THE FORMATION OF NERVOUS CONNECTION BETWEEN EYE AND BRAIN DURING NEUROGENESIS

LYNDA DENT TARALA

THE FORMATION OF NERVOUS CONNECTION BETWEEN EYE AND BRAIN DURING NEUROGENESIS

by

Lynda Dent Beazley

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SUMMARY

This study investigates the anatomy, development and regeneration of the ipsilateral visual projection in Anurans, using electrophysiological mapping techniques.

The ipsilateral projection was thought to involve an initial projection to the contralateral tectum; this was confirmed by carrying out various localised lesions in adult Xenopus. The intertectal fibres of the ipsilateral projection were found to travel along the optic tract.

Embryonic operations were carried out to alter the positional relations between the eye and the optic centres; the results of these operations were investigated when the animals became adult. In animals with one rotated eye, a double nasal compound eye or left-to-right transplanted eye, the ipsilateral projection from the abnormal eye was normal, while that from the normal eye reflected the abnormality of the positionally temporal part of the other eye. In animals enucleated during early development, the ipsilateral projection was abnormal in that it was disorganised, and diffuse. Various prefunctional mechanisms of neuronal specification such as biochemical specificity and modulation, are considered as explanations of these results but are found to be inadequate. Points on the two tecta receiving visual information from the same part of visual space via the two eyes are found to have become functionally joined; this happened despite the abnormality of one of the eyes. A hypothesis is put forward that regions on the two tecta receiving similar spatio-temporally organised inputs become

functionally joined; this hypothesis of binocular interaction is consistent with the results from each group of embryonic operations.

The determination of the pattern of the ipsilateral projection was found to be taking place at or after stage 65/66 in Xenopus; however the ipsilateral projection has been fixed when the animal is 2 months post-metamorphic. Ipsilateral visual responses were recorded in a stage 62 Xenopus juvenile.

The ipsilateral visual projection was found to regenerate following section of the intertectal fibres; the regenerated maps were normal. The formation of the ipsilateral connections by regenerating intertectal fibres was found to involve reformation of previous neuronal connections, rather than a binocular interaction between the two eyes as takes place during development.

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INTRODUCTION

General Introduction

Behaviour is directly dependent on the orderly arrangement of neurons in the nervous system into appropriate patterns of interconnections. An understanding of the development of behaviour at a physiological level therefore must be based on studies into the growth and death of nerve cells, their migrations relative to other cells and their differentiation to form mature neurons connected to others or to effector organs. The factors governing these processes have been the subject of much interest and dispute since the beginning of this century. This thesis concerns one of the mechanisms by which growing nerve cells select the cells with which they make connections.

The initial problem of neurogenesis is the extent to which neural connections are either preformed by embryological processes of induction and cell differentiation or are determined by functional regulation through experience. This maturation-versus-learning question has been the subject of much controversy. In 1931 Holt adopted one extreme view and argued that no neuronal connections are embryologically determined. He considered that the nervous system developed as a series of random connections in an equipotential network. As random movements of the organism took place, dendrites were stimulated to grow to axons which were conducting impulses at that time. In this way, he believed all neuronal connections including even simple reflexes were formed.

Since that period, when function was considered to preceed form, information has accumulated to support the idea of the genetic determination of certain parts of the nervous system. The primary sensory pathways, especially the visual system, have been studied in some detail, and the evidence here seems to show that inter-neuronal arrangements are organised without the learning process being involved. Within the last 5 years the experiments carried out to elucidate the mechanisms underlying the particular innervation of individual muscles have been interpreted as supporting the idea that prefunctional specificity determines the formation of neuro-muscular connections (Mark 1969).

However, despite the acceptance of the importance of genetically determined factors in the development of neuronal connections, there is some evidence, at first purely behavioural and more recently also electrophysiological that functional deprivation disturbs normal development of the nervous system.

Both Von Senden (1960) and Gregory (1966) discuss cases of adults blind from birth who in later life gain their sight by an operation for cataract. Although these authors are not in complete agreement,they both stress the lack of depth perception in such people. Von Senden claimed that the majority of the patients were unable to use previously learnt haptic information to identify objects visually. In other words, they could not transfer information from one modality to another.

Hubel and Wiesel (1963, 1965, 1970) have examined electrophysiologically the properties of visual units in kittens and cats. They found that the units in the visual cortex are of the normal adult type at birth, but if normal vision is interferred with from birth to 3 months, there is a critical period during which the connections break down and the units become highly abnormal. Visual deprivation at later stages is without this effect.

The development of visuo-motor co-ordination under conditions of motor deprivation was studied by Held & Hein (1963). In these experiments, pairs of kittens were placed in a roundabout; both animals could see the environment but only one could move his legs to rotate the apparatus. After 72 days, the kittens were tested for visuo-motor skills and it was found that the kitten with motor deprivation during visual experience was functionally blind, and recovered to normal only after several days of normal activity.

It seems clear therefore that function is important for the development of certain functional neural connections and maintenance of normal function of the nervous system. In Anura, the primary visual pathway seems to be genetically determined; it has no functional dependence, Sperry (1951), Szekely (1954) Gaze (1960). However there is a second visual projection in these animals, and there seemed to be some evidence that this projection might not be determined in the same way as the primary one (Keating 1968) but might be dependent on the incoming visual information. It was decided to investigate this phenomenon in more detail electrophysiologically to establish the exact role function plays in the development and regeneration of this system.

Development of the Visual System

The anatomical development of the visual system in amphibia is as yet not fully documented. The development of the eye itself is, however, better understood than that of the tectum, because the eye was amongst the first organs to be studied from the point of view of developmental mechanics.

After the neural plate has closed, both lateral walls of the neural tube, thus formed, evaginate in the region of the prospective fore-brain to give the primary eye vesicles. These protusions have at first a wide communication with the lumen of the neural tube but soon this becomes constricted so that, by the tail-bud stage, the eye vesicles are connected to the brain by narrow eye stalks. The outer region of the vesicles induces an ectodermal lens placode which then invaginates and as a lens vesicle disengages itself from the ectoderm. At the same time, the eye vesicle also invaginates to form a double-walled eye cup. The inner wall of this cup becomes thicker and develops into the retina; the outer wall remains thin and forms the pigment epithelium. Optic fibres then grow out from the retina, along the optic stalk, and cross over at the chiasma, reaching the contralateral tectum (Nieuwkoop and Faber 1956).

The optic tectum is a part of the mesencephalon formed by evagination of the original neural tube. At first it consists of undifferentiated cells; overt differentiation begins when the first axons from the retinal ganglion cells reach the rostral pole of the tectum and cells in the periventricular grey zone migrate outwards finally forming the various layers of the tectum described by Larsell (1931) and Kollros (1953).

The arriving optic axons cross the surface of the optic lobe for various distances before turning ventrally to terminate in layers 7 and 8 immediately below the superficial opticus layer 9, which consists of the incoming retinal axons, axons and dendrites from cells in the deeper layers and a small number of cells. The layering of the tectum begins at stage 47 (Nieuwkoop & Faber, 1956) at the rostral pole of the tectum and gradually spreads caudally, being complete by stage 55 (Nieuwkoop & Faber 1956). Presumably correlated with this innervation and layering of the tectum from the anterior to the posterior pole, is the finding that there is similarly directed gradient of cholinesterase development (Boell et al., 1955).

Differentiation of the tectum seems to depend partly on the incoming optic axons, and it fails to develop properly if this innervation is lacking. Enucleation at an early stage, in the frog, has been shown to result in hypoplasia both of the inner and outer layers of the contralateral tectum. There was found to be a 56% size decrease in the outer layers, 7, 8 and 9, and a smaller but not inconsiderable loss of 38% in the inner layers, 1-6 (Kollros 1953). This size decrease seemed to be due to the absence of optic fibres, the reduction in cell numbers and the incomplete development of some of the cells which remained.

McMurray (1954) carried out repeated nerve crush in Xenopus tadpoles and found that there was a 45% decrease in cell numbers in the contralateral tectum. The extent of this loss was reduced if the optic nerve was allowed to regenerate into the tectum.

Xenopus tadpoles first show consistent visuo-motor responses at stage 49 (Gaze & Peters 1961) when stratification of the optic lobe has started. These authors using the electron microscope made counts of the optic nerve fibres at this stage. They found that there were 5,000 fibres of which less than 10 were seen to be myelinated; in later stage tadpoles, the numbers increased until in the adult 28,250 unmyelinated and 6,000 myelinated fibres were counted.

This increase in fibre number reflects the increase in the size of the eye and tectum as the animal grows. The source of tectal cells is not yet known, but there is autoradiographic evidence that retinal growth takes place at the ciliary margin in the developing Xenopus eye (Straznicky & Gaze 1970). During regeneration of the adult newt retina (Gaze & Watson, 1967) cell division takes place at the ciliary margin and cells migrate towards the centre of the retina. Cronly-Dillon recorded electrophysiologically the retinotectal projection from the regenerating retina in the adult newt (1967). He found that the first part of the visual field to become normally organised was the centre, while the responses from the periphery were still abnormal. This result taken together with that of Gaze and Watson suggests that the retina regenerates from cells at the ciliary margin, which migrate towards the centre of the retina and only then do they differentiate completely to form normal neuronal connections.

Anatomical studies of the projection of the retina onto the optic tectum

Histological techniques which selectively stain degenerating fibres have been used to trace the connections between the retina and the optic tectum. Early workers traced the Marchi degeneration after enucleation of one eye. In this way it was found that in the sub-mammalian vertebrates, the optic fibres decussate completely at the optic chiasma since degeneration was found only in the contralateral tectum (Bellonci 1880; Wlassak 1893; Ramon Y Cajal 1952-55). Marchi studies never revealed degenerating fibres on the ipsilateral side. Recently, however, using Nauta staining techniques ipsilateral fibres have been seen but these appear to terminate not in the tectum but in the hypothalamus and the thalamus (Roye 1965).

Experiments such as those mentioned above in which one eye was removed cannot give any indication whether there is any retinotopic organisation of the optic fibres terminating in the tectum. One technique for investigating the retinotectal organisation is to make lesions in the retina and study the resultant pattern of degeneration. Quadratic lesions of the retina are too gross to show a point-to-point projection of the retina onto the tectum, but such methods used on various species of teleost fish do support it. (Lubsen 1921; Stroer 1939; Akert 1949; Legissa 1955; Attardi & Sperry 1963). These authors found that the superior temporal retinal quadrant projects to the rostro-lateral part of the contralateral tectum; the inferior temporal quadrant projects to the rostro-medial part of the tectum; the inferior nasal quadrant projects to the caudo-medial part; and the superior nasal quadrant projects to the caudo-lateral part.

Stroer (1940) found a similar visual projection in the newt Triturus taeniatus, using the same degeneration technique, and was also able to trace optic nerve fibres from the retina to the tectum. In 1963, Attardi and Sperry used a Bodian-Protargol technique staining regenerating nerve fibres to trace the retino-tectal projection in goldfish. They found that the projection agreed with previous findings. This experiment is discussed later from another point of view (p. 26)

Herrick attempted to trace individual fascicles of nerve fibres from the retina to the tectum in Necturus (1941) and Amblystoma (1942), as Stroer had done using Salmo and Triturus. In most amphibia, including Necturus and Amblystoma but not Triturus, the optic nerve fibres remain fasciculated for only a short distance after leaving the eye; the nerve fibres become randomly interlaced throughout their passage to the tectum (Maturana, 1958). This explains why Herrick was unsuccessful in establishing the retino-tectal projections in the species he studied, using the technique of tracing the path of fascicles of optic nerve fibres. A negative result in no way implies that an orderly retino-tectal projection does not exist in either Necturus or Amblystoma.

Quadrantic lesions followed by degeneration studies have been carried out in many mammals, and the majority suggest strongly that there is at least an area to area projection from the retina to the superior colliculus (reviewed by Hamdi & Whitteridge 1953).

Physiological evidence for the topographical projection of the retina onto the optic tectum

The first studies of the electrical activity of the superior colliculus were not concerned with localisation, potentials being evoked in response to illumination of the whole field of view (Wang 1934; Bernhard 1940) or to electrical stimulation of the optic nerve (Bishop & O'Leary, 1941 & 1942).

Talbot and Marshall (1941) introduced the technique of mapping the central representation of the visual fields, by recording action potentials from a determined position on the visual cortex, evoked in response to a small area light in the visual field. Both cat and Rhesus monkey were used in these experiments. A similar method was used by Apter (1945) to map the projection of the visual fields onto the superior colliculus of the cat. She found that for any position of the electrode, the evoked potentials were of minimum latency and maximum amplitude only at one localised position of the light in the visual field, and so she was able to map the visual projection in detail.

Buser and Dusardier (1953) stimulated the retina with bipolar electrodes 1 mm apart and recorded the potentials evoked in the contralateral optic tectum of the catfish, tench and carp. This method cannot show a detailed projection of the retina onto the tectum, but these workers were able to find a separate projection of retinal quadrants in the carp and the tench which conformed with the scheme of representation found in the bony fish by histological degeneration techniques. In the catfish for technical reasons, it was possible to distinguish only a separated projection from the nasal and temporal half retinae.

The representation of the visual field on the optic centres has been mapped in several species including the pigeon (Hamdi & Whitteridge 1953 & 1954) and the rabbit (Hamdi & Whitteridge 1953) the goat (Cooper, Daniel and Whitteridge 1953).

Gaze (1958) and Matturana, Lettvin, McCulloch and Pitts (1959) established that there is a point-to-point representation of the visual fields onto the optic tectum of the frog. It was found that only the superior half of the visual field is represented on the dorsal surface of the tectum; the inferior half is represented on the ventro-lateral surface and can be reached only by passing the electrode through the tectum. The visual mapping of the frog was therefore restricted to the dorsal surface of the tectum and hence to the part of the visual field above the horizontal meridian. The naso-superior, naso-inferior, tempero-inferior and tempero-superior quadrants were found to project to the rostromedial, rostro-lateral, caudo-lateral and caudo-medial areas of the optic tectum respectively.

Gaze also mapped the retino-tectal projections of Xenopus laevis (1959) and goldfish (Jacobson and Gaze 1965). The newt has been mapped by Burgen and Grafstein (1964) and by Cronly-Dillon (1967); Schwassman and Kruger (1965) have investigated the retino-tectal projection of some fresh water fish. All these species show essentially similar maps.

Gaze and Jacobson (1962) found that in addition to the direct contralateral projection, in the frog there was another retinotopic projection, from the binocular (nasal) part of the visual field (temporal retina) to the rostral area of the ipsilateral tectum. Measurements of the latency of the visual responses to one tectal position through the contralateral and ipsilateral eyes were carried out. These showed that the latencies of the ipsilateral responses were greater than those of the contralateral. For this and for other reasons, the ipsilateral pathway was therefore presumed to be a polysynaptic one. By mapping the ipsilateral and contralateral visual projections before and after localised tectal lesions, it was found that the ipsilateral projection involved initial passage through the contralateral tectum. The intertectal linkage to complete the ipsilateral pathway was not successfully investigated by these authors. The ipsilateral pathway is considered further in the discussion (p. 88).

Regeneration of the optic nerve and recovery of vision

Regeneration of the optic nerve and subsequent recovery of vision was first demonstrated by Matthey (1925a,b). He cut the optic nerve distal to the chiasma in a series of 37 Triton cristatus. Three to five months later the 5 surviving animals seemed to behave normally. He proved that true visual recovery had taken place by removing the normal eye and showing that the animal could still follow a small lure moved outside the tank, and would attempt to attack a worm lowered into the water of their tank inside a glass jar. Dissection revealed that the optic nerve had entered the brain in all 5 animals. Histological examination of 2 of the animals confirmed this.

In the following year (1926) Matthey carried out experiments in which he exchanged left eyes between pairs of adult Triton cristatus. Of the 86 animals operated only 4 survived, of which 3 showed return of vision through the transplanted eye.

Since these original eye transplantations, Stone and his co-workers have carried out not only successful autoplastic eye grafts in urodeles, but also homoplastic and heteroplastic transplants (Stone and Usher 1927; Stone 1930; Stone and Zaur 1940; Stone and Ellison 1940; Stone and Chace 1941; Stone and Cole 1943).

Other experiments demonstrating the phenomenon of optic nerve regeneration are discussed later.

The hypothesis of neuronal specificity

Harrison had in 1910 postulated that growing nerve tips select the appropriate path by chemo-taxis and that certain biochemical properties of the neuron determine the nervous connections made by that cell.

In the 1930's however it was thought that nerves regenerated at random and formed random connections, since it was argued that it was inconceivable that nerve fibres chaotically scrambled at the site of nerve section could regain their original connections. There was also evidence that chemical attraction could not be demonstrated in vitro, whereas mechanical guidance of growing nerve fibres was known to occur both in vivo and in vitro.

Weiss (reviewed 1955) carried out a series of limb transplantations in urodeles and found that if the transplanted limb was sufficiently close to the original one, then the two moved in synchrony. This happened even if the transplanted limb was reversed and therefore its activity was inappropriate in terms of the animal's behaviour. To explain these results Weiss put forward the hypothesis of modulation, which proposed that when regenerated fibres made contact with a muscle, information passed up the nerve from the muscle to the nerve cell body causing changes in the synaptic contacts of the cell. These changes were thought to be such that the nerve stimulated the muscle to function appropriately. By extrapolation, it was assumed that modulation took place during the development of the entire nervous system. As mentioned earlier (p.1) another explanation of these results, namely the initial formation of specific connections during development and their reformation during regeneration, now seems more likely (Mark 1969).

