaneously and gradually in adult life was compared with that in animals in which a rotated eye was suddenly de-rotated by surgical means in adult life. A preliminary account of this work has already appeared (Keating 1975*b*).

METHODS

Larval *Xenopus laevis* between stages 50 and 57 (Nieuwkoop & Faber 1967) were anaesthetized in a 1:2000 solution of MS 222 (Tricaine-methanesulphonate). One eye was freed from the periorbital tissue with tungsten dissection needles, rotated by either 90 or 180° about the optic axis and held in its new position for some minutes until the periorbital tissue exudate gelled, maintaining the eye in that position. The state of eye rotation was checked two months after the animals had metamorphosed. For the purposes of this experiment the animals were divided into five groups.

Group 1

'Control' animals in which the eye remained rotated until the terminal recording experiment one year after metamorphosis.

Group 2

Animals in which the eye rotation when checked at 2 months after metamorphosis was found to be between 70 and 110°. At this time the eye was surgically de-rotated to the normal position. The animals were then mapped one year after metamorphosis.

Group 3

Animals in which the eye rotation when checked at two months after metamorphosis was found to be between 150 and 180°. At this time the eye was partially de-rotated to a position of some 90° rotation. The animals were mapped one year after metamorphosis.

Group 4

Animals in which the eye rotation when checked two months after metamorphosis was found to be between 80 to 90°, but which then spontaneously de-rotated in the subsequent 10 months to approximately zero degree rotation. The animals were mapped one year after metamorphosis.

Group 5

Animals in which the eye rotation when checked 2 months after metamorphosis was found to be between 140 and 180° but which then spontaneously de-rotated in the subsequent

10 months to between 80 and 100° rotation. The animals were mapped one year after metamorphosis.

Surgical de-rotation

The de-rotations 2 months after metamorphosis were performed in the same fashion as were the larval eye rotations, but the eyes were sutured in the final position with ophthalmic sutures (Ethicon Limited) between the sclera and surrounding skin.

Electrophysiological mapping of the visuo-tectal projections

The animals were anaesthetized with ether and the dorsal surface of the optic tecta were exposed and covered with mineral oil. The animal was decerebrated and 0.5–1.0 mg *d*-tubocurarine was administered intramuscularly. The optic tecta were photographed at a magnification of 50 times and a 1 cm rectangular grid was superimposed on the photograph. The animal was then set up for recording at the centre of an 'Aimark' projection perimeter with the optic axis of the rotated or de-rotated eye centred on the fixation point of the perimeter arc.

The visual projections were mapped by placing a recording micro-electrode serially on positions corresponding to grid intersections on the tectal photograph, and for each tectal position determining the optimal region of the visual field, within which visual stimuli evoked unitary activity recorded through the micro-electrode. The latter consisted of a metal filled glass pipette tipped with gold and platinum, of tip diameter 2–3 μm. Conventional means of amplification and display of the unitary potentials were used (Gaze, Keating & Chung 1974).

RESULTS

The contralateral and ipsilateral visuotectal projections from both eyes, as revealed by electrophysiological recording from a normal animal, are illustrated in figure 1. The four projections are all plotted on one coordinate system, a polar coordinate system centred on the optic axis of one eye, in this case that of the right eye. Plotting the visuotectal projections from both eyes on a common coordinate system permits the direct comparison of those loci of visual space projecting to one tectal locus, since the four visual field projections in any given illustration represent a common area of visual space. The normal congruence of visual input to one tectum through both eyes may be seen by noting, on the field charts for the two eyes, the similarity of the field positions projecting to one tectal locus. It is our practice to select, as the origin for our coordinate system, the optic axis of that eye which has been subjected to surgical interference. Some of the subsequent figures consist therefore, of visuotectal projections centred on the optic axis of the left eye. Left-eye centred projections from a normal animal may be imagined as a left-right mirror reversal of the field charts in figure 1, and such projections are illustrated in Fig. 1 of Keating *et al.* (1975). In fact, the visuotectal projections illustrated in figure 5 of this present paper, although obtained from an experimental animal, do represent normal visuotectal projections with the left eye of the animal centred on the projection perimeter.

Group 1

This 'control' group consisted of eight animals in which one eye had been rotated by 90 or 180° between larval stages 50 and 54. The degree of rotation 2 months after metamorphosis was noted and this rotation was maintained until the terminal recording experiment.

The visuotectal projections of all but one of these animals showed the phenomenon that we described in earlier papers as 'binocular interaction' (Gaze *et al.* 1970; Keating *et al.* 1975). The results are summarized in table 1 and it may be seen from this table that in all cases the contralateral visuotectal projection from an eye reflected either its normality or the degree to which that eye was rotated. The ipsilateral visuotectal projections of all animals except one (NDR 8) 'followed' the contralateral projection from the *other* eye, so that the ipsilateral visuotectal projection from the normal eye was rotated while that from the rotated eye was normal. Thus, despite the rotation of one eye, the two visual projections at either tectum are still congruent. The results in one such animal, NDR4, in which the right eye had been rotated by 90° at stage 53 are illustrated in figure 2. Results obtained in two animals with one

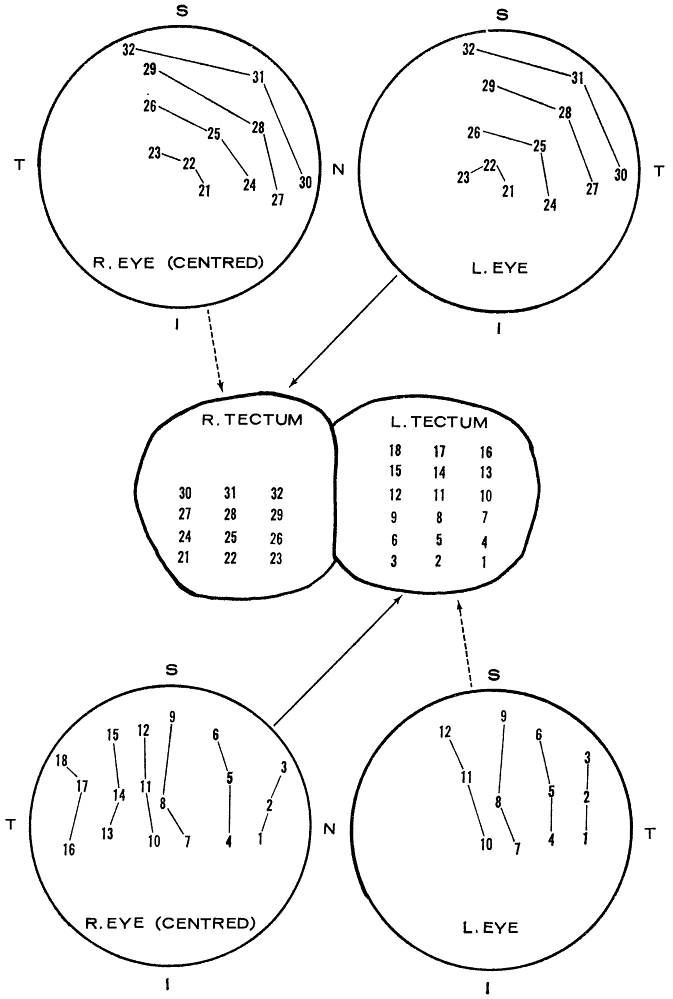

FIGURE 1. Normal visuotectal projections in *Xenopus laevis*. The projection of the visual fields to both optic tecta were mapped with the optic axis of the right eye centred on the perimeter. The tectal diagrams represent dorsal view with the rostral aspect of the tectum directed towards the foot of the page. The numbers on the tectal diagrams represent electrode positions. For each electrode position a corresponding visual field position(s), stimulation of which evoked unitary activity at that tectal position, is indicated by the appropriate number on the perimetric chart representations of the visual field. Contralateral visuotectal projections are indicated by arrows with continuous lines, ipsilateral visuotectal projections by arrows with dotted lines. Since all four projections are mapped with the animal in one position the perimetric charts represent superimposable aspects of visual space, R, right; L, left; N, S, T, I, nasal, superior, temporal and inferior aspects of the visual field of that eye.

TABLE 1. RESULTS FROM EXPERIMENTAL GROUP 1

animal	operated eye	stage of original operation	degree and direction of original rotation	rotation present at 2/12 p.m.	final rotation	contralateral visuotectal projections: operated eye	contralateral visuotectal projections: normal eye	ipsilateral visuotectal projections: operated eye	ipsilateral visuotectal projections: normal eye
NDR 1	left	50	90° c	80° c	80° c	r 80° c	n	n	r 80° c
NDR 2	left	50	90° c	90° c	90° c	r 90° c	n	n	r 90° c
NDR 3	right	51	90° a	100° a	100° a	r 100° a	n	n	r 100° a
*NDR 4	right	53	90° c	90° c	90° c	r 90° c	n	n	r 90°
NDR 5	left	54	90° a	90° a	90° a	r 90° a	n	n	r 90° a
NDR 6	right	50	180°	150° a	150° a	r 150° a	n	n	r 150° a
NDR 7	left	52	180°	180°	180°	r 180°	n	n	r 180°
NDR 8	right	54	180°	180°	180°	r 180°	n	r 180°	n

* Results of this animal illustrated in figure 2.

c, clockwise; a, anticlockwise; r, rotated; n, normal; p.m., post metamorphosis

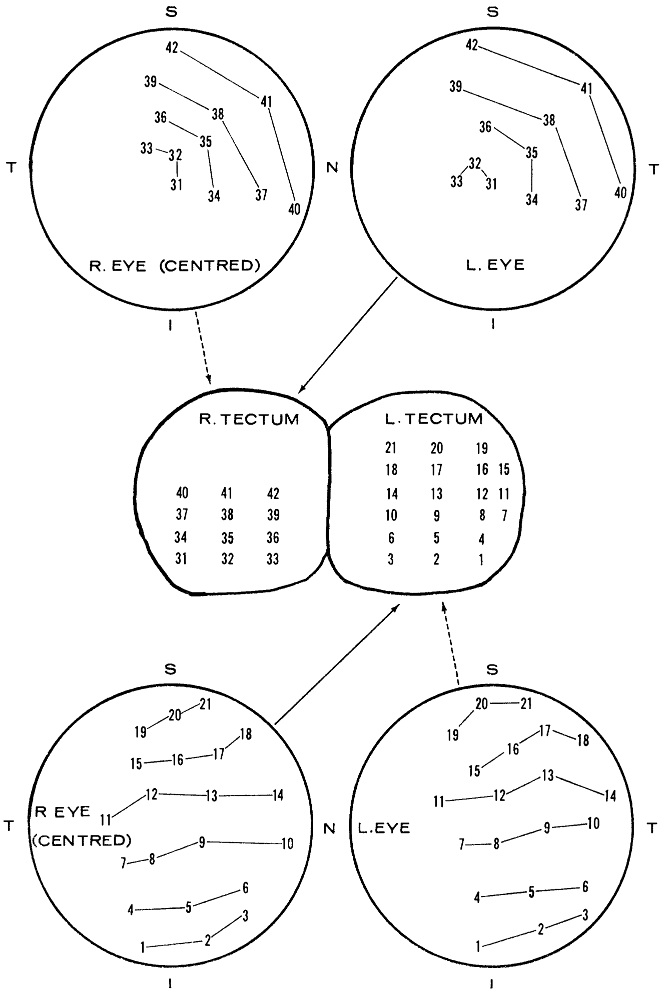

FIGURE 2. Visuotectal projections, with the right eye centred, in animal NDR 4. In this animal the right eye had been rotated by 90° in a clockwise direction at stage 53 of larval life. The animal was mapped one year after metamorphosis. Conventions as in figure 1. Despite the eye rotation the two visual projections to each tectum are still in register, indicating a changed pattern of intertectal connections.

eye rotated 180° between stages 50 and 54, and in which the phenomenon of binocular interaction was observed, gave results similar to those previously described (Keating *et al.* 1975).