In the early 1940's Sperry carried out a series of experiments to investigate regeneration in the visual system of Amphibia. The experiments were designed to study the mechanism by which the optic nerve regenerates, resulting in recovery of vision. Sperry inferred the pattern of neural connections in the visual system by testing the behaviour of the animal. He used both anurans and urodeles, since these animals can normally locate accurately and capture a lure within their visual field. Another useful behavioural test is the optokinetic response. This is a response to movement of vertical stripes tempero-nasally across the visual field, in which the animal turns his head and sometimes also his body to follow the movement of the stripes; if stripes are moved naso-temporally no response is elicited. When horizontal stripes are moved in the dorso-ventral axis, the animal responds by moving his head in the same direction as the stripes.

Sperry's experiments were designed to investigate the effect, on the pattern of regeneration of neuronal connections, of altering the positional relationship between the eye and the brain. As a control, therefore, he needed to establish the effect of such an alteration without cutting the optic nerve.

Sperry's first experiment was therefore to rotate one or both eyes in adult animals and to examine the behaviour over several months. The animals used were Triturus viridescens, and it was found that responses both to a lure and to moving stripes were reversed in both the dorso-ventral and naso-temporal axes and no correction took place over 4½ months, despite the maladaptive nature of the responses. When eyes were restored to their normal orientation, the animals behaviour was immediately corrected. Stone (1953) carried out a similar experiment in which he rotated an eye and kept the animal for 4½ years before derotating the eye. During this 4½ year period, no recovery of normal optokinetic responses, nor proper localisation of a lure took place. However immediately the eye was rotated back to its normal position, normal visual behaviour returned.

These experiments show that there is no functional plasticity in the newt visual system to allow it to adjust to the reversed positional relationship between the eye and the brain. The next experiment was therefore to rotate an eye and cut its optic nerve to find out if during regeneration of the nerve a central re-allocation of synapses could take place. Such experiments were carried out using adult Triturus viridescens (Sperry 1943b). The animals were divided into 2 groups. In one group the left eye was rotated by 180° and 2 days later the optic nerve was cut; in the other group the left optic nerve was cut but the eye was not rotated. Visual responses in those animals with normally orientated eyes were normal after periods varying from 30-95 days. Sperry suggested that in the animals with a slower rate of recovery, neural degeneration and regeneration of the retina had taken place. The animals with rotated eyes behaved as if their visual field were inverted, and showed no sign of recovery of normal function over a period of 50 days following return of vision.

If re-allocation of central connections were taking place following random regeneration of the optic nerve, it would be expected that normal vision would result. It is not reasonable to expect that central plasticity mechanisms would lead to an ordered but inverted visual field such as Sperry found, since after eye rotation and optic nerve regeneration the animal showed inverted visual behaviour. He therefore concluded that when the optic axons regenerated they reformed their original connections.

As further tests of this hypothesis Sperry carried out 2 other series of experiments, which were designed to alter the relationship between the eye and the tectum in other ways than by complete inversion. One was to carry out left-right eye transplants. Depending on the orientation of the transplanted eye, it is possible to keep the dorso-ventral or the naso-temporal axis unchanged but the other is necessarily inverted. These transplants were done in a series of anurans and the urodele, Triturus viridescens (Sperry 1945).

In another series Sperry uncrossed the chiasma and directed the cut nerves into the ipsilateral side of the brain in several species of Anura. This operation does not alter the relation of the eye to the orbit but results in each eye innervating the wrong (ipsilateral) tectum. The effect of this is to invert the visual input along the mid-sagittal plane of the animal.

The results of these 2 sets of operations also supported the hypothesis that the regenerating nerves chose the correct area of the tectum with which to form connections. In all cases of eye transplants the animals behaved as would be expected from the altered polarity. Thus if the naso-temporal axis of the eye was reversed the optokinetic responses to moving stripes in the naso-temporal axis of the body were reversed but those to stripes moving in the dorso-ventral axis were normal.

In the animals with uncrossed optic nerves, optokinetic responses were reversed about the mid-sagittal plane of the animal. These animals were able to locate accurately only small objects in the mid-sagittal plane; in all other positions the animals mislocated the objects at the corresponding position on the opposite side of the midline.

Sperry (1944) also attempted to show by making localised lesions in the tectum that regenerating fibres return to their original position. In normal frogs Sperry found that lesions in the dorsal part of the optic lobe caused blindness in the superior visual field; a lesion in the anterior pole of the tectum led to blindness in the nasal field and destruction of the posterior tectum to blindness in the temporal field. In animals with regenerated nerves a similar distribution was found, while in animals with rotated eyes and regenerated nerves the representation of the visual field on to the optic centres was found to be reversed.

These experiments seemed to show clearly that on regeneration optic nerve fibres reform their original connections in the optic lobe. The processes of relearning and relocation of synapses, modulation, cannot explain the restoration of reversed vision. It was not possible that fibres were merely growing back along the paths of the degenerating nerve fibres for several reasons. Sperry (1943) showed that following optic nerve section the regenerating fibres become a tangled mass as they penetrate the scar tissue at the site of the cut. Stone in 1930 had shown that following transplantation of an eye in Amblystoma, the stump of the optic nerve proximal to the brain had degenerated before regenerating nerve fibres grew back.

Sperry therefore put forward a hypothesis to explain the mechanisms by which regenerating fibres sort themselves out and find their correct tectal position. This hypothesis of Biochemical Specificity (Summarised by Sperry 1951) states that each retinal ganglion cell including its axon, has a certain unique biochemical property, different from that of any other ganglion cell. The tectal cells each have a unique biochemical label comparable with one or a small number of retinal ganglion cells. Sperry suggested that as each optic nerve fibre regenerates it puts forth many branches and by this "shot-gun" approach, finally makes contact with its partner tectal cell and forms stable synaptic connections. The unsuccessful branches then degenerate leaving synaptic contact with only one tectal locus.

Further evidence for the Biochemical Specificity in the visual system

Sperry relied on the behavioural and lesion experiments to establish the hypothesis of the specificity of neuronal connections in the amphibian visual system. Further evidence that regenerating fibres reform their original connections came in 1959 when both Gaze and Maturana et al. mapped electro-physiologically the visual projections of animals in which the optic nerve had been cut and the corresponding eye had been rotated some months previously. These authors found that the map of the visual field from the rotated eye on the optic tectum was itself rotated.

Gaze and Jacobson (1963) recorded the visual projections in a series of frogs at various times after the optic nerve had been severed. By this means, they hoped to establish the temporal sequence of events leading to the restitution of a normal visual projection. Unfortunately for technical reasons it was not possible to record from an animal, then allow it to recover and re-record at a later date. It was necessary therefore to carry out a large number of experiments and to assume that the results from animals recorded at shorter periods following nerve sections represented earlier stages in regeneration than those recorded after an interval of several months.

Four patterns of nerve regeneration were apparent in the results. Pattern 1, which occurred earliest after nerve section consisted of disorganised regeneration from small regions of the nasal and/or temporal retina. The tectal responses recorded consisted of abnormally wide inconsistent multi-unit receptive fields and therefore a detailed map could not be constructed. All parts of the tectum were innervated, the lateral area gave responses from the nasal visual field, and medial from the temporal field. Pattern 1 could possibly be identified with a stage, such as that postulated by Sperry, when the regenerating axons have far-reaching terminal arborisations covering abnormally large areas of tectum; at present this is merely conjecture. The segregation into lateral and medial tectal areas may depend on the correct choice of the lateral or medial optic tracts by the regenerating fibres. There is histological evidence that such a choice is made as fibres regenerate in the goldfish visual system (Attardi and Sperry 1963).

Pattern 2 was similar to pattern 1 except at the rostral pole of the tectum. The projection to this part of the tectum came from the far nasal visual field and was organised across only one axis of the tectum (medio-lateral) and across the corresponding retinal axis (supero-inferior). If this is indeed a stage towards recovery of a normal projection, that it seems that not only do organised neural connections develop first at the rostral pole of the tectum, but that the retinotectal projection is organised in the medio-lateral axis of tectum before the rostro-caudal one.

If the animals were left for longer periods between cutting the nerve and recording the visual projections, patterns 3 and 4 were recorded. Pattern 3 was the normal visual projection while pattern 4 consisted of the normal or abnormal visual maps and, in addition, 2 extra visual projections.

In all the animals it was the left optic nerve that had been cut. The type of regeneration termed pattern 4 consisted of a normal or abnormal contralateral projection from the left eye to the right tectum along with an anomalous projection from the peripheral nasal part of the left visual field to the rostro-lateral area of the right tectum. In some experiments latency measurements were made. It was found that the latencies of the responses comprising the peripheral nasal projection were significantly longer than those of the more central contralateral projection.

In some animals the projections from the left eye to the left tectum were mapped. It was found that there was an extra ipsilateral projection, which was retinotopically similar to the normal contralateral projection, but a comparison of the latency of responses to the right and left tecta showed that there was a significant difference between those of the anomalous ipsilateral and contralateral projections. The latency of an ipsilateral response was shorter than that of the contralateral one. Further, a lesion of a small area of the ipsilateral tectum receiving an anomalous projection from a certain part of the visual field destroyed the response of the contralateral tectum to stimulation from the same part of the visual field. This led the authors to conclude that some of the cut optic nerve fibres had regenerated ipsilaterally to give a contralateral type of projection on the ipsilateral tectum. This projection had itself then given rise to an intertectal linkage which was manifested by the anomalous contralateral projection mapped from the peripheral nasal field. This conclusion was supported by the observation in histological preparations of some overt ipsilateral fibre growth.

Recently Gaze and Keating (in preparation) have shown that pattern 3 is the end product of regeneration when the optic nerve is crushed leaving the nerve sheath intact, whereas when the nerve is cut and the nerve fibres are splayed out then some direct ipsilateral growth takes place and pattern 4 results.

These experiments to investigate the early states of optic nerve regeneration indirectly support the hypothesis of neuronal specificity in several ways. They provide further evidence that the regenerating fibres return to their previous tectal loci. The existence of patterns 1 and 2 show that the development of a normal visual projection does not depend merely on the sequence in which fibres re-entered the tectum. Further, pattern 4 suggests that any specificity retained by one tectum is exactly mirrored in the other, as Sperry had assumed in his experimental animals with uncrossed chiasmas.

There is further evidence that specific reconnection can occur following regeneration of the optic nerve via highly abnormal paths. Gaze (1959) found that in a Xenopus tadpole the optic nerve had regenerated via the oculomotor nerve route to the tectum. This animal gave no optokinetic responses but some points of a visual projection were mapped. In a more extensive series of Xenopus tadpoles, Sperry and Hibbard (1967) directed the cut optic nerve in the oculomotor route and found that the nerve regenerated along this route, decussated at the level of the third root and again in the region of the optic chiasma. Since the optic nerve therefore entered the ipsilateral tectum, if a normal establishment of neuronal connections were to take place, this would lead to reversed optokinetic responses. This in fact was the result found by Sperry and Hibbard and it adds to the evidence that optic nerve fibres can regenerate and reform their original or homologous connections irrespective of the rate at which they regrow or the sequence in which they re-enter the tectum.

The hypothesis of biochemical specificity predicts that if part of the retina or part of the tectum is destroyed then following regeneration of the optic nerve there will not be any reallocation of the remaining tectal loci.

Westerman (1965) removed 3 retinal quadrants in the goldfish eye by electrocoagulation; he also sectioned the optic nerve. After a sufficient length of time to allow the nerve to regenerate, he stimulated the retina optically or electrically and recorded the tectal responses. He found that the responses from the remaining quadrant of the visual field connected with the appropriate tectal area. The remainder of the tectum was devoid of optic nerve fibres.

Other results which support the hypothesis of a strict place specificity were found by Jacobson and Gaze (1965). They created a size disparity between the retina and the tectum in adult goldfish. In one series they allowed an entire optic nerve to regenerate into either the lateral or medial half of the tectum, in the other series half an optic nerve regenerated into a whole tectum. These authors found, by electrophysiological mapping that no compression or expansion of the visual maps had taken place in these experiments.

However, recently Gaze and Sharma (1970) have found evidence that strict place specificity does not exist in the rostro-caudal axis of the tectum. They removed the caudal half of the tectum in a series of adult goldfish and in some animals the corresponding optic nerve was cut. The visual projections were later mapped electrophysiologically. It was found in fishes with tectal lesion but no nerve cut that the visual field projection which would normally have projected to the caudal tectum was partially restored in an appropriate retinotopic manner to the rostral tectum. In several of those animals with both tectal lesion and nerve cut, compression of the projection of the whole visual field onto the remaining part of the tectum had occurred.

The results of Gaze and Sharma suggest that the concept of a precise point-to-point specificity put forward by Sperry may be an oversimplification of the mechanisms of connection selectivity taking place when the optic nerve regenerates.

Maturana et al. (1960) have identified a number of types of retinal visual units in the frog. These different units terminate at characteristic depths in the superficial tectum. Gaze and Keating (1968) have found that when the optic nerve regenerates this depth distribution is maintained, showing that the optic nerve fibres are specified not only in terms of the antero-posterior and medio-lateral axes of the tectum but also in terms of the relative depth component.

One experimental series which might be thought to discredit the neuronal specificity hypothesis was carried out by Burgen and Grafstein (1962). The eye of adult newts was transected in a plane parallel to the corneo-scleral junction approximately around the equator of the bulb. Most of the retina was removed from both parts of the eye and the pigment epithelium was removed from the central portion in some cases; the front part was then replaced either in its original orientation or rotated by 180°. After 4-7 months the projection of the retina onto the tectum was mapped electrophysiologically. These authors, found that the operations could result in a number of different projections. In some the central part of the visual field projected to an abnormal position on the tectum; in the majority of cases, however, the orientation of the representation of the peripheral area of the visual field corresponded to that of the central part, whether or not it had been rotated. Assuming that the central retina is derived from central pigment epithelium, Burgen and Grafstein's results would suggest that the hypothesis of neuronal specificity does not apply to the visual system of newt.

However the autoradiographic studies by Gaze and Watson (1967) show that the retinal cells of the adult newt regenerate at the ciliary margin of the eye and migrate towards the centre of the retina to their final position; in other words, peripheral pigment epithelium gives rise to central retina. This result explains the consistency of rotation of the central and peripheral parts of the visual projection that was found by Burgen and Grafstein. A further criticism of the experimental technique employed by Burgen and Grafstein is that they may not have allowed a sufficient period of time to elapse between carrying out the operations and mapping the regenerated visual projections. Gaze and Jacobson (1963) found that early stages of regeneration were probably quite different from the final normal visual projection, and since work of Gaze and Watson suggests that regeneration had probably not reached completion in the period allowed by Burgen and Grafstein, then these workers may have been recording early and incomplete stages of regeneration.

The experiments designed to test the hypothesis of neuronal specificity described above examined the growth of regenerating fibres by electrophysiological methods. It is also possible to test the hypothesis by examination of the histological picture of a regenerated system in an attempt to trace nerve fibres to the point at which they form synaptic connections. Both approaches are valid, but each has certain disadvantages. The technique of electrophysiological mapping is extremely valuable since it detects functional connections whereas this cannot be done by histological techniques. It is also possible to be certain that the fibre has made functional synaptic contacts by electrophysiological methods which is not by histological techniques. Electrophysiological techniques of the sort used here however have the disadvantage that they do not allow identification of the path taken by the nerve fibres to reach their destination.

The histological investigation of the regenerating visual system was first carried out successfully by Attardi and Sperry in 1963. They removed specific portions of the goldfish retina and sectioned the optic nerve. After varying periods of time the fish were sacrificed and examined histologically using a modification of the Bodian—protargol technique, which was found to stain normal fibres black and newly regenerated ones a reddish colour. When the dorsal half of the retina was removed the fibres from the remaining ventral half regenerated to occupy only the dorsal tectum; conversely fibres from the dorsal retina entered only the ventral tectum. If the peripheral retina was cut away leaving only the central part, then the regenerated fibres could be seen to cross the inappropriate areas of the tectum, without entering the synaptic layers, and terminating in the appropriate central area of the tectum.

This experiment was especially important because not only did it demonstrate that regenerating fibres regain their original position in the tectum but that the fibres seem to regenerate selectively along their original paths to reach the tectum. For example, fibres from the ventral part of the retina, which normally pass via the medial optic tract to the dorsal surface of the tectum, regenerated among this tract and did not enter the lateral optic tract at all. It seems, therefore, that the hypothesis of the specification of the retina and tectum must be extended to include at least parts of the visual pathway.

Further evidence to support this finding comes from the work on the visual system of cichlid fish. Arora and Sperry (1962) crossed the lateral and medial optic tracts and later tested for the recovery of visual function; they also examined the histology of the regenerated system. Normal vision was found to have returned when the nerve regenerated. The histological picture revealed that the fibres had not grown into the tract ahead of them, but had crossed back and entered the tectum by the normal route.