These findings indicate that, in these animals, the pattern of intertectal connections was quite different from the normal. The altered pattern was that which was necessary to maintain the congruence of the two visuotectal projections to each tectum.

TABLE 2. RESULTS FROM EXPERIMENTAL GROUPS 2–5

						contralateral visuotectal projections		ipsilateral visuotectal projections	
animal	operated eye	stage of original operation	degree and direction of original rotation	rotation present at 2/12 p.m.	final rotation	operated eye	normal eye	operated eye	normal eye
group 2									
ADR 1	right	50	90° c	80° c	0°	n	n	r 80° a	r 80° c
*ADR 2	left	50	90° c	90° c	0°	n	n	r 90° a	r 90° c
ADR 3	right	52	90° c	110° c	0°	n	n	r 110° a	r 110° c
ADR 4	right	52	90° a	80° a	0°	n	n	r 80° c	r 80° a
ADR 5	left	53	90° c	90° c	0°	n	n	r 90° a	r 90° c
ADR 6	left	54	90° c	70° c	0°	n	n	r 70° a	r 70° c
ADR 7	right	54	90° c	70° a	0°	n	n	r 70° c	r 70° a
ADR 8	left	56	90° c	100° c	0°	n	n	r 100° a	r 100° c
group 3									
ADR 9	right	51	180°	180°	60° c	r 60° c	n	r 120° a	r 180°
ADR 10	right	51	180°	160° c	120° c	r 120° c	n	r 40° a	r 160° c
ADR 11	left	53	180°	150° c	110° c	r 110° c	n	r 40° a	r 150° c
*ADR 12	right	54	180°	170° c	90° c	r 90° c	n	r 90° a	r 180°
group 4									
CDR 1	left	51	90° c	80° c	15° c	r 15° c	n	n	r 15° c
*CDR 2	left	52	90° c	90° c	0°	n	n	n	n
CDR 3	right	57	90° c	90° c	0°	n	n	n	n
group 5									
CDR 4	left	50	180°	180°	100° a	r 100° a	n	n	r 100° a
CDR 5	right	50	180°	140° c	80° c	r 80° c	n	n	r 80° c
CDR 6	right	51	180°	150° c	90° c	r 90° c	n	n	r 90° c
*CDR 7	right	53	180°	160° a	100° a	r 100° a	n	n	r 100° a
CDR 8	right	56	180°	160° a	80°a	r 80° a	n	r 80° a	n

a, anticlockwise; c, clockwise; r, rotated; n, normal; p.m., post metamorphosis

* Animals the results of which are illustrated in Figures.

In one animal, NDR 8, in which the right eye had been rotated by 180° at stage 54, no binocular interaction occurred. Thus the ipsilateral visuotectal projection through the normal eye was normal, whereas that through the rotated eye was rotated. Similar animals in which no modification of the intertectal connections occurred following larval eye rotation were found as a minority group in our earlier series (Keating *et al.* 1975).

Group 2

In each of eight animals in which one eye had been rotated between stages 50 and 56, that eye was observed to be rotated between 70 and 110° at 2 months after metamorphosis and was at that time surgically de-rotated to the normal position. The eye remained stable in this position in the ensuing 10 months until the terminal recording experiment. The visuotectal projections in these animals are described in table 2. All eight cases gave similar results. The contralateral visuotectal projections through both eyes were normal. The ipsilateral visuotectal projection through the *normal* eye was *rotated* and the degree and direction of the rotation was similar to that of the operated eye at 2 months post-metamorphosis before its surgical derotation. The ipsilateral visuotectal projection from the *surgically de-rotated* eye was also *rotated* and the degree and direction of the rotation was similar to that involved in the de-rotation procedure itself. The two visuotectal projections to each tectum were not congruent; two different loci of visual space projected, through the two eyes, to each tectal locus.

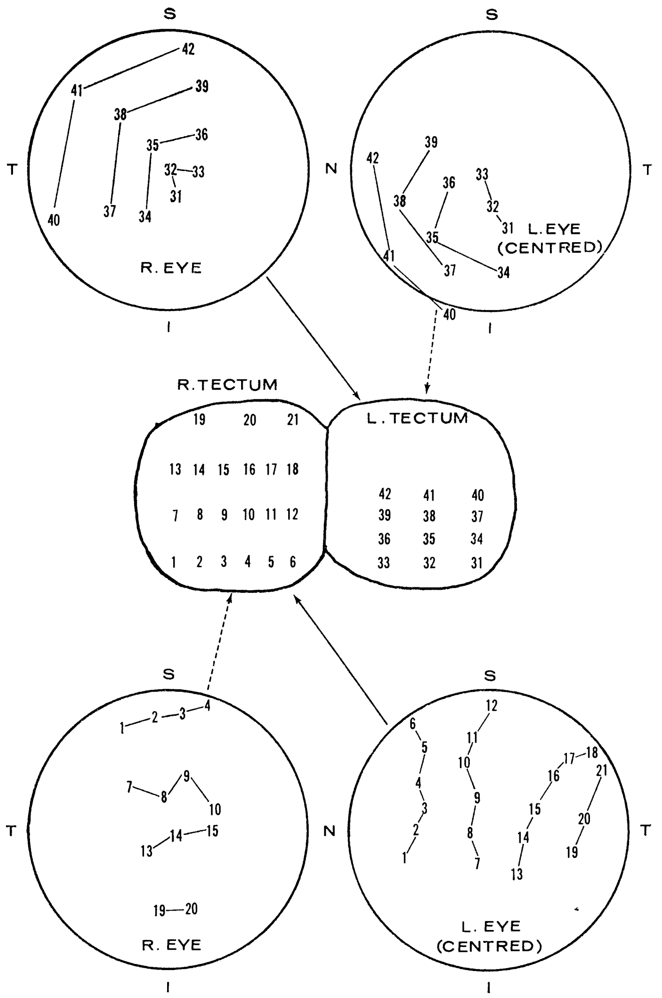

FIGURE 3. Visuotectal projections, with the left eye centred, in animal ADR 2. In this animal the left eye had been rotated by 90° in a clockwise direction at stage 50 of larval life. At 2 months after metamorphosis the left eye was still in a position of 90° rotation and was, at this time, surgically de-rotated by 90° in an anticlockwise direction so as to resume its normal orientation. The animal was mapped one year after metamorphosis. Conventions as in figure 1. The de-rotation has produced a disparity between the two visual projections to each tectum and this has not been corrected.

These effects may be seen in figure 3 which illustrates a typical result obtained in this group of animals. In this animal, ADR 2, the left eye had been rotated by some 90° in a clockwise direction at stage 50 and was still in this position 2 months after the animal metamorphosed. At this time the eye was surgically de-rotated 90° in an anticlockwise direction to return the eye to its normal orientation. The visuotectal projections with the experimental left eye centred on the perimeter are shown in figure 3. The contralateral visuotectal projections through both eyes are normal. The ipsilateral visuotectal projection through the normal, right, eye was rotated 90° in a clockwise direction, and the ipsilateral visuotectal projection through the de-rotated left eye was rotated at 90° in an anticlockwise direction.

These results indicate a pattern of intertectal connections which had changed in response to the initial larval rotation of the eye but had not altered following the surgical de-rotation of the eye at 2 months after metamorphosis.

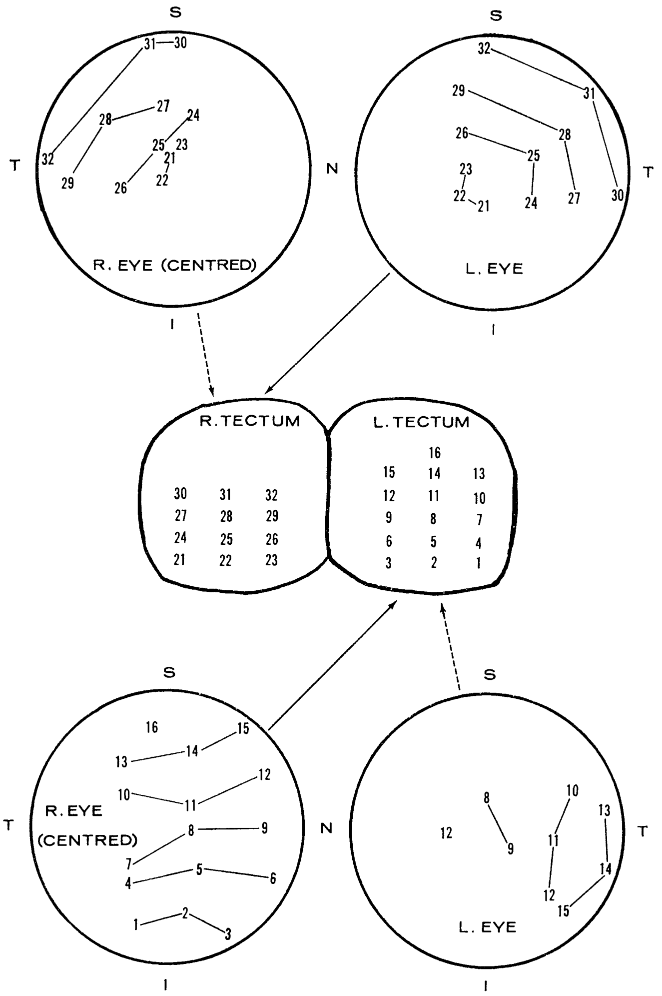

FIGURE 4. Visuotectal projections, with the right eye centred, in animal ADR 12. In this animal the right eye had been rotated by 180° at stage 54 of larval life. At 2 months after metamorphosis the eye was observed to be in a position of 170° rotation in a clockwise direction and was, at this time, surgically de-rotated by 80° in an anticlockwise direction so as to produce a final position of some 90° rotation in a clockwise direction. The animal was mapped one year after metamorphosis. Convention as in figure 1. The partial de-rotation has produced a disparity between the two visual projections to each tectum and this has not been corrected.

Group 3

In each of four animals one eye had been rotated by 180° between stages 51 and 54, the eye was observed to be rotated between 150 and 180° at 2 months after metamorphosis. At this time the eye was surgically de-rotated to 90°. The eyes remained fairly stable in this state of partial de-rotation till the terminal recording experiment 10 months later. The visuotectal projections in these animals are described in table 2. All four animals gave similar results.