Arora (1963) repeated this series of cross-union experiments taking care that the fibres could not return to their original tract by implanting them firmly in the foreign tract.

Histological examination revealed that the fibres had grown across the part of the tectum in their path but had not formed synaptic contacts; the fibres had regenerated back to their former tectal positions before entering the synaptic layer. Thus further evidence had been obtained that fibres choose the appropriate division of the optic nerve before entering the tectum.

Development of the specificity of the visual system

In 1942 Sperry inferred that the optic nerve fibres arising from different parts of the retina must be distinguished from one another according to the position of their ganglion cell bodies. Sperry thought that differences were physico-chemical in nature and were induced in the cells as the optic cup differentiated during early development. He further qualified this in 1945 by suggesting that retina becomes specified along two separate axes, the naso-temporal axis being specified first and later the dorso-ventral one. He based this prediction on the fact that similar temporally separated events seem to occur to determine polarity in other anlage such as the ear and limbs (Harrison 1936).

Stone (1948) found that it was possible to rotate an eye in embryonic animals without affecting the subsequent behaviour. If the rotation were carried out at a slightly later stage, vision was permanently reversed. Szekely (1954) in similar experiments was able to show that the eye is indeed polarised first in the naso-temporal axis and then in the dorso-ventral one. This has also been shown electrophysiologically by Jacobson (1968). He rotated the eye anlage in Xenopus tadpoles at various stages of development and recorded the retino-tectal projection from that eye when the animal had metamorphosed and matured. The visual projection from a rotated eye was normal if the rotation had been carried out before stage 30 (Nieuwkoop and Faber, 1956) and completely reversed in animals operated upon at stage 32 and later. Eye rotation at stage 30-31 led to a reversal in the naso-temporal axis of the retino-tectal projection but no abnormality in the dorso-ventral axis. Thus Sperry's prediction that the specification of the eye anlage takes place in two stages has been proved correct.

Sperry assumed that a similar and parallel specification of the developing tectum took place during development. Attempts to discover whether the tectum does become independently specified at a certain stage of development in Amphibia have proved unsuccessful (Crelin 1952; Weimer 1955). These authors employed the technique of tectal grafting to rotate part of the tectum. Later the animals were tested behaviourally and a histological examination of the tecta was made. Crelin used Amblystoma punctatum embryos; he found that during the neurula stage the grafting was successful and normal vision resulted. However, if the graft was made at tailbud stages and later, stages before the optic nerve fibres have grown into the tectum, then the grafted tissue did not survive and visual responses when tested in mid-larval life were poor. By consideration of the stages at which the eye is polarised, it would seem unlikely that tectal specification occurs at the neurula stage; the interesting stages, in terms of tectal specification, were not successfully investigated.

An alternative explanation of the contralateral retinotectal projection does not require a similar and parallel specification of both the retina and the tectum. It is possible to envisage a mechanism whereby retinal axons from different parts of the eye grow from the eye and reach the tectum at different times. The first axons arriving at the tectum would, according to this hypothesis, occupy the first tectal area available to them; fibres arriving later would be forced to continue their growth until reaching an un-innervated area of the tectum. However such a hypothesis would seem to be inadequate, for it cannot explain the result of rotating the eye before the stage at which the nerve fibres have reached the tectum. Despite the eye rotation the fibres seem to reach their proper places in the tectum, showing that the tectum must be labelled in some way before the retinal fibres have reached it.

Delong and Coulombre (1965) using chicks were able to show that in this species the eye and the brain are separately specified before the optic fibres reach the tectum. They established the stage at which the optic fibres made contact with the tectum by a histological study using silver impregnated specimens. The first optic fibres were seen to reach the tectum at day 6; by day 12 the tectum was completely innervated. Quadrantic ablations of the retina at stages before day 6 were then carried out and at day 12 the embryos were examined histologically. It was found that if ablations were carried out at day 4 or 5 localized areas of the tectal surface were not later innervated, those areas corresponding to the ablated retina, being devoid of fibres. Ablation at day 3 resulted in a normal fibre distribution at day 12. These authors were therefore able to show that the retina and tectum are independently specified in the chick before day 6, the stage at which the fibres enter the tectum.

The results of Delong and Coulombre suggest that strict place specificities develop in the retina and tectum resulting in non-innervated tectal areas if part of the retina is absent. It seems that the situation is somewhat different in the case of the Amphibia. Gaze, Jacobson and Szekely (1963) investigated electrophysiologically the retino-tectal projection from a "compound" eye, which contained only nasal or temporal retina but was specified normally in the dorso-ventral axis. The nasal or temporal half of the eye was removed and replaced by half an eye of the opposite embryological origin. By using a donor eye from the opposite side of the body, it was possible to keep the dorso-ventral axis of the compound eye normal; thus it was possible to construct both double nasal and double temporal compound eyes. When the retino-tectal projections of such compound eyes were mapped it was found that the fibre projection from each half eye, had spread to cover the entire tectal surface, as if a "gradient" existed across the tectum and the fibres had become spaced out to cover the entire tectum.

Doubt was cast on this interpretation of these results when it was suggested that the apparently normal tectum might in fact consist of a half tectum hypertrophied to the size of a normal tectum as a result of receiving a double innervation;

the non-innervated half tectum, simultaneously, failing to develop. This interpretation has been proved to be inadequate as a result of an experimental series carried out by Gaze, Keating and Strazincky (1969) in which they allowed animals with one normal eye and one compound eye to develop and metamorphose. The optic chiasma was then uncrossed and the fibres forced to grow into the ipsilateral tectum. It was found that a normal visual projection had regenerated from the normal eye to the tectum which had previously received its innervation from the compound eye. The tectum was therefore normal, not a hypertrophied half tectum, and the original explanation put forward by Gaze, Jacobson and Szekely is probably correct.

Binocular interaction

Another interesting feature of the visual projections from the animals with one compound eye, was the nature of the ipsilateral projections. Especially revealing were the ipsilateral projections from animals with one double nasal compound eye. It was found that the ipsilateral projection from the compound eye was normal and that from the normal eye was consistently abnormal. To explain a compound eye giving rise to a normal ipsilateral projection, in specificity terms, would involve postulating some form of re-specification of retino-tectal connections since embryologically nasal retina does not normally form an ipsilateral projection. It is not possible to explain how a normal eye projects abnormally to its ipsilateral tectum, in terms of embryological specificity mechanisms as described by Sperry.

Keating (1968) noticed that the ipsilateral projection from the normal eye always reflected the abnormality in the contralateral projection from the compound eye. In every animal it was noticeable that any point in visual space projected through both eyes to one tectal locus. Thus the projection from the compound eye to the contralateral tectum is abnormal and the ipsilateral from the normal eye is abnormal in the same respects. Similarly just as the projection from the normal eye to the contralateral tectum is normal so is the ipsilateral from the compound eye.

Keating hypothesised that the mechanism to produce this congruance of visual projections was that when the ipsilateral projection developed, the nerve fibres grew towards and made connections with tectal cells receiving similar visual inputs, via the contralateral pathway and thus firing with similar temporal patterns. Thus a binocular interaction between the functional inputs via the two eyes was postulated.

Hubel and Wiesel (1963)(1965) have studied the importance of function in the development and maintenance of visual connections in the cat. They found by electrophysiological recording that visual responses in the neonate kitten are strikingly similar to those of adult cats; the receptive fields of cortical units were simple or complex, with definite field orientation and cells with similar orientations were found to be arranged in columns. The vast majority of cells were binocularly driven. It seemed therefore that inductive growth processes were solely responsible for the development of these neuronal connections in the cat visual system.

It was found however that normal function was essential for the maintenance of normal connections. In kittens with monocular deprivation from birth to 3 months, although most cells of the lateral geniculate nucleus had normal receptive field properties, the cortical responses were vastly altered. It was found that very few cells were driven by the deprived eye and even then the receptive field properties were abnormal. Other series of experiments which were variations on monocular deprivation, were the introduction of an artificial squint by cutting an eye muscle and deprivation by use of an occluder to exclude pattern vision. These experiments gave similar results to those obtained by monocular deprivation.

In the light of these experiments it would seem reasonable to expect that animals binocularly deprived from birth would be found to have a cortex consisting of cells unresponsive to either eye. This was found not to occur. Over 50% of the cells recorded had normal receptive field properties and the majority of cells were binocularly driven. It would seem that the change in dominance found in the monocularly deprived animals must be adaptive to the animal, allowing more efficient use of the remaining eye.

In their original experiments, Hubel and Wiesel found that monocular deprivation of the adult, even for long periods of time was without effect. The critical period for the disruption of the neuronal connection must have been some time in the first three post natal months.

Recently similar experiments have been carried out, but the time of both eye closure and the reopening of the eye were varied in an attempt to pinpoint the critical period. The animals were tested not only physiologically in a terminal experiment as before (Hubel and Wiesel, 1970) but before this they were also tested behaviourally (Dews and Wiesel, 1970). The results of both testing methods were in agreement, and showed that susceptibility starts suddenly near the beginning of the fourth week, and remains high until between the 6th and the 8th week, when it declines, and finally disappears completely at the end of the 3rd month.

Ganz, Satterberg and Fitch (1968) have monocularily deprived cats for 1 to 6 months after birth and recorded electrophysiologically from the cortex. Their results support those of Hubel and Wiesel. Before carrying out electrophysiological mapping these animals were tested behaviourally (Ganz and Fitch, 1968). The cats showed initial deficiencies in perceptual motor co-ordination, depth estimation and ability to discriminate visual forms when using only the deprived eye, despite relatively normal visual acuity. Some improvement in depth estimation over 14 days was noticed if the period of deprivation was 4 to 8 weeks. 12 weeks deprivation prevented any slight improvements.

Another experiment which suggests that function is essential to maintain normal nervous connections and indicates the effect of one system on another was carried out by Sterling and Wickelgrem (1969). These authors monocularly deprived kittens from birth for several months and then in some animals surgically removed the cortex. 4-6 weeks later the receptive field properties of the collicular cells of the monocular deprived cats were investigated electrophysiologically. In animals with no cortex the field properties of collicular cells were found to be similar to those of normal cats. However in cats in which the cortex had not been removed the cells of the colliculus exhibited abnormal receptive field properties. This result suggests that the cortex affects in some way certain abnormalities during monocular deprivation.

These various experiments using monocularly deprived cats suggest the importance of function in maintaining normal neuronal connections, themselves formed by mechanisms of inate growth processes. The development of the ipsilateral visual projection in Anura is postulated to depend not on such growth factors but on an interaction between nerve cells. The observation that responses via the two eyes reach the same tectal locus, as Keating noticed in the case of the compound eye animals, is tested here in animals with various eye abnormalities such as eye rotation, cross transplantation and enucleation. The development of the ipsilateral visual responses and the regeneration of the system is also investigated in this thesis and various hypotheses to explain the results are discussed.

METHODS

Induced breeding in Xenopus laevis

Pairs of adult Xenopus laevis were kept in plastic buckets, half filled with water. The night before eggs were required, 1·0 ml of a saline solution of gonadotrophin, 600IV/ml were injected subcutaneously into the dorsal lymph sac of the female and 0·5 ml into the male. The pair were then placed in cold water and left undisturbed in a dark room overnight. Since ovulation takes place as the temperature rises above 22°C, the room was maintained thermostatically at about 23-24°C. The eggs were removed the next day and kept in glass crystallising dishes containing Holtfreters solution to a depth of about 1-2 cm. The optimal number of eggs seemed to be 100 per 250 mls of solution. The composition of the solution was:-

Sodium chloride.............	$NaCl$.......	0·35 gm
Potassium chloride.........	KCl........	0·005 gm
Calcium chloride............	$CaCl_2$......	0·01 gm
Sodium hydrogen carbonate...	$NaHCO_3$.....	0·02 gm
Distilled water..........................		100 ml

Embryonic operations

Eye rotations were carried out in Xenopus laevis embryos and tadpoles at various stages between 26 and 58 (Nieuwkoop and Faber 1956). The animal was first placed in several changes of Holtfreters solution in an attempt to minimise the risk of infection. Then it was anaesthetised, in a 1/3000 solution of MS222 (Sandoz) for about 15 seconds until it ceased moving, and transferred in a pipette to an operating dish containing Holtfreters solution. The operating dish was a small Petri dish containing a layer of plasticene. A depression to accommodate the embryo neatly was made in the plasticene using the round tip of a moulding rod and the creature was manoeuvred into it using a fine hair loop set in the end of a glass tube. A narrow glass bridge, cut from a coverslip, was lowered over the tadpole to hold it in place. Under a binocular microscope, using irridectomy scissors, fine tungsten needles, and watchmakers forceps, the eye was cut away from its surrounding tissue, rotated by 180°, so that its ventral part became dorsal and its nasal aspect became temporal, and replaced. The rotated eye was held in place by a glass bridge for 20-30 minutes to allow the tissue to heal, and the animal was then carefully transferred to a clean crystallising dish containing Holtfreters Solution. The degree of rotation in embryos of stage 32 and older could easily be checked by noting the position of the optic cleft which in the normal eye is situated ventrally. In tadpoles beyond stage 40, it was necessary to carefully cut the eye muscles while leaving the optic nerve and the hyaline and ophthalmic blood vessels intact.

Contralateral eye transplants were carried out at stages 38 and 45. Two tadpoles were anaesthetised at the same time; the right eye was then removed from one, and the left eye from the other. The right eye was then placed in the left orbit of the other animal and vice versa. In these transplanted eyes either the naso-temporal or the dorso-ventral axis was aligned correctly; in other words the eye cleft was positioned ventrally or dorsally in the orbit.

Enucleations were performed at various stages, the depression formed by removing the eye being allowed merely to close up a little before taking the animal out of the operating dish.

Using similar techniques, G. Szekely carried out embryonic operations to construct compound nasal eyes in embryos at stage 30/31.

Rearing the Xenopus tadpoles

The tadpoles were kept in Edinburgh tap water in crystallising dishes at a density not greater than one tadpole to 500 ml of water. The temperature was maintained at 23°C. Feeding begins in Xenopus at stage 45 when the mouth breaks through. The animals are filter feeders and were fed on a fine suspension of Heinz Beef and Liver Baby Soup in tap water. Sufficient "Soup" was given each day so that the water was cleared of food in about 24 hours. After operation animals were left without food for 2 days.

At stage 61, when the head begins to shrink, soup feeding was discontinued. The tadpole was transferred to shallow tap water and a lid was put over its container. It was fed Tubifex which was left permanently in the water. When metamorphosis was complete, the toad was placed in deeper tap water again and fed on both Tubifex and finely chopped beef heart. Each toad was allowed 2 litres of water which was changed every few days.

Eye rotations in juveniles

Young adult Xenopus at stages 65 and 66 were used for a series of eye rotations. The animals were anaesthetised lightly in a solution of 1/3000 solution of MS222 (Sandoz) in tap water, and then placed on a cork board and the body covered by a piece of wet tissue paper to keep the animal moist. The skin immediately surrounding the eye was cut with scissors and the eye muscles were severed. The eye was then rotated between 90° and 180°. One or two toes of the animal were removed to distinguish it from the others in the series, and the head was photographed to show the position of the eyes. Each photographic plate was labelled carefully to show the number of toes removed from that animal. The operated animals were left in very shallow water until they had recovered from the anaesthetic and only then were replaced in deeper water.

Eye rotations in adult frog and Xenopus

Eye rotations were carried out in a series of adult Xenopus laevis and Rana esculenta. In both species the technique was similar.

A little liquid paraffin was dropped onto the eyes and the animal was then anaesthetised in an atmosphere of anaesthetic ether until it just stopped twitching. The anaesthetised animal was pinned out onto a cork board by a pin through its nose, with its chin resting on a mound of plasticene. A wet tissue was placed over the body to prevent it becoming too dry. Under a binocular microscope the eye lid was cut away as completely as possible and then the skin severed from the eye ball. The eye muscles were then cut one at a time being careful not to injure any blood vessels to the eye or within the orbit. The eye was rotated through about 45°-90°. Greater rotation resulted in an immediate cessation of the iridial circulation and eventual atrophy of the eyes. In the first operations, the eye was held in place with a stitch of surgical thread joining the edge of the eyeball and the surrounding skin. This was found to be unnecessary, however, if all the eye muscles had been completely cut. The retinal circulation was checked over the next few days, using an ophthalmoscope, and those animals with poor retinal blood supply were discarded. The frogs were kept in containers with ½" of tap water in the bottom and a brick or stone was provided as a ledge, allowing the animals to sit above the water; they were fed 3 times a fortnight with finely chopped beef heart, or liver, which was placed in their mouths.

Transection of the optic nerve or optic tract in adult Xenopus and frogs, nerve crushes in frogs

The animal was anaesthetised as for eye rotation and then placed on a cork board dorsal surface downwards with the head pointing away from the operator. The mouth was opened and a pin pushed through the upper jaw into the cork board. An S-shaped pin was hooked onto the lower jaw and an elastic band was stretched from the pin to another at the caudal end of the animal to keep the mouth open. The skin on the roof of the mouth was cut and a flap lifted back to expose the sphenopalatine bone. In frogs, the optic chiasma could then usually be seen beneath the bone; a window was cut in the bone using a scalpel and a small square of bone removed. The underlying cartilage was then cut and deflected away, to uncover the chiasma below. The optic nerve or tract was then cut with scissors, or the optic nerve crushed with forceps. The cartilage and skin were then replaced, apposing the surfaces as far as possible.

In Xenopus especially ones several years old, the sphenopalatine bone was thick and opaque. It was necessary to estimate the position of the chiasma and use a dental drill to remove the bone. The animals were not fed for at least 10 days after the operation.

Mapping the projection of the visual fields onto the optic tectum

1. Adults

The animal was anaesthetised in an atmosphere of ether, having first coated the eyes with a layer of paraffin. To expose the optic tecta and the cerebral hemispheres the skin covering the dorsal cranium was removed and the parietal and frontal bones drilled away. The animal was decerebrated by destroying the cerebral hemispheres with forceps, and then an injection of 0·2 mg tubucurarine was given intramuscularly. Liquid paraffin was poured onto the tectum at frequent intervals to keep it moist. The meninges were removed with fine forceps while the tecta were covered by paraffin. The eyelids were cut away. The tecta were photographed and an enlargement made at X 50 magnification. A centimetre grid was superimposed on the X 50 enlargement, so that each square on the photograph represented a 0·2 mm X 0·02 mm area on the tectum. The presence of the vivid melanophore pattern on the tectal surface, together with the grid, permitted accurate placing of the electrode on the tectum.

The animal was positioned on the platform of a micromanipulator with one eye approximately centred on an AIMARK projection perimeter with a radius of 33 cm. A moist tissue was placed over the animal's body. Accurate alignment of the optic axis of the eye with the axis of the perimeter was done in the following way:- a narrow beam of light from an ophthalmoscope was projected from the centre of the perimeter through a hole in the centre of a plane mirror lying on the axis of the perimeter and inclined at an angle of 45° to it. The position of the eye was adjusted so that the light beam entered the eye and was reflected in the mirror as a red glow emerging from the pupil. Since there is no tapetum in the frogs eye, the reflection is faint and rotation of the eye by only a few degrees from the axis of the perimeter caused the glow to fade and disappear.

Under vision through a binocular microscope, a microelectrode was guided by means of a micromanipulator to positions on the tectum corresponding to the intersections on the grid of the photograph. The electrode was lowered onto the tectum until electrical contact was made.

Mapping was performed in a dimmed light. Action potentials in the tectum, evoked in response to a small circular black disc in the visual field were recorded between the microelectrode on the tectum and a pin in one of the frog's hind limbs, acting as the indifferent electrode. Evoked potentials were amplified by an RC coupled amplifier with a time constant of 2 msecs., displayed on an oscilloscope and monitored with a loudspeaker. The position of the black disc was adjusted to give a maximal response, and then marked on the perimeter chart, if necessary the electrode was lowered slowly into the tectum to a depth of 300μ to improve the quality of the responses.

The visual projections from both eyes to both tecta were mapped in each animal. At the beginning of the experiment the animal was set up so that the operated eye was centred on the perimeter. In the case of enucleate animals the remaining eye was centred. Once the animal had been positioned, care was taken not to move it during the experiment. It was extremely important to know through which eye the responses were being recorded, the eye not being investigated at that time was completely covered therefore by an opaque shield. To check the adequacy of the shielding at intervals during the experiment both eyes were simultaneously covered and visual responses sought in the usual way.

The electrodes used were of two types; tungsten needles with a tip diameter of 1μ and glass pipettes filled with Woods metal and tipped electrolytically with platinum, to give a tip size of between 3 and 5μ.

After completion of the mapping experiment, the whole head was fixed for histology, in all animals operated on as tadpoles pre stage 58, and in those animals with sectioned optic nerves or tracts. In other words, all animals except those with simple eye rotation either in the adult or juveniles at stage 65/66. The fixed head was embedded in paraffin wax, cut in transverse serial sections at 15μ and silver impregnated by Holmes method.

2. Tadpoles

The procedure for mapping tadpoles was essentially similar to that used with adults. One difference was that the animals were anaesthetised by allowing them to swim in a 1 in 3000 solution of MS222 until immobilised; the experiment was terminated before the animal recovered. Also it was found unnecessary to use a dental drill to remove part of the skull, a scalpel blade being sufficient. The only other difference was that the tecta were drawn using a camera lucida, carefully marking the position of the blood vessels, since the melanophores had not fully developed and it was found that the veins were not reproduced clearly on a photograph.

Lesions to determine the ipsilateral visual pathway

In this series of animals, normal adult Xenopus were prepared for recording the visual projections in the usual way, and the chiasma was then exposed using the method previously described. The animal was then set up in the perimeter, with one eye centred and responses were evoked at several points on each tectum, to stimulation through each eye. The optic tract was then cut, and the same eye as before was centred. Responses were then sought through both eyes to several points on both tecta, to find out which projections had been destroyed and which survived. The animals were sent for histology. In other normal Xenopus the contralateral and ipsilateral projections via one eye were mapped. A small localised lesion was then made in the contralateral tectum with a heated metal rod. The contralateral and ipsilateral projections were then remapped, being careful that the position of the eye remained unaltered throughout.

RESULTS

Normal animals

The visual projections of 11 normal adult animals were mapped; 7 of these animals were Xenopus and 4 were Rana. In all the normal adults, the animal was set up so that one eye was centred on the centre of the perimeter at the beginning of the experiment, and was then left in place throughout. The visual projections were then mapped through each eye to each tectum.

The visual maps recorded from a normal Rana with the left eye centred on the perimeter was shown in Fig. 1. They were similar in all those normal animals which were mapped. With parallel rows of recording points on the tectum, the maps consisted of approximately parallel rows of field positions with no overlapping between the rows. Four visual projections were mapped, and in each case all the points were found to be located in the superior field.

One projection was contralateral, from the centred left eye to the right tectum (Fig. 1) in which the most nasal field positions projected to the rostral pole of the tectum and the most temporal positions to the caudal tectum. The least superior visual field projected to the lateral edge of the tectum, and the most superior to the medial tectal areas.

The rows of field positions ran approximately in the superior-inferior axis and in this animal the rows were tilted so that the most superior points in any one row were more temporal than those in the inferior field. The angle at which these rows were placed was found to vary within 45°

limits between different animals, depending on the positioning of the eye relative to the perimeter and on slight differences in the direction of the lines of tectal points which were mapped.

Fig. 1 also shows the projection from the non-centred right eye to the right tectum, the centred left eye being covered; visual responses could be recorded only from the rostral part of the tectum. It can be seen clearly from Fig. 1 that the rows of points in the visual field corresponded through both eyes, when the same tectal positions were mapped. Thus the position in visual space of row 1-2-3-4 recorded from the most rostral part of the tectum, was the same for both eyes. Unfortunately the right eye being non-centred means that the labels nasal and temporal as marked on the diagram merely indicate the direction of this axis and not the true position relative to the optic axis of the right eye. Figure 2 indicates the situation when the animal is mapped with one eye centred throughout the experiment. Nevertheless it is important to note that the points in visual space projecting to the caudal tectum (13 to 20) are more nasal in the field of the right eye but more temporal in that of the left. This is because the more nasal a point is in visual space relative to one eye the more temporal it is relative to the other. Similarly row 1-2-3 of visual points is the most temporal row mapped through the right eye but the most nasal for the left.

The visual projections to the left tectum are shown in Fig. 1. The contralateral projection from the non-centred

<u>Figure 1</u>. Normal visual projections (Rana).

The projection of the visual field through both eyes to the left and right tecta. The numbers on the tectal diagram represent electrode positions. The field position corresponding to each tectal position is indicated by the appropriate number on the perimetric chart representation of the visual field. In this experiment and in all others the rows of tectal positions are numbered from lateral to medial with the lowest numbers rostrally and the highest numbers caudally.

The field projections in this experiment through both eyes are plotted with the left eye centred.

<u>Key</u>:- N = nasal pole
T = temporal pole
S = superior pole
I = inferior pole

The above key applies to all the diagrams of visual field projections.

Figure 1

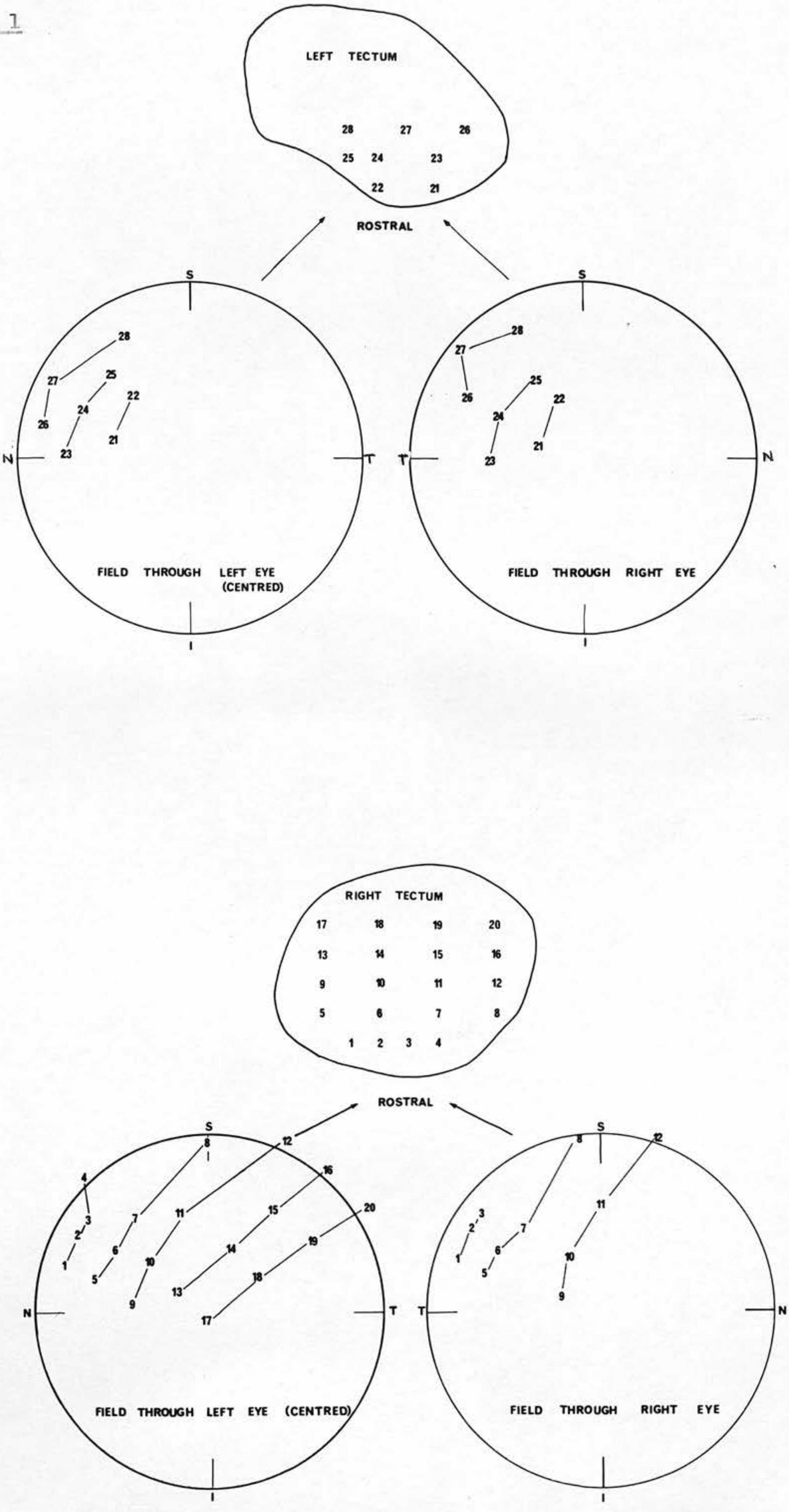
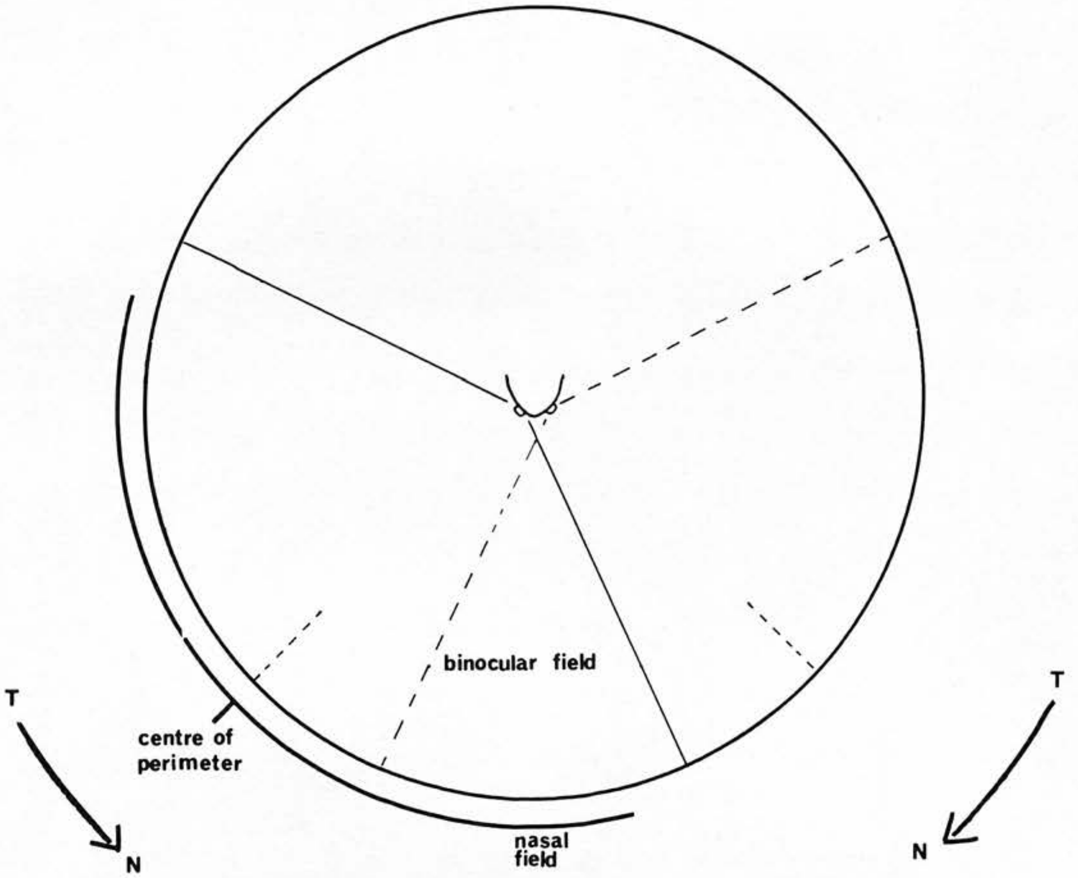

LEFT TECTUM
28 27 26
25 24 23
22 21
ROSTRAL
S
N
T
I
28
27
25
22
26
24
21
23
FIELD THROUGH LEFT EYE (CENTRED)
S
T
N
I
28
27
26
25
22
24
21
23
FIELD THROUGH RIGHT EYE
RIGHT TECTUM
17 18 19 20
13 14 15 16
9 10 11 12
5 6 7 8
1 2 3 4
ROSTRAL
S
N
T
I
8
12
4
16
3
7
11
15
20
2
1
6
10
14
19
5
18
13
9
17
FIELD THROUGH LEFT EYE (CENTRED)
S
T
N
I
8
12
3
2
11
7
1
6
5
10
9
FIELD THROUGH RIGHT EYE

Figure 2

This diagram shows the extent of the visual fields of both eyes in an Anuran, such as Xenopus. The limits of the visual field of the right eye are shown by the solid lines; those of the visual field of the left eye by the broken lines. The optic axes of the eyes are marked as short broken lines. In this diagram the right eye is centred on the visual perimeter. It is apparent that the nasal part of the visual field of the left eye can be charted on the perimeter. The tempero-nasal axes of the eyes are also marked on their appropriate sides.

Figure 2



right eye appears to be rotated compared to the contralateral projection from the left eye to the right tectum, the points running in a naso-temporal rather than a supero-inferior direction. This misalignment is not due to any basic differences between the two contralateral projections but to the non-centring of the right eye.

The visual maps through the right and left eyes to the left tectum are similarly arranged. Thus the most rostral tectal points 11 and 22 receive an input from similar positions in visual space through the two eyes in the nasal part of the left visual field. As explained earlier the terms nasal and temporal when applied to the non-centred right eye are charted relative to the left eye and do not imply that the contralateral projection from the right eye arises from the temporal field.

The other three Rana mapped in this series gave similar results to those described although in one case the animal died before all the projections had been mapped. Table 1 lists the visual projections mapped in each animal.