The contralateral visuotectal projection through the normal eye was normal, and that through the rotated eye was rotated to the same degree as the eye itself in its final position, between some 60 and 120°. The ipsilateral visuotectal projection through the *normal* eye was *rotated* by about 180° while that through the *operated* eye was *rotated* by between 40 and 120° in an anticlockwise direction.

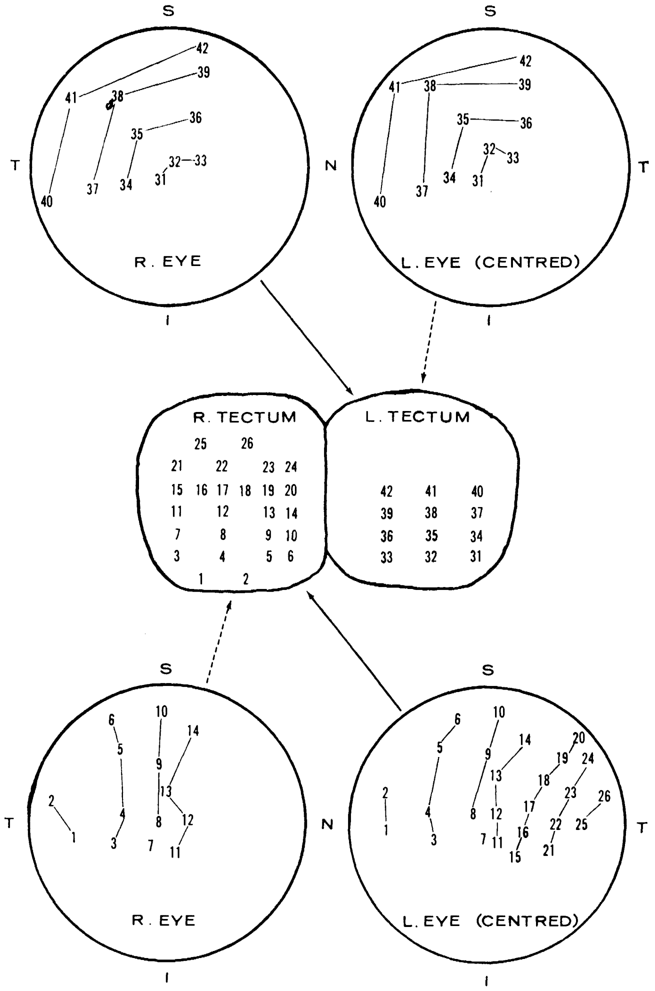

FIGURE 5. Visuotectal projections, with the left eye centred, in animal CDR 2. In this animal the left eye had been rotated by 90° in a clockwise direction at stage 52 of larval life. At 2 months after metamorphosis the eye was noted to be in a position of 90° rotation, but during the subsequent 10 months the eye spontaneously and gradually de-rotated to the normal position. The animal was mapped one year after metamorphosis. Conventions as in figure 1. Following the gradual de-rotation of the eye the two visual projections to each tectum are in register. This indicates that the intertectal connections have changed during the gradual de-rotation.

A typical result is shown in figure 4, which represents the visuotectal projections obtained in animal ADR 12. The right eye had been rotated 180° at stage 54 and at two months after metamorphosis the right eye was observed to be rotated by some 170° in a clockwise direction. At this time the eye was surgically de-rotated to a position of 90° clockwise rotation with reference to the normal position. The contralateral visuotectal projection from the normal eye was normal and that through the rotated eye was rotated clockwise by 90°. The ipsilateral projection through the *normal* eye was *rotated* by 180° while that through the *rotated* eye was *rotated* by some 90° in an anticlockwise direction. The two visuotectal maps at each tectum were not congruent.

As in the previous group, the intertectal connections which had changed following the larval eye rotation, did not appear to undergo a further change following the surgical de-rotation of the eye at two months after metamorphosis.

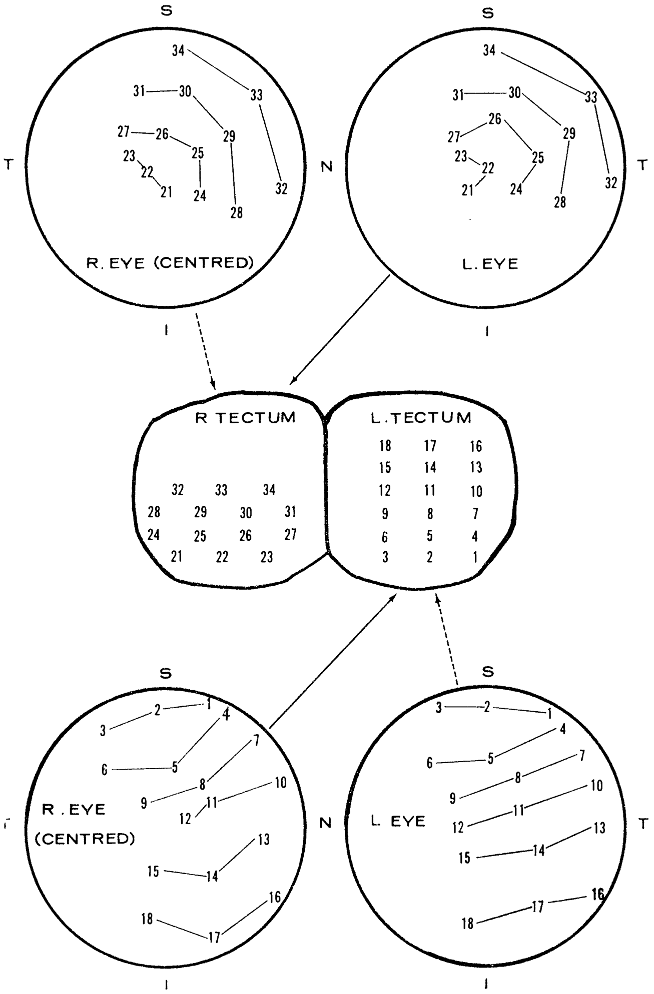

Figure 6. Visuotectal projections, with the right eye centred, in animal CDR 7. In this animal the right eye was rotated by 180° at stage 53 of larval life. At 2 months after metamorphosis the eye was observed to be in a position of 160° rotation in an anticlockwise direction. During the subsequent 10 months the eye spontaneously and gradually de-rotated to a position of 100° anticlockwise rotation. The animal was mapped 1 year after metamorphosis. Conventions as in figure 1. Following the gradual partial de-rotation of the eye the two visual projections to each tectum are in register. This indicates that the intertectal connections have changed during the gradual partial de-rotation.