TABLE 1

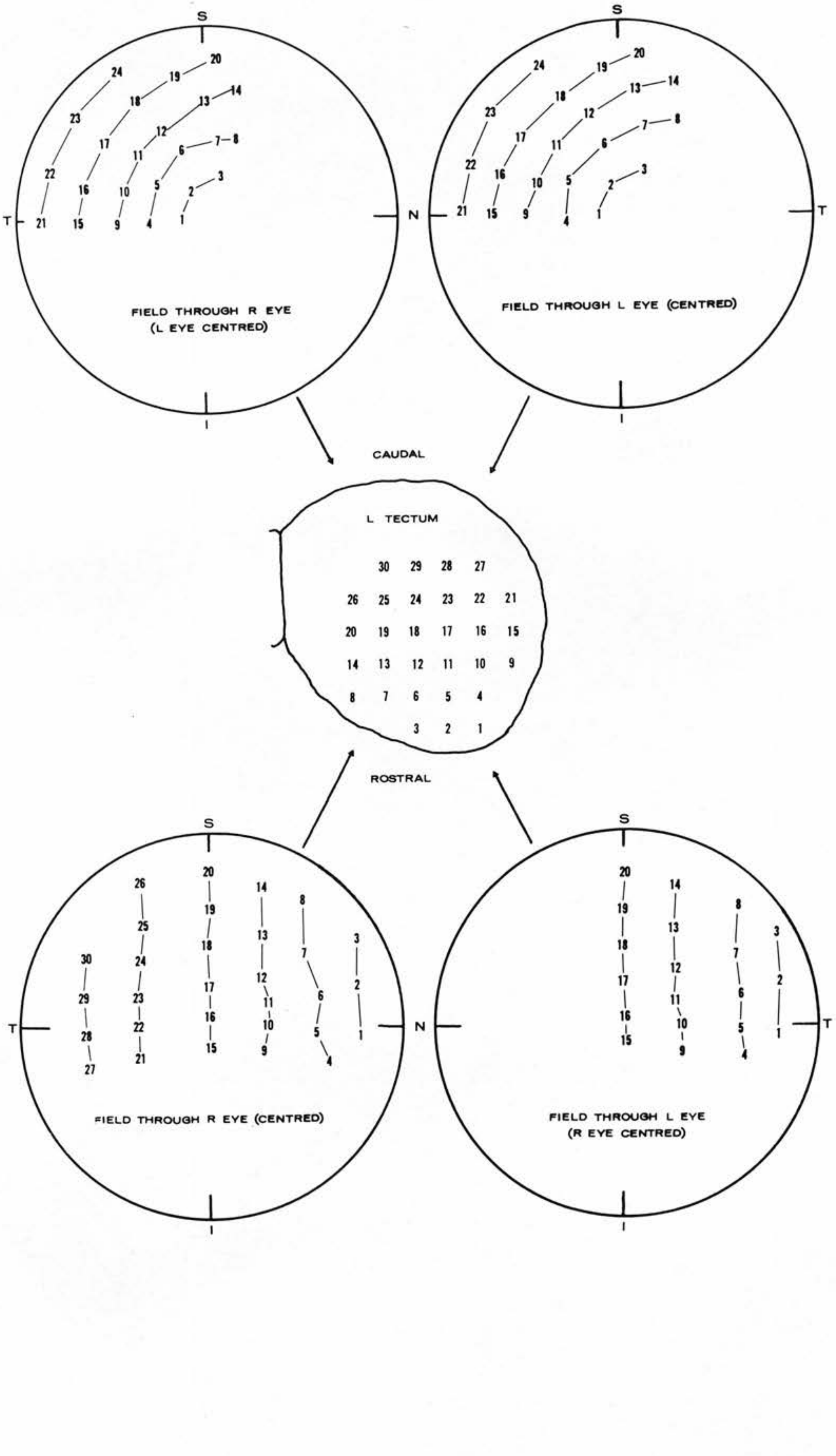
Animal	Centred Eye	Projections Mapped
NRE3	R	R eye to L tectum
NRE9	L	All 4
NRE7	L	All 4
NRE6	L	All 4

Visual maps recorded from Xenopus were similar to those from Rana except that the ipsilateral responses were usually

Figure 3. Normal visual projections (Xenopus)

The projection of the visual field through both eyes to the left tectum. In the upper two charts, the left eye was centred on the perimeter; in the lower charts, the right eye was centred.

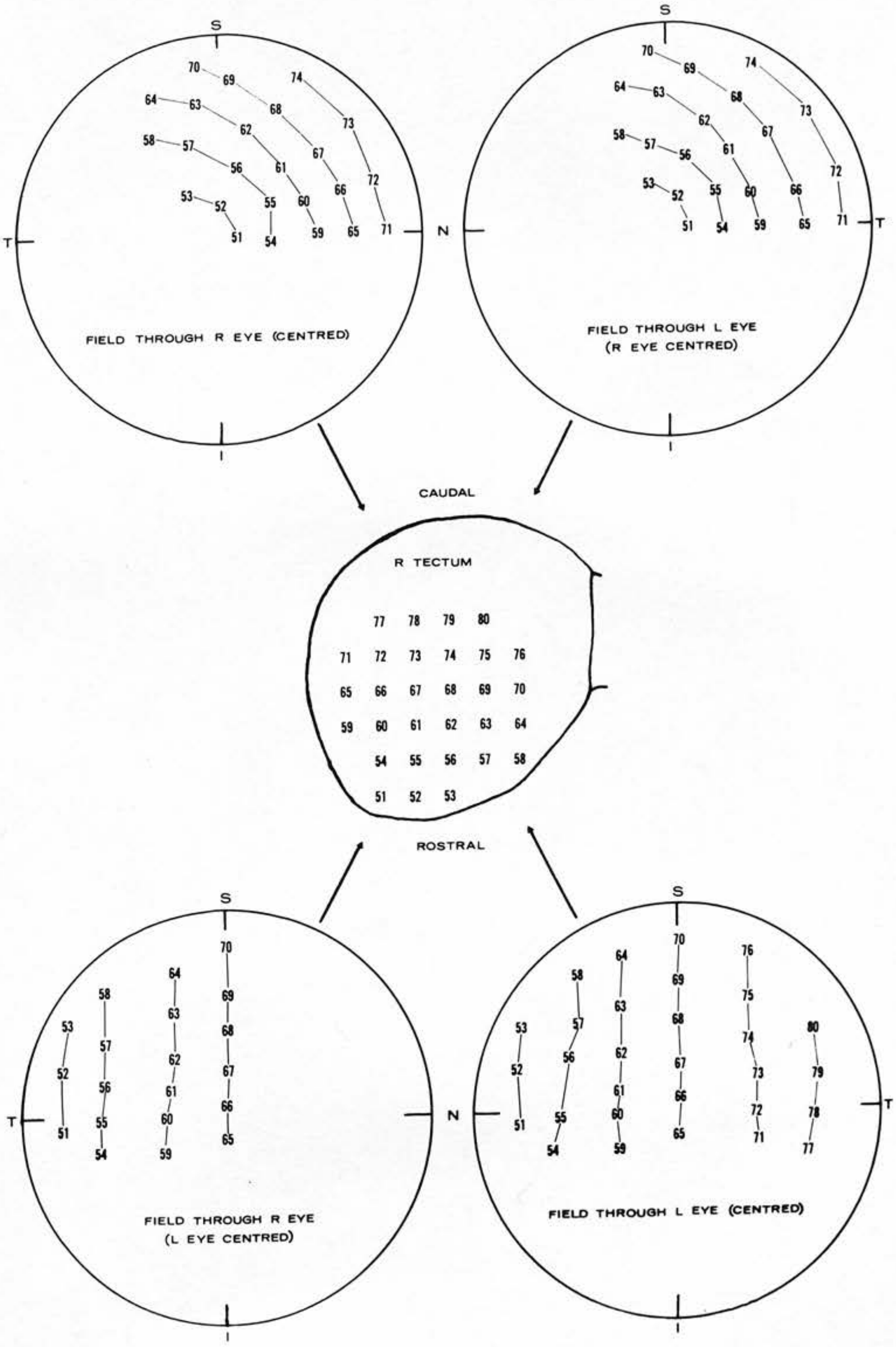
Figure 3


S
T
N
I
FIELD THROUGH R EYE
(L EYE CENTRED)
S
N
T
I
FIELD THROUGH L EYE (CENTRED)
CAUDAL
L TECTUM
30 29 28 27
26 25 24 23 22 21
20 19 18 17 16 15
14 13 12 11 10 9
8 7 6 5 4
3 2 1
ROSTRAL
S
T
N
I
FIELD THROUGH R EYE (CENTRED)
S
N
T
I
FIELD THROUGH L EYE
(R EYE CENTRED)

<u>Figure 4</u>. Normal visual projections (Xenopus)

The projection of the visual field through both eyes to the right tectum. In the two upper charts the right eye was centred on the perimeter; in the lower charts the left eye was centred.

Figure 4


S
T
N
I
70 69 74
64 63 68 73
62 67
58 57 61 72
56 66
53 52 55 60
51 54 59 65 71
FIELD THROUGH R EYE (CENTRED)
S
N
T
I
70 69 74
64 63 68 73
62 67
58 57 61 72
56
53 52 55 60 66
51 54 59 65 71
FIELD THROUGH L EYE
(R EYE CENTRED)
CAUDAL
R TECTUM
77 78 79 80
71 72 73 74 75 76
65 66 67 68 69 70
59 60 61 62 63 64
54 55 56 57 58
51 52 53
ROSTRAL
S
T
N
I
70
64 69
58 63 68
53 57 62 67
52 56 61 66
51 55 60 65
54 59
FIELD THROUGH R EYE
(L EYE CENTRED)
S
N
T
I
70 76
64
58 69 75
63
53 57 68 74 80
56 62
52 67 73 79
61 66
51 55 60 65 72 78
54 59 71 77
FIELD THROUGH L EYE (CENTRED)

recorded from at least the rostral $\frac{2}{3}$ of the tectum instead of the rostral $\frac{1}{3}$ as in Rana. Figure 3 represents the visual projections from a Xenopus to the left tectum and Figure 4 shows the maps obtained to the right tectum. In Table 2 are listed the visual projections mapped in each animal.

TABLE 2

Animal	Centred Eye	Projections mapped
X N L 2	L	L eye to R tectum
X N L 1	L	L eye to R tectum
		R eye to R tectum
X N L 8	L	All 4 projections
X N L 3	L	L eye to R tectum
X N L 4	L	L eye to L tectum
		L eye to R tectum
X N L 5	L	L eye to L tectum
		L eye to R tectum
X N L 6	L	L eye to R tectum
X N L 7	R	All 4 projections

Enucleations

The hypothesis of interaction would predict that either no ipsilateral projection would develop, if the other eye were removed before the stage at which interaction occurs, or that the ipsilateral projection would be abnormal. A hypothesis involving a form of neuronal specificity would predict the development of a normal ipsilateral projection. Enucleations were therefore carried out as a test of the binocular interaction hypothesis.

Five Xenopus from which one eye had been removed before the larva had reached stage 29 (Nieuwkoop and Faber 1956) were mapped when adult with the remaining eye centred on the perimeter. The contralateral projection from this eye was found to be normal in every animal; the multi-unit receptive fields of the contralateral projection seemed normal, although this was not examined in detail.

The ipsilateral projection, in each animal was abnormal in that the multi-unit receptive fields recorded at any tectal locus were wider than in the normal animal being 45°-50° in diameter. This meant that it was not possible to construct a meaningful map. Ipsilateral responses were recorded from the caudal pole of the tectum which in the normal animal receives no ipsilateral visual input.

In each enucleate animal mapped, it was noted at the time of removing the skull that the tectum ipsilateral to the remaining eye was approximately $\frac{1}{3}$ smaller than the contralateral one. The histologically stained sections of these tecta showed a marked difference between the thickness of the tecta on the two sides. The remaining optic nerve, despite the absence of the partner crossed the midline to enter the contralateral tectum. Fig. 5 shows the optic chiasma of a normal Xenopus and Fig. 6 illustrates the "chiasma" region in an enucleate animal.

These enucleations were carried out in larvae at a stage at which the eye consists merely of a cup-shaped evagination of the neural tube. Fig. 7 shows a stained transverse

Figure 5.

Transverse section through region of optic chiasma in normal adult Xenopus. Optic nerves can be seen approaching chiasma. Holmes silver stain. Bar represents 0·1 mm.

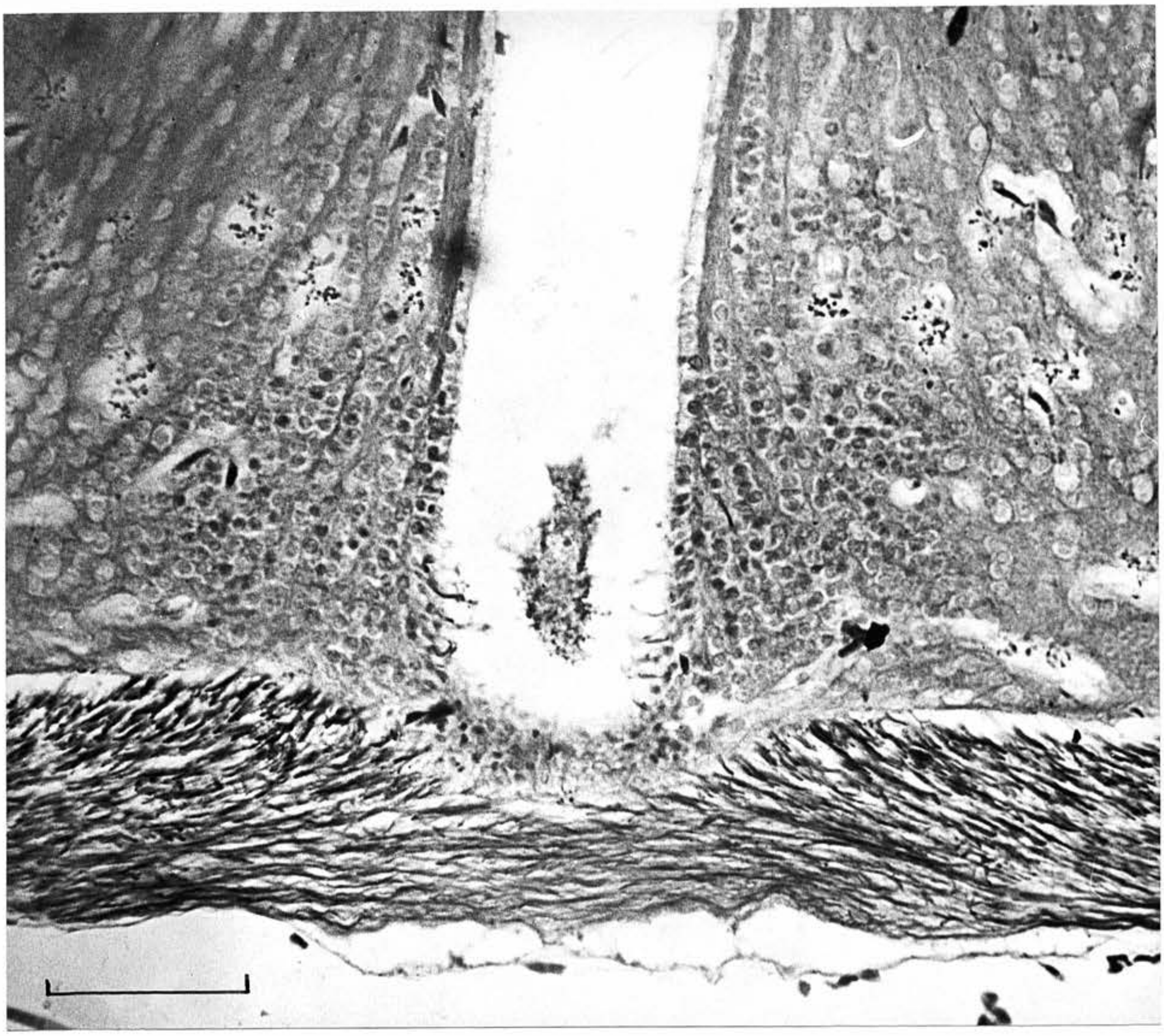


Figure 6.

Transverse section through chiasma region in adult Xenopus; in this animal one eye had been removed before stage 29. The remaining optic nerve can be seen approaching the region where chiasma is normally found. Holmes silver stain. Bar represents 0·1 mm.