Group 4

In each of three animals one eye was rotated by 90° in a clockwise direction between stages 51 and 57 of larval life and that eye was observed to be rotated between 80 and 90° at 2 months after metamorphosis. No surgery was performed at this time but during the subsequent 10 months the rotated eye gradually de-rotated so that by 1 year after metamorphosis the operated eye had either actually or almost reassumed its normal orientation. At this time the visuotectal projections were mapped and the results are tabulated in table 2.

All three animals gave similar results. The contralateral visuotectal projections through both eyes were normal. The ipsilateral visuotectal projections through both eyes were normal. The result obtained in one animal of this group, CDR 2, is illustrated in figure 5.

Group 5

In each of five animals in which one eye had been rotated by 180° at a stage between 50 and 56 of larval life, the operated eye was observed to be rotated by between 140 and 180° at 2 months after metamorphosis. No surgery was performed at this time but during the subsequent 10 months the eyes underwent a gradual spontaneous partial de-rotation, so that by 1 year after metamorphosis the eyes were rotated only some 80–100° from the normal position.

Four of the five animals gave similar results. In these four the contralateral visuotectal projection through the normal eye was normal and that through the rotated eye was rotated by 80–100°. The ipsilateral visuotectal projection through the *normal* eye was *rotated* to the same degree as the rotated contralateral visuotectal projection through the *rotated* eye. The ipsilateral visuotectal projection through the *rotated* eye was *normal*. The two visuotectal projections to each tectum were in register in these animals. A result of this type, obtained from animal CDR 7, is illustrated in figure 6.

One of the five animals in this group, CDR 8, gave a different result. In this animal the right eye was observed to be rotated some 160° in an anticlockwise direction 2 months after metamorphosis and this anticlockwise rotation gradually reduced to approximately 80° by one year after metamorphosis. The contralateral visuotectal projection in this animal were the same as in the others of this group. The ipsilateral visuotectal projection from the *normal* eye was *normal* and that from the rotated eye was *rotated* in an anticlockwise direction by 80°. At each tectum the two visuotectal projections were not in register.

DISCUSSION

The experiments reported in this paper were designed to investigate the possibility of a readjustment of intertectal connections in adult life of *Xenopus laevis* when the interocular geometry of the two eyes changes. Previous work (Keating & Feldman, in preparation) had indicated that surgical changes in interocular geometry represented by an acute rotation of one eye by more than 30° in the adult animal did not result in any subsequent modification of intertectal neuronal connections. All these experiments, however, involve rotation of the eye away from its normal position and hence a change in the intertectal connections away from the normal pattern. It may be that a change in intertectal connections from an abnormal pattern to a normal pattern can take place in adult life over a greater range of change of interocular geometry than would a change from a normal pattern to an abnormal pattern. A predilection of the intertectal connections for their normal pattern might be indicated by the finding that an approximately normal pattern of intertectal connections develops in dark-reared *Xenopus* (Feldman, Gaze & Keating 1971; Keating & Feldman 1975).

In the first group of our experimental animals, the so-called 'control', it was confirmed that eye rotation by 90 or 180° in mid-larval life may be followed by a modification of intertectal neuronal connections. The question asked of the group 2 animals was whether the abnormal pattern of intertectal neuronal connections, occurring in response to a 90° rotation of the eye at larval stage 50–56, could be induced to return to the normal pattern by the surgical restoration of the normal interocular geometry. This restoration was achieved by de-rotating to the normal position the previously rotated eye, the de-rotation being performed two months after metamorphosis. The success of the de-rotation was indicated both by the normal position of the ventral fissure in that eye and by the normal orientation of the contralateral visuotectal projection through the previously rotated eye. If, following this de-rotation, the modified intertectal connections had returned to their normal pattern, then the ipsilateral visuotectal projections from both eyes should have been normal. This condition was not found in any of the eight animals of group 2. The ipsilateral visuotectal projections from both eyes were rotated and there was no congruence of the two visuotectal projections to each tectum. The results obtained were those which one would have expected on the basis that the modified pattern of intertectal connections existing at 2 months after metamorphosis did not change in response to the acute de-rotation of the previously rotated eye. It was seen then, that the intertectal system of a post-juvenile *Xenopus* cannot alter its connections in response to a sudden large change in interocular geometry even if the alteration required were in the direction of normality.

The three animals which constitute group 4 of the experimental series underwent a similar 90° de-rotation of a previously rotated eye in adult life, but in these cases the de-rotation occurred spontaneously and very gradually over a prolonged period of time. The normality of all four visuotectal projections in each of these three animals (see figure 5) indicates that the pattern of intertectal connections at 1 year after metamorphosis in these animals was normal. Since these animals had received a larval eye rotation of 90° similar to those of both group 1 and group 2 animals, it seems reasonable to assume that their intertectal connections 2 months after metamorphosis were of the modified pattern. If this were so, then the discovery, one year after metamorphosis, of a normal pattern of intertectal connections indicates that the adult animal is indeed capable of rearranging its functional intertectal connections. Such plasticity seems, however, to be triggered only by a very gradual change in interocular geometry even though over a period of time the cumulative change in interocular geometry may be considerable.