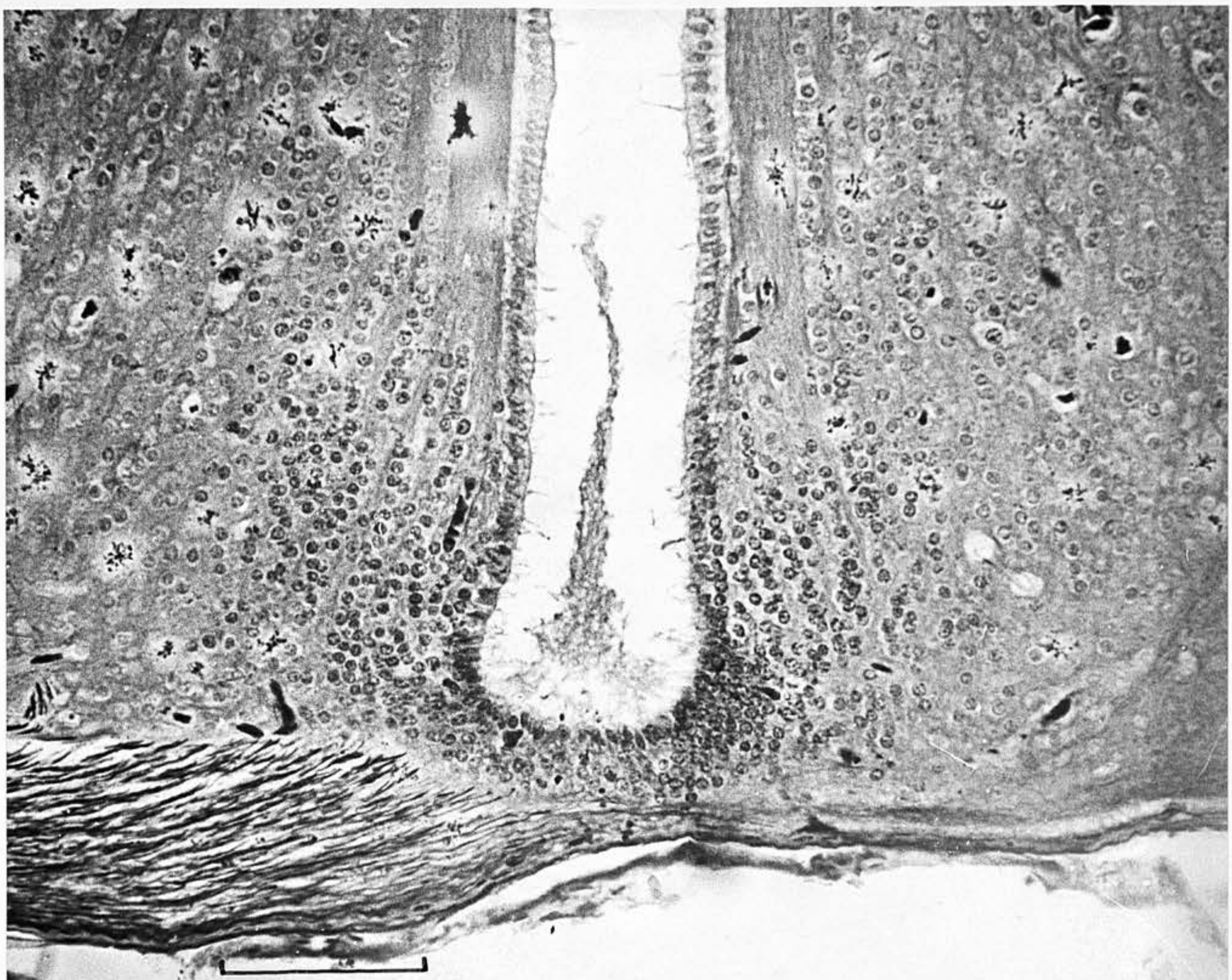
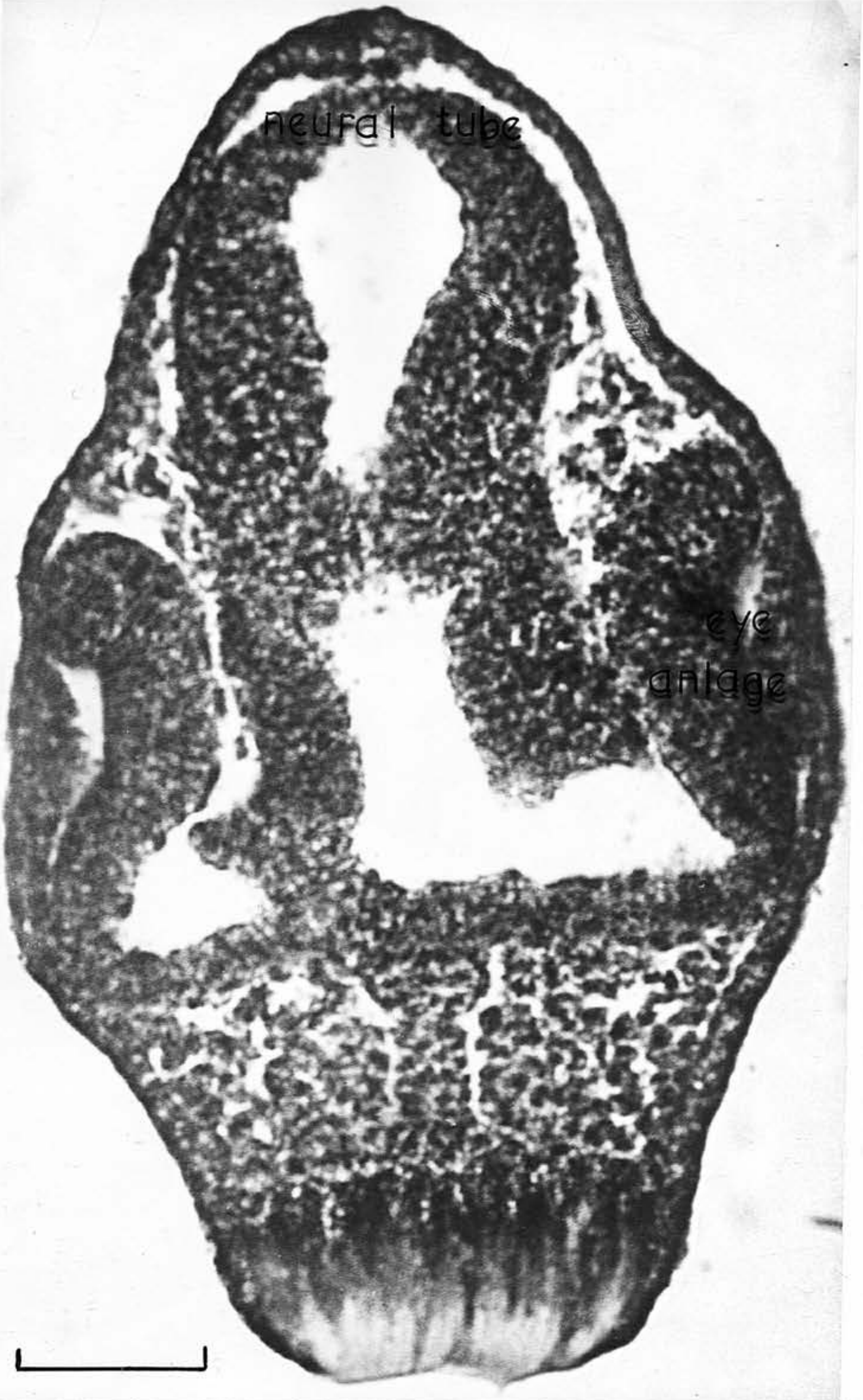


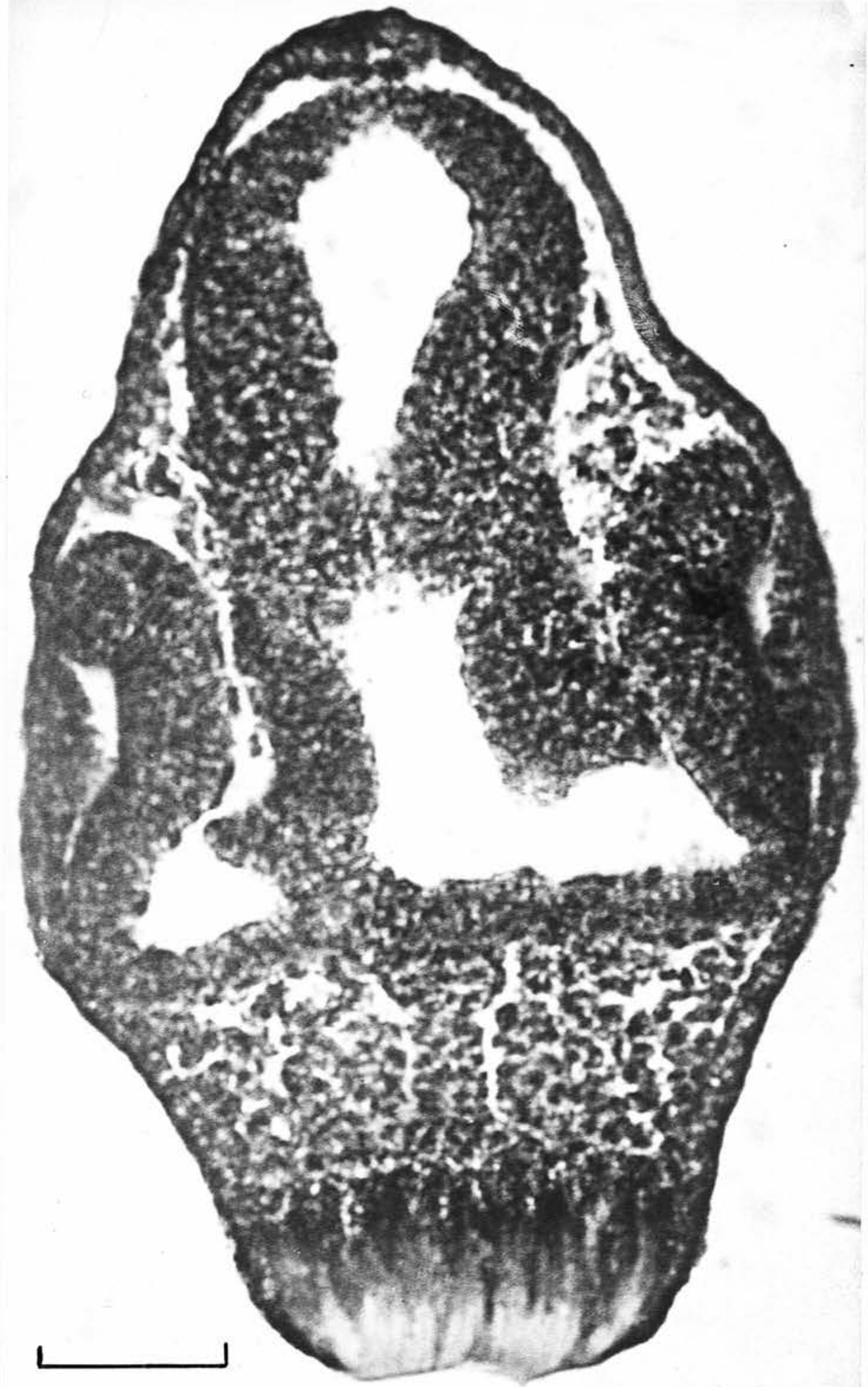
Figure 7.

Transverse section through the head of a stage 29 Xenopus larva; neither theoptic anlage nor the neural tube exhibit any overt differentiation. At this stage the eyes are joined to the brain by a narrow optic stalk, which is not seen in this section. Holmes silver stain. Bar represents 0·1 mm.

neural tube

eye
anlage


neural tube
eye
anlage



section of a stage 28 (Nieuwkoop and Faber 1956) at the level of the eye enlage. At this stage, the eye appears as an undifferentiated mass of cells. The growth of the optic nerve fibres from the retina to the tectum takes place between stages 34 and 39. In Figs. 8 and 9 the region of the chiasma in a stage 34 tadpole can be seen; the appearance of the eye in a stage 39 tadpole is shown in Fig. 10. Thus in these enucleate animals we know that the optic fibres must have grown along their normal pathway despite the absence of fibres from the other eye.

Eye rotations in tadpoles

The hypothesis of binocular interaction would predict that if one eye were rotated before the time at which interaction takes place, then the ipsilateral projection from the rotated eye will be normal since it is interacting with the normal contralateral projection. For a similar reason the ipsilateral projection from the normal eye would be predicted to be rotated. Predictions based on a hypothesis of specificity are quite different. If the rotation were done before the stage of specification then the maps would be normal; if the operation were carried out after specification of the ipsilateral projection then the ipsilateral projection from the normal eye should be normal and that from the rotated eye should be similarly rotated. It was therefore decided to carry out a series of eye rotations in Xenopus tadpoles and to map the visual projections in the adult.

Figure 8

Transverse section through the head of a stage 35 Xenopus larva. At this stage 3 distinct layers are visible in the retina. Optic nerve fibres have left the eye and are growing towards the brain, in larvae at this stage. Holmes' silver stain. Bar represents 0·1 mm.

neural tube

eye anlage

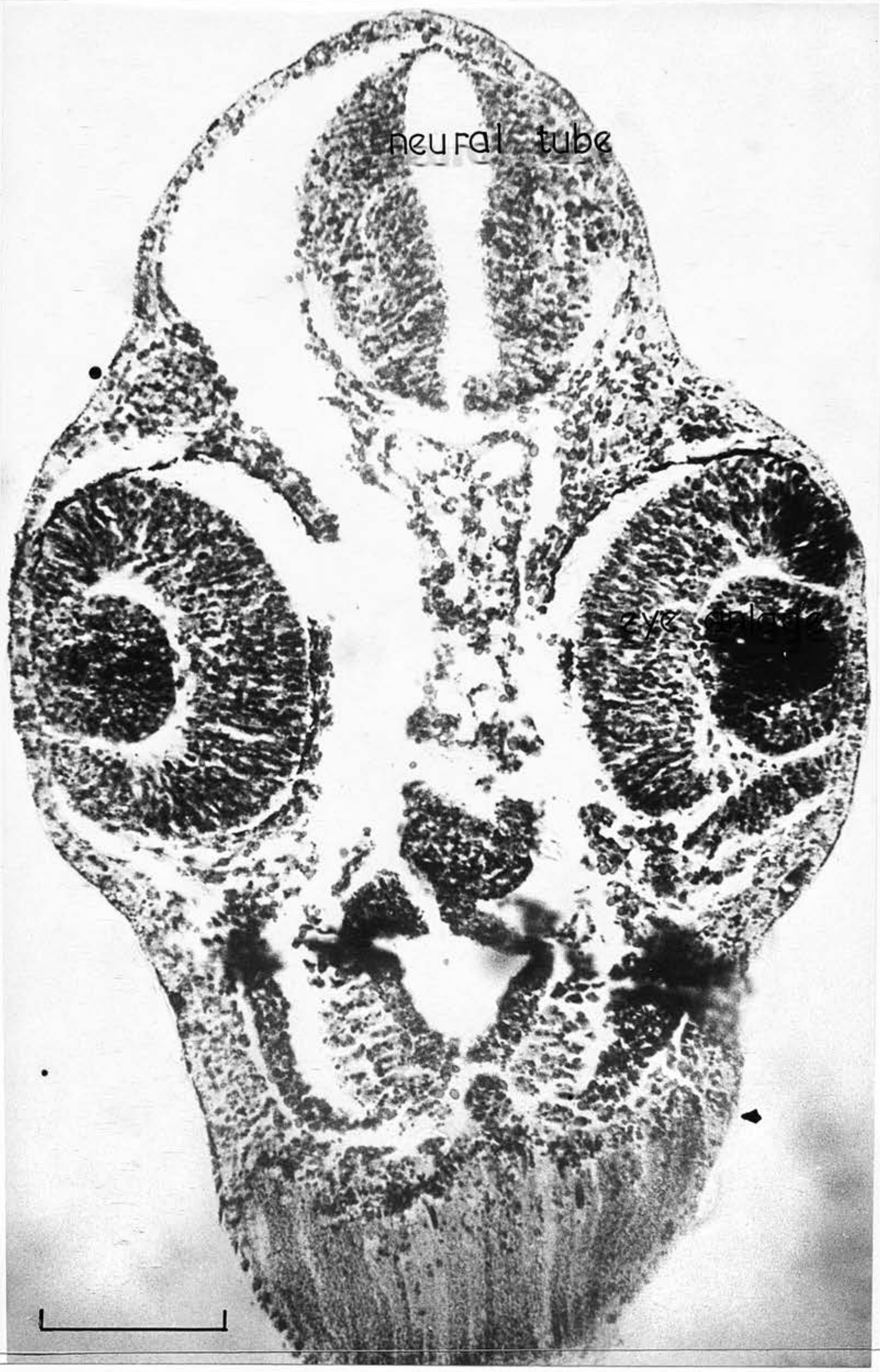

neural tube
eye anlage

Figure 9

Longitudinal section through the head of a stage 35 Xenopus. Holmes' silver stain. Bar represents 0·1 mm.

neural tube

notochord

eye anlage

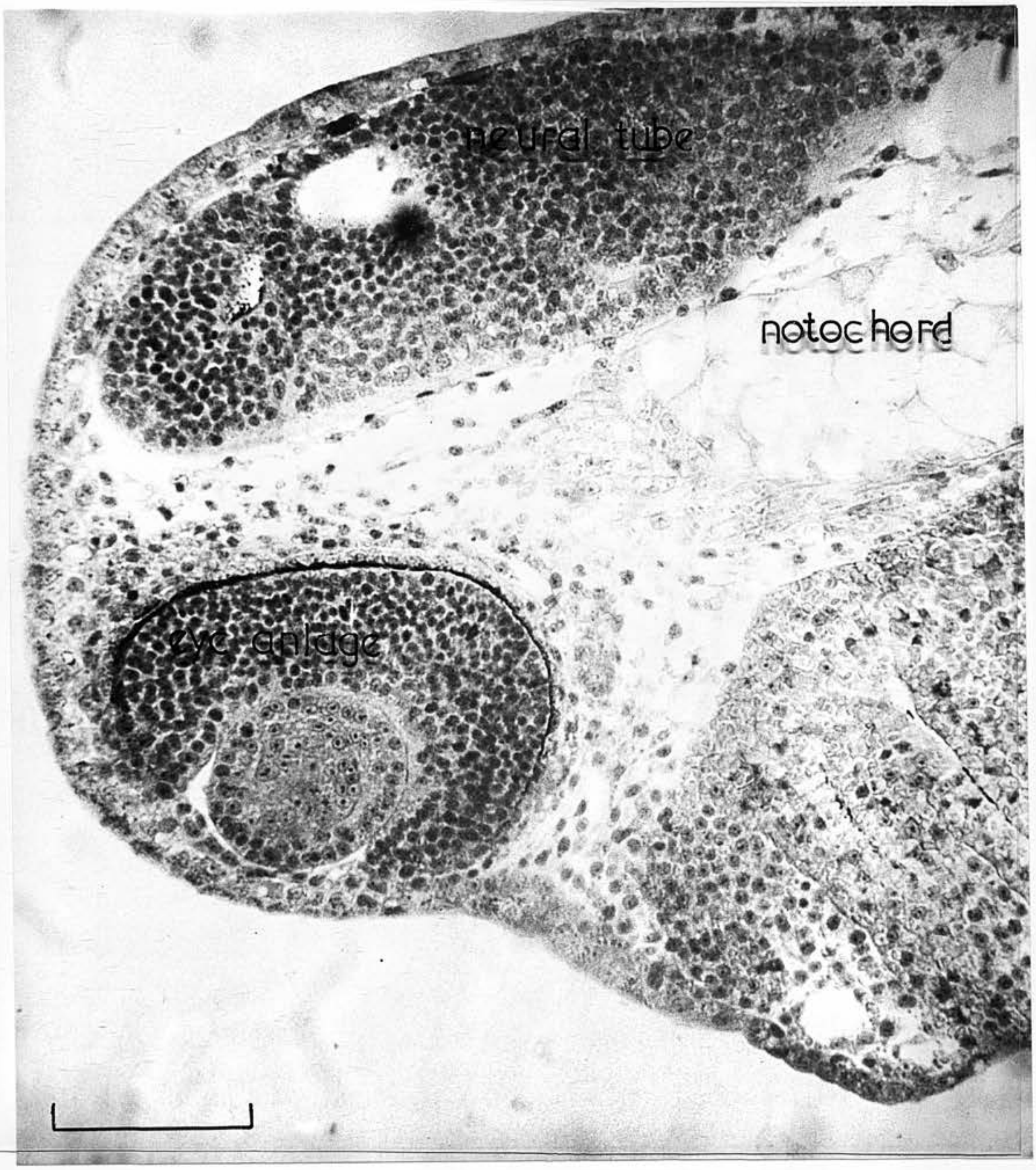
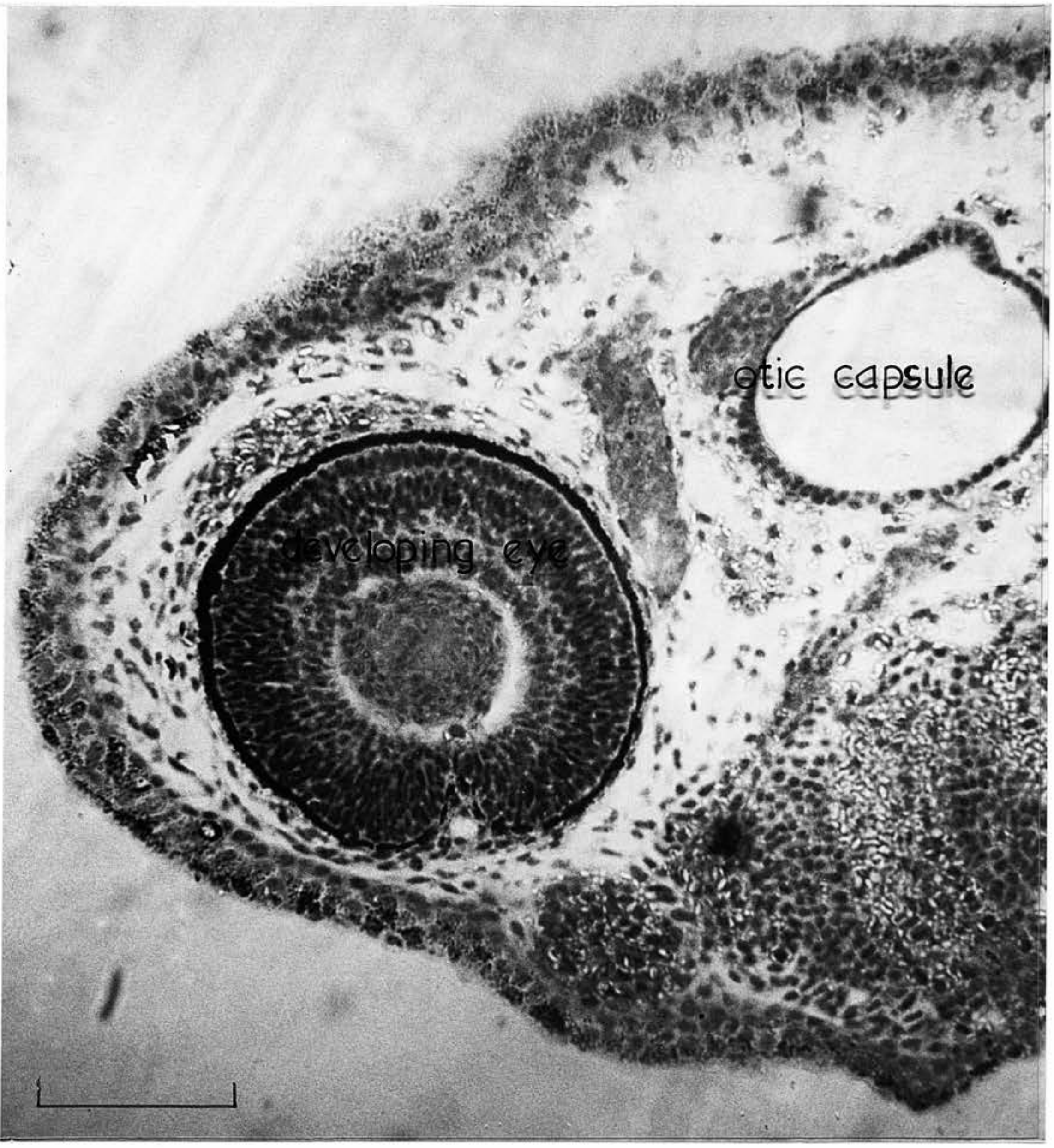

neural tube
notochord
eye anlage

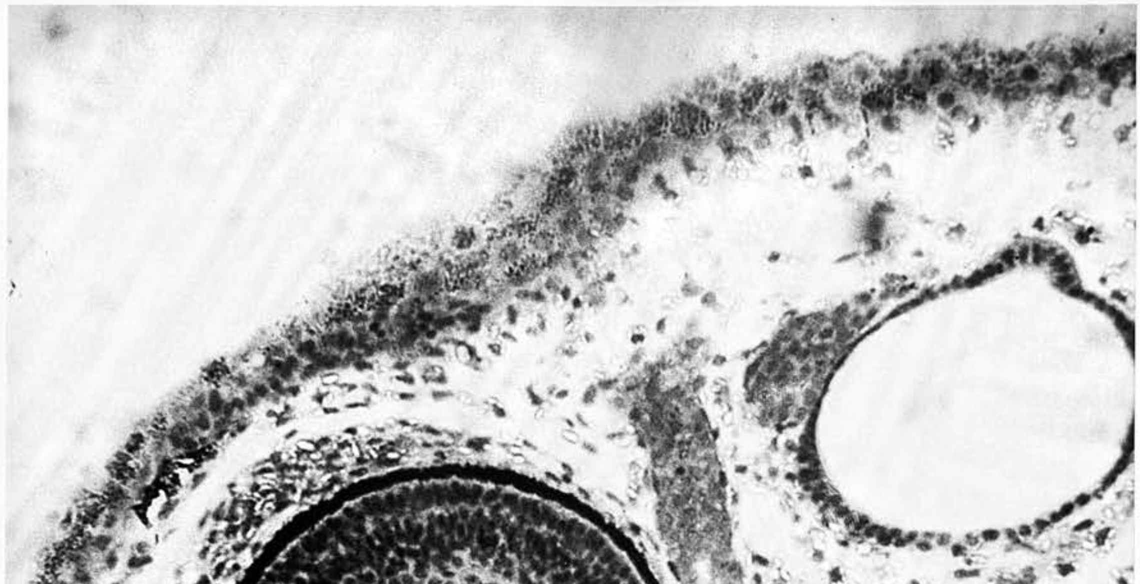
Figure 10

Longitudinal section through the head of a stage 39 Xenopus larva, showing the developing eye. At this stage, the eyes have become situated lateral to the neural tube; it is therefore not possible to include both the eye and the brain in one longitudinal section. Holmes' silver stain. Bar represents 0·1 mm.

otic capsule

developing eye


otic capsule
developing eye



Of the 7 animals recorded in this group, 5 gave essentially similar results. A typical result was animal XRE 7, in which the right eye had been rotated at stage 45 (Nieuwkoop and Faber 1956) and which was mapped with the right eye centred. It can be seen from Fig. 11 that the contralateral projection from the rotated right eye to the left tectum is rotated anti-clockwise by about 90° compared with the normal animal (Figs. 3 and 4). The rows of the visual field positions run in a naso-temporal rather than a supero-inferior direction, with the most medial tectal points 12, 16, 19 responding to stimuli in the temporal visual field and the most lateral tectal points to stimuli in the more inferior parts of the field.

Although only 2 rows of field positions were mapped from the normal left eye to the left tectum, the points were well organised and showed that the map was abnormally oriented. The map rotated to the same extent as the map from the right eye to the left tectum, the field positions to one tectal locus being no more than 10-15° apart when mapped through the two eyes.

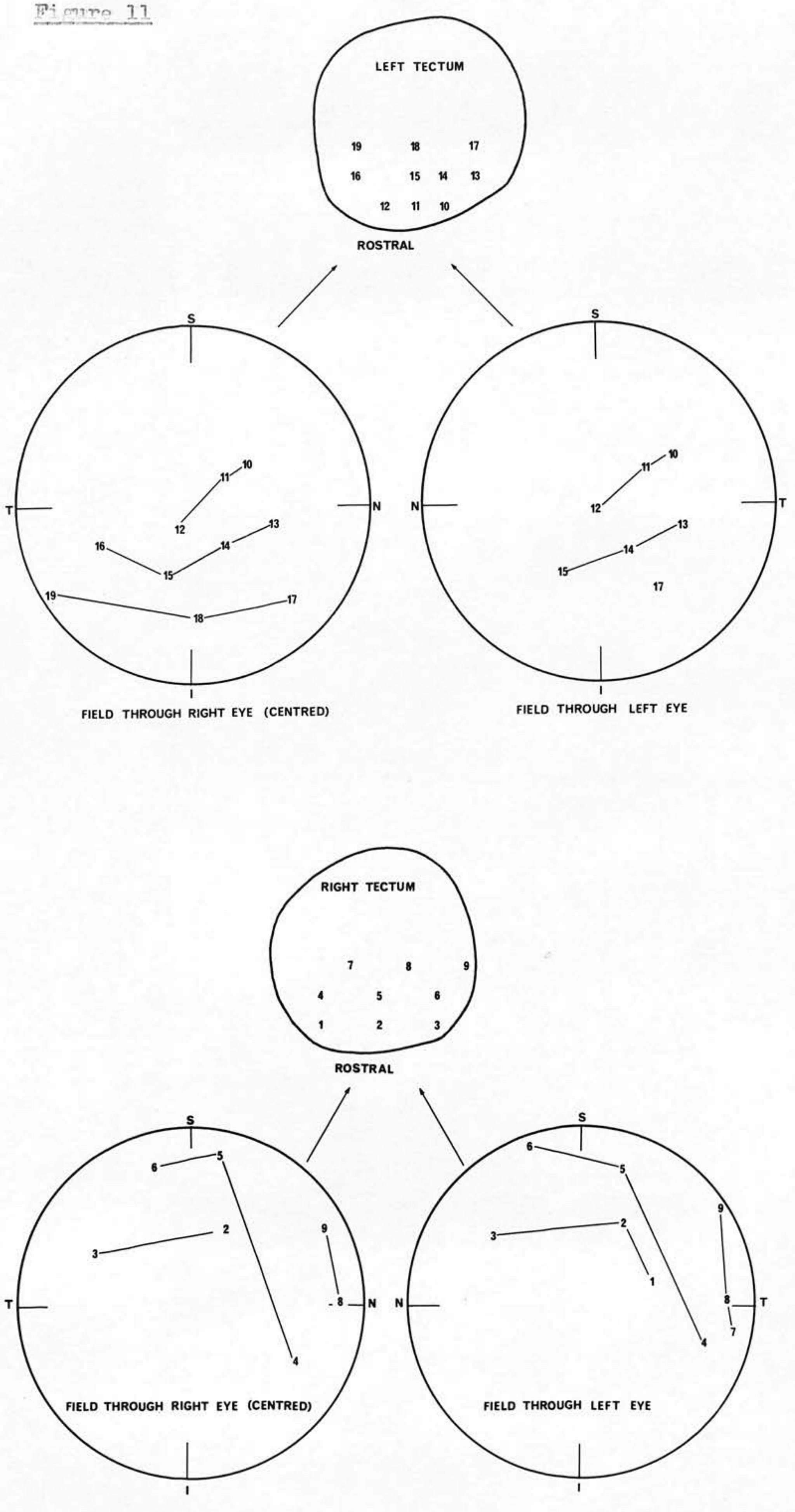
The map of the contralateral responses through the left, non-centred eye to the right tectum are shown in Fig. 11. By comparison with Figs. 3 and 4. it can be seen that this map is normal. The ipsilateral projection from the right eye to the right tectum (Fig. 11) can be seen also to be normal despite the rotation of the eye itself, and to be similar to the contralateral projection to this tectum.

EXPT XRE7.

Figure 11

Visual projections to the left and right tecta from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 90° anti-clockwise, and the left eye was normal.

Figure 11

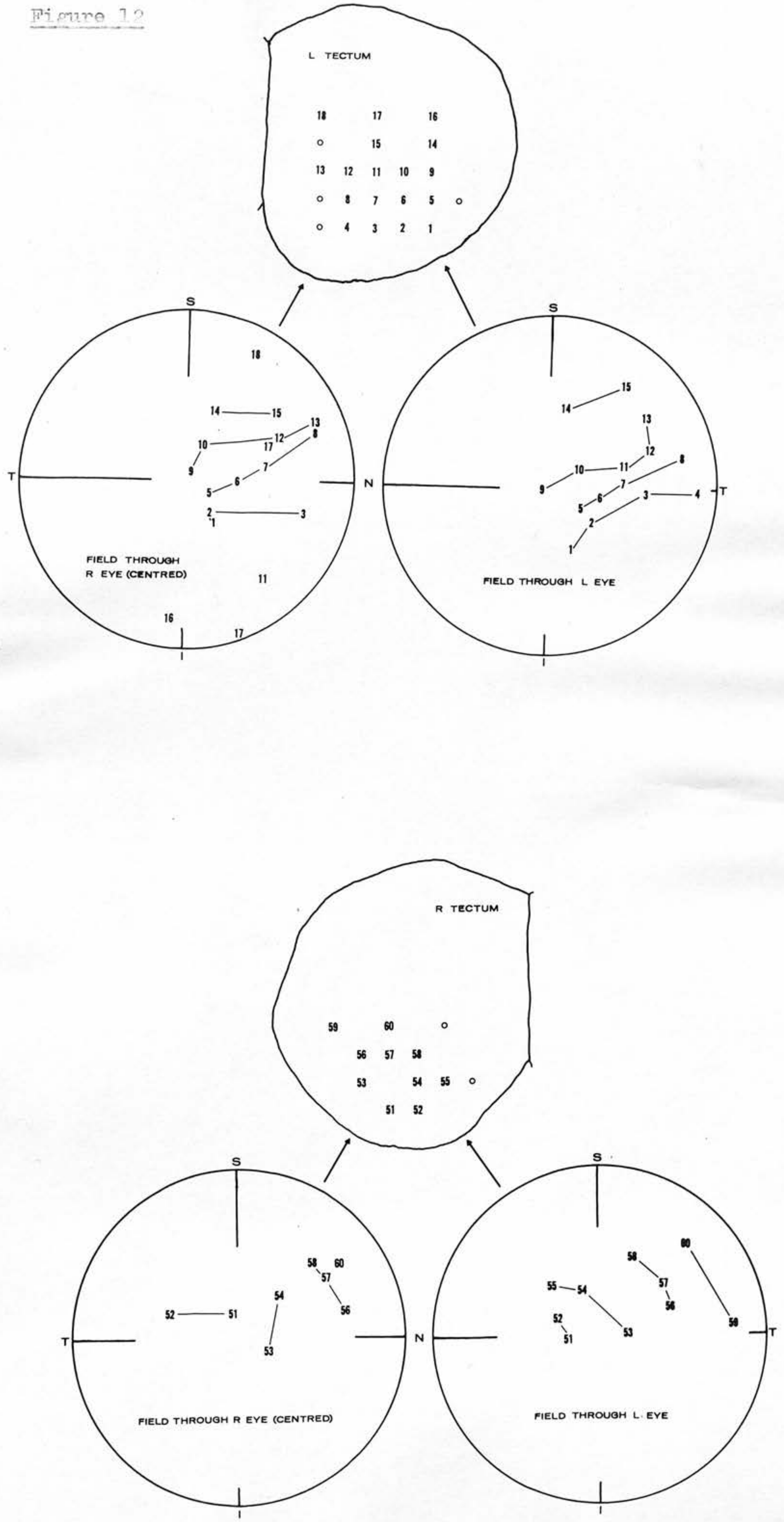

LEFT TECTUM
19 18 17
16 15 14 13
12 11 10
ROSTRAL
S
T
N
I
10 11 12 13 14 15 16 17 18 19
FIELD THROUGH RIGHT EYE (CENTRED)
S
N
T
I
10 11 12 13 14 15 17
FIELD THROUGH LEFT EYE
RIGHT TECTUM
7 8 9
4 5 6
1 2 3
ROSTRAL
S
T
N
I
2 3 4 5 6 8 9
FIELD THROUGH RIGHT EYE (CENTRED)
S
N
T
I
1 2 3 4 5 6 7 8 9
FIELD THROUGH LEFT EYE

EXPT XRE1.