The above conclusion does, however, depend upon the assumption that in the three animals of group 4 the intertectal connections at 2 months after metamorphosis were of a modified pattern. The ideal experimental series would have involved monitoring, by electro-physiological recording, the nature of the intertectal connections in all animals two months after metamorphosis, and recording from each animal again after a further 10 months. In our hands the damage the tectal structures received during the lengthy recording session necessary to obtain all four visuotectal maps has proved too great to permit reliably double-recording of this sort. In addition, of course, one had no foreknowledge at 2 months after metamorphosis, of those particular animals in which the rotated eye would spontaneously de-rotate.

Since we do not possess direct evidence that these animals of group 4 had a modified pattern of intertectal connections at 2 months after metamorphosis, and because such an assumption is central to our interpretation, we must examine further the validity of this assumption. It is possible to construct an explanation for the findings in the group 4 animals which does not require this assumption. It could, for example, be proposed that in a small proportion of animals subjected to a 90° larval rotation of one eye, the intertectal connections do not readjust in response to such a rotation, but remain normal in pattern. (Although no animals of such a nature were observed in our group 1, one of the animals with 180° rotation of a larval eye did not produce a modified intertectal system.) Furthermore, in such animals the continued existence of a normal pattern of intertectal connections might of itself be a causal mechanism in the spontaneous de-rotation of the eye that occurred in adult life. In other words the spontaneous de-rotation was a secondary readjustment to a failure of the intertectal connections to change away from the normal pattern. No such spontaneous de-rotation of an eye was seen in those animals of our earlier series (Keating *et al.* 1975) in which a normal pattern of intertectal connections persisted following larval rotations of one eye. The suggestion of a causal relation between binocular visual neuronal connections and interocular alignment is, nevertheless, certainly tenable and one for which in the mammalian system there is some evidence (Berman & Cynader 1972; Sherman 1972*a*, *b*; Cool & Crawford 1972; Blake & Crawford 1974; Blake, Crawford & Hirsch 1974; von Noorden 1974).

If the explanation for the post-metamorphic spontaneous de-rotation of the eye, in animals of group 4, was the failure of the intertectal connection to change away from the normal pattern following larval eye rotation, then such an explanation should also hold for other animals in which spontaneous de-rotation of an eye occurred in post-metamorphic life. Of interest, therefore, are animals in which spontaneous de-rotation was only partial so that at the time of the terminal recording experiment the initially rotated eye was still rotated but to a considerably less degree than it had been at 2 months after metamorphosis. If, in these animals, the intertectal connections were normal then the ipsilateral visuotectal projection from one eye should reflect the normality or abnormality of the contralateral visuotectal projection from the *same* eye. The animals of group 5 were mapped to see if this prediction held.

Each of five animals in group 5 underwent a 180° rotation of one eye between stages 50 and 56 of larval life and the rotated eye maintained, more-or-less, this degree of rotation until 2 months after metamorphosis. During the subsequent 10 months the rotated eye spontaneously and slowly underwent a gradual partial de-rotation to between 80° and 100° rotation from the normal position. The visuotectal projections in four of these animals, CDR 4, 5, 6 and 7, yielded results indicating that the intertectal connections at the terminal recording experiment were not normal but were appropriate to the final position of partial de-rotation adopted by these eyes. The pattern of intertectal connections was similar to that which was seen in animals in which the eye had been initially rotated by 90° in larval life (group 1). This result may be interpreted as indicating that the intertectal connections had undergone an initial modification appropriate to 180° rotation of one eye, but during the subsequent gradual partial de-rotation, the intertectal connections had undergone a gradual further modification appropriate to the changing interocular geometry.

It is difficult to construct an alternative explanation for these Group 5 animals. One cannot reasonably suggest that these animals underwent a spontaneous de-rotation because their intertectal connections had not been modified in the first instance, since one would then have to explain why in the final instance the intertectal connections are those appropriate to a 90° rotation. Animals in which an initial eye rotation of 90° is produced 2 months after metamorphosis do not modify their connections from a normal pattern to that appropriate to the 90° rotation. The animals of group 5 would have achieved a 90° rotation much later than 2 months after metamorphosis and it is unlikely that their intertectal connections had changed at this later stage if they had not responded earlier.

The findings in these four animals of group 5 furnish support for the interpretation advanced initially to account for the group 4 results. A gradual change in interocular geometry occurring in adult life in *Xenopus* is able to evoke changes in the functional pattern of intertectal connections. The four animals of group 3 were animals in which a similar change in interocular relationship to that which took place in group 5 was induced acutely by surgical de-rotation. One larval eye had been rotated by 180° between stages 51 and 54. Examination of the rotated eyes at 2 months after metamorphosis revealed that the eyes were still rotated by between 150 and 180°. At that time the eyes underwent a surgical partial de-rotation so that they became rotated at 90° in a clockwise direction from normal. The final position of the eye noted one year after metamorphosis, just before the terminal recording experiments, was approximately 90° as may be seen from table 2. Analysis of the visuotectal projections in these four animals indicated that the intertectal connections had indeed undergone a modification in response to the 180° rotation performed in larval life, but had not undergone a subsequent remodification appropriate to the partial de-rotation induced surgically 2 months after metamorphosis. This result is therefore comparable with that obtained from group 2 animals in which an acute surgical de-rotation of about 90° 2 months after metamorphosis did not induce a further modification or restoration towards normal of the intertectal connections. The results in group 5 animals, when compared with those obtained in group 3, produce a similar conclusion to that which emerges from a comparison of experimental groups 4 and 2. This conclusion is that the adult intertectal system in *Xenopus laevis* may respond to considerable changes in interocular geometry provided that these changes take place gradually. In the animals of groups 4 and 5 in this series, the average rate of de-rotation of the eye was of an order equivalent to one third of a degree per day. A similar overall change in interocular geometry taking place suddenly, during the course of one operation, does not produce a similar change in intertectal neuronal connections.

Two animals, ADR 8 and CDR 8, in both of which one eye had been rotated by 180° in larval life, yielded results best interpreted as indicating that no change in intertectal connections occurred in response to such a change in interocular geometry. In previous experiments we have also observed that following larval rotation of an eye in some cases the intertectal connections do change while in others they do not. The reasons for this divergence of results are not entirely clear. It may be that with a degree of rotation of 180°, where one is testing the capacity of the intertectal system to adjust to its limit, one requires optimal conditions which do not always obtain in the laboratory. In the related amphibian *Rana pipiens*, Jacobson (1971), Jacobson & Hirsch (1973), and Skarf (1973) observed no modification of intertectal connections after larval rotation of one eye even when the rotations were much less than 180°. Similar, as yet unidentified, factors may be operating to produce an interspecies difference of results. In the majority of cases in our laboratory in which one larval eye in *Xenopus* is rotated by 180°, and the animal subsequently reared through metamorphosis under normal laboratory conditions, the intertectal connections undergo a modification.

The neuronal connections linking the two optic tecta of *Xenopus laevis* thus seem to provide an interesting example of neuronal plasticity. The system has some unsatisfactory features in that the plasticity is not demonstrable on every occasion under apparently similar experimental conditions. The failure to observe the phenomenon in *Rana pipiens* is disturbing, although the changes in interocular geometry occurring in this animal during and after metamorphic climax are considerably less than those occurring in *Xenopus* ,and it may be that the capacity for plastic readjustments to these connections is much lower in *Rana* than in *Xenopus*. The demonstration of plasticity in *Rana* may require similar gradual changes in interocular geometry to those that have been described in this paper. Beazley (1975) has recently described a further modification of intertectal connections in *Xenopus* following the substitution of one eye by another eye taken from the opposite side of a donor animal.

There are formidable developmental problems facing a system of neuronal connections that has to integrate information from a sensory space which is perceived by sensory receptor areas the interrelationship of which changes geometrically during growth (Bower 1970, 1974; Keating 1974). One possible solution to the problem of integrating binocular visual information would be to pre-wire a system of connections appropriate to the final adult form but which would therefore be spatially inappropriate to the intermediate developmental forms. An alternative, and that which seems to be adopted by *Xenopus*, is to produce a pattern of intertectal connections which modifies itself as an ongoing process in response to the changing interocular geometry. Information about the requirements for such a modification, and the extent required of such a modification, would be available to the nervous system if it had some means of directly identifying those central loci being stimulated from one locus of visual space. Binocular visual function could permit this identification since, as such central loci are continuously activated by common visual stimuli, their spatio-temporal patterns of neural activity should be similar. If the developing system were able to monitor and use this information, and to remould the functional pattern of intertectal connections so as to maintain in register the visual inputs from both eyes to one tectum, then changes in interocular geometry associated with growth would result in an automatic compensation in binocular visual connections in response to such changes.

The general picture emerging from these studies on the intertectal system in *Xenopus* is that a process independent of visual experience produces an approximation to the normal pattern of intertectal connections, but that ongoing processes, which require binocular visual experience, may cause a 'fine-tuning' of these intertectal connections rendering them appropriate to each of the intermediate metamorphic, juvenile, early and late adult situations in each of which the detailed interocular relationship is different. The much more bizarre rearrangement of intertectal connections following surgical manipulation of the developing visual system may represent merely an exaggerated response to abnormal interocular relationships by a mechanism that exists normally only to produce much smaller modifications.

Binocular vision appeared during vertebrate evolution at a level much earlier than that of the mammal. It is nevertheless intriguing to note that it is in the mammalian binocular visual system that the most striking experimental evidence has been obtained for a developmental role of experience dependent modulation of synaptic connections (Hubel & Wiesel 1965; Blakemore & Van Sluyters 1975). Various workers have suggested that the normal role of this developmental plasticity in the mammalian visual system is to adjust to changes in interocular geometry during growth (see Grobstein & Chow 1975; Daniels & Pettigrew 1975). It may well be, therefore, that in species as far apart as *Xenopus* and the cat, and perhaps even man, the nervous system uses a common class of solution to solve the developmental problems associated with binocular vision. There are situations other than binocular vision in which growth produces alterations in the geometry of relationships between the receptor areas or between receptors and detectors. Examples include the auditory system and that integrating sensory-motor coordination. It will be interesting to see whether in these situations the nervous system also opts for a functionally dependent modulation of synaptic connections in order to generate neuronal systems optimally adaptive at all postnatal growth stages.

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Discussion

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The remarkable plastic changes you have demonstrated in the *Xenopus* visual system would seem to be based in the main upon static, somatotopic observations. Bearing in mind that (1) *Xenopus*'s behavioural response is, I believe, greater to moving than static visual stimuli and (2) the animal normally shows marked optokinetic and vestibular stabilizing responses, do you think that retinal image movement and/or motor-induced movement of eye and head has any part to play in the adaptive processes you described?

M. J. Keating We have no information on this interesting point. It is certainly true in other cases of experience-dependent neural maturation that motor responses are a necessary part in the plastic modification that takes place. It would be interesting indeed if this were also the case in the system that I have described.