Figure 12.

Visual projections to the left and right tecta from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 90° clockwise, and the left eye was normal.

Figure 12

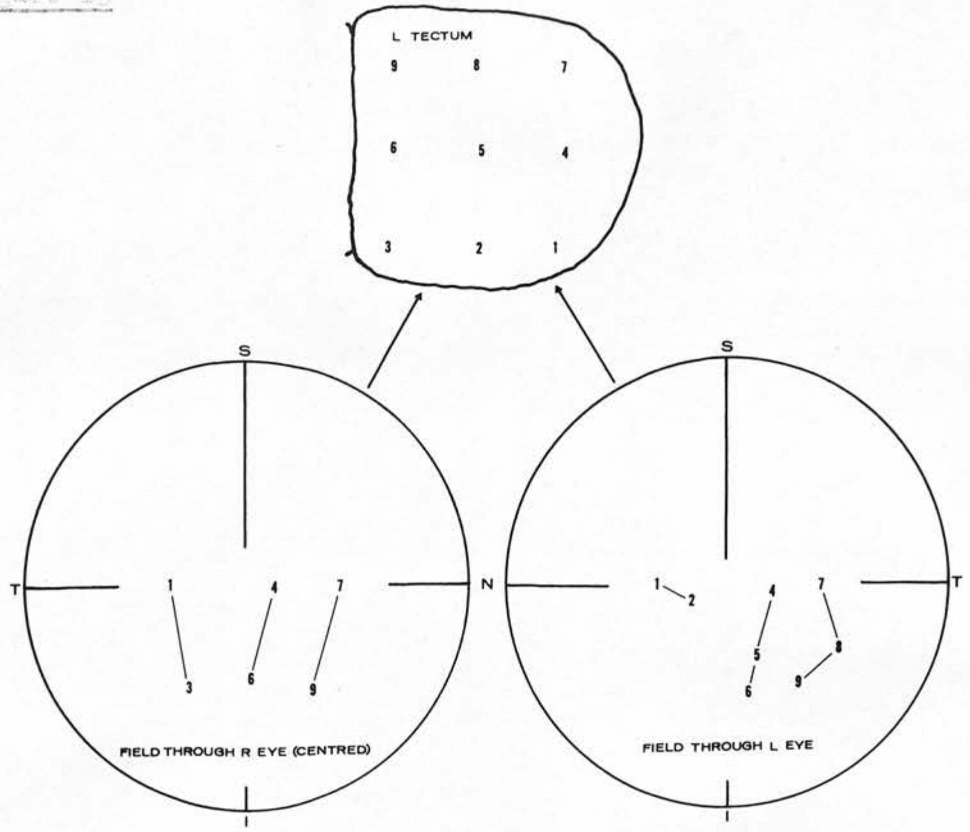

L TECTUM
18 17 16
○ 15 14
13 12 11 10 9
○ 8 7 6 5 ○
○ 4 3 2 1
S
T
N
I
18
14 15
13
10 12 8
17
9 7
5 6
2 3
1
FIELD THROUGH R EYE (CENTRED)
11
16
17
S
T
I
15
14
13
12
10 11 8
9 7
5 6 3 4
2
1
FIELD THROUGH L EYE
R TECTUM
59 60 ○
56 57 58
53 54 55 ○
51 52
S
T
N
I
58 60
57
54
56
52 51
53
FIELD THROUGH R EYE (CENTRED)
S
T
I
60
58
57
55 54
56
59
52
51
53
FIELD THROUGH L. EYE

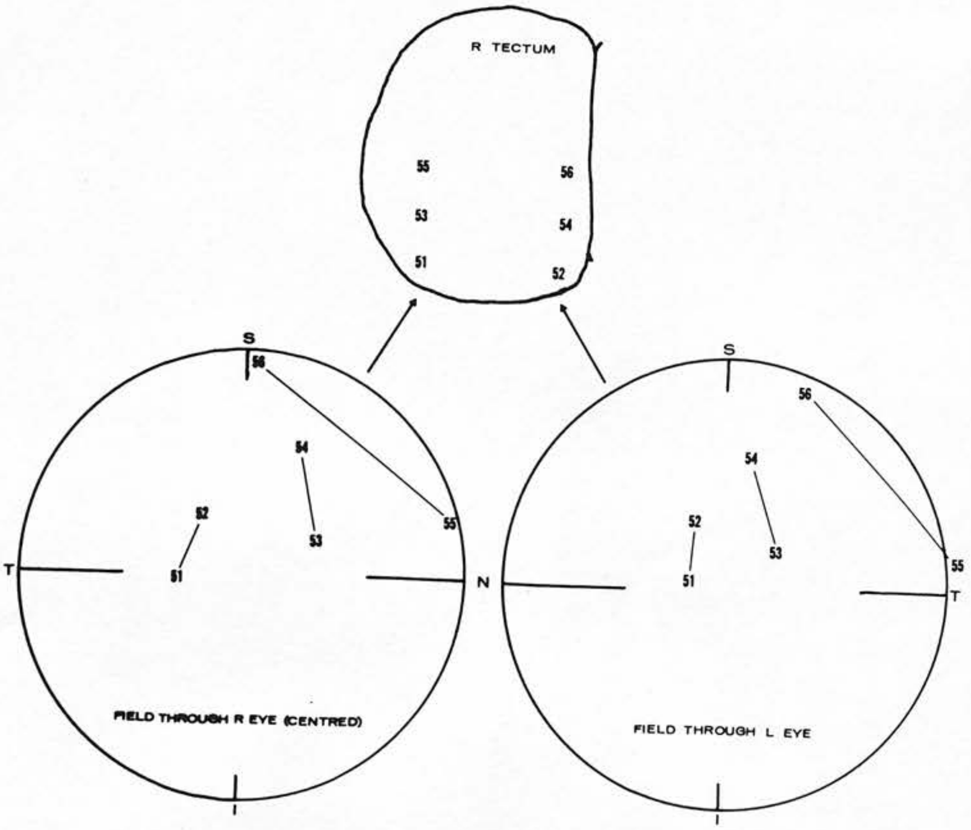
EXPT XRE2

Figure 13

Visual projections to the left and the right tecta from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 180°, and the left eye was normal.

Figure 13


L TECTUM
9
8
7
6
5
4
3
2
1
S
T
N
1
4
7
3
6
9
FIELD THROUGH R EYE (CENTRED)
I
S
N
T
1
2
4
7
5
8
6
9
FIELD THROUGH L EYE
I

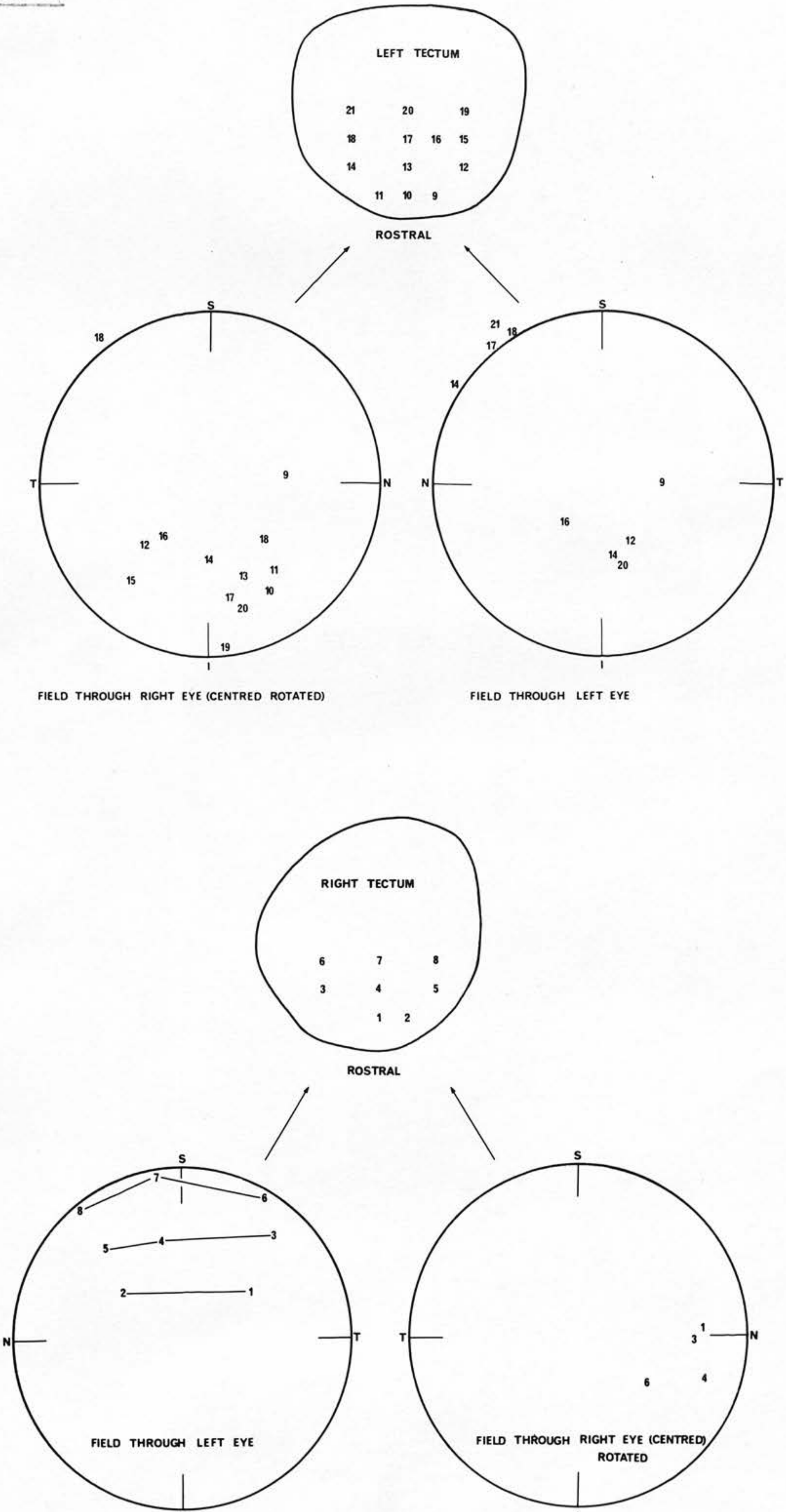

R TECTUM
55
56
53
54
51
52
S
56
54
52
55
53
T
51
N
FIELD THROUGH R EYE (CENTRED)
I
S
56
54
52
53
55
51
T
FIELD THROUGH L EYE
I

EXPT XRE9

Figure 14

Visual projections to the left and right tecta from both eyes, mapped with the right eye centred. The right eye was rotated approximately 180°, and the left eye was normal.

Figure 14


LEFT TECTUM
21 20 19
18 17 16 15
14 13 12
11 10 9
ROSTRAL
S
T
N
I
18
9
16 12
18
14
15
13
11
17
10
20
19
FIELD THROUGH RIGHT EYE (CENTRED ROTATED)
S
N
T
I
21 18 17
14
9
16
12
14
20
FIELD THROUGH LEFT EYE
RIGHT TECTUM
6 7 8
3 4 5
1 2
ROSTRAL
S
7
8
6
5
4
3
2
1
N
T
I
FIELD THROUGH LEFT EYE
S
T
N
I
1
3
6
4
FIELD THROUGH RIGHT EYE (CENTRED) ROTATED

The other 4 animals in this series which gave similar results are summarised in the table below. The visual maps of 2 of these animals, XRE1 and XRE2 are shown in Figs. 12 and 13 respectively.

TABLE 3

Xenopus	Stage at which eye rotated	Degree of rotation	R eye to L tectum	R eye to R tectum	L eye to R tectum	L eye to L tectum
XRE1	32	90° clockwise	rotated 90° clockwise	normal	normal	rotated 90° clockwise
XRE2	32	180°	rotated 180°	"	"	rotated 180°
XRE6	58	"	"	"	"	"
XRE8	44	90° anti-clockwise	rotated 90° anti-clockwise	"	"	rotated 90° anti-clockwise

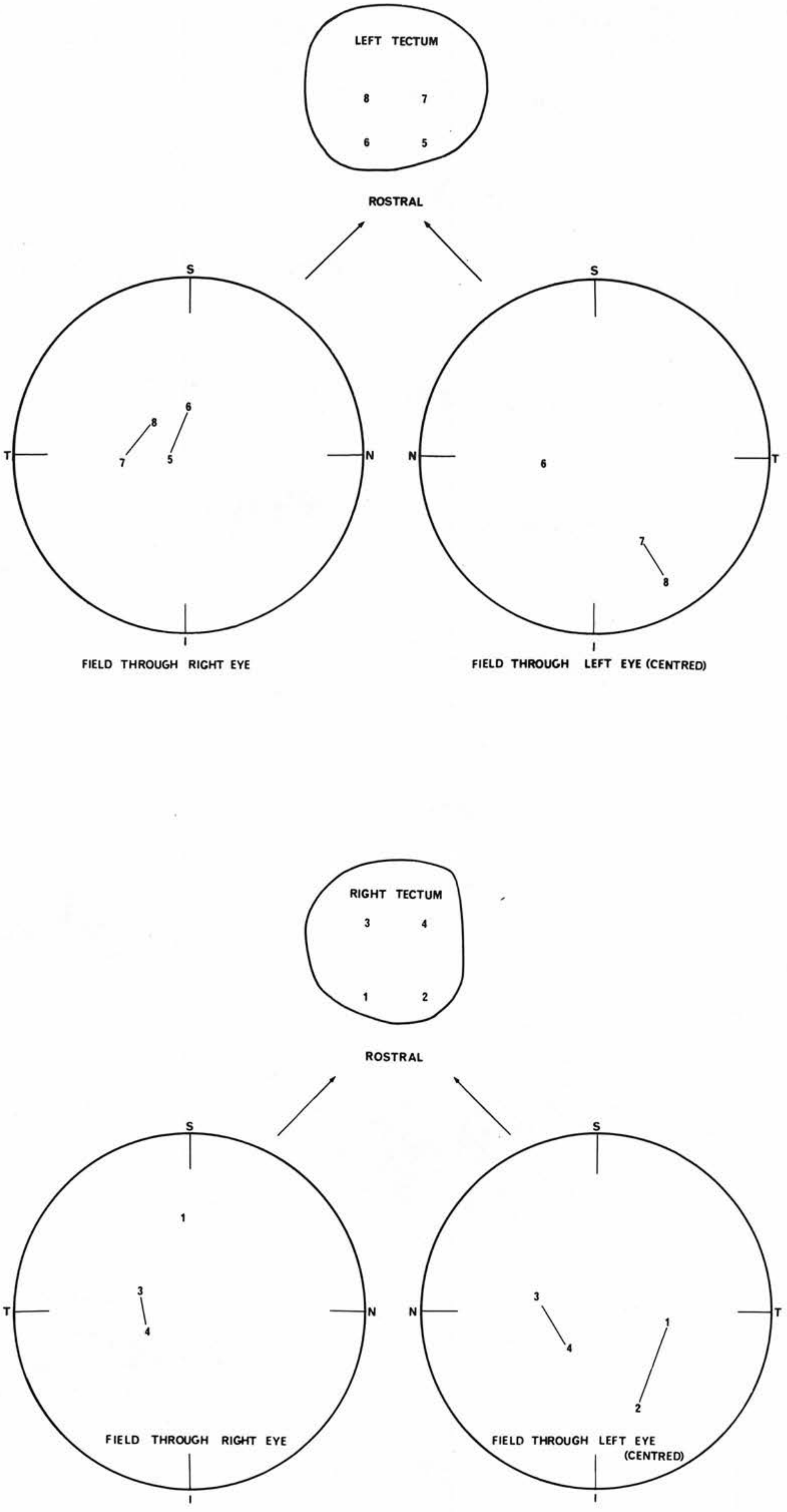
In Xenopus XRE9 the right eye was rotated through 180° at stage 38 of larval life. The visual projections of the adult animal were mapped with the right eye centred on the perimeter. Fig. 14 shows the visual projection from the rotated right eye to the left tectum. It can be seen that this projection arises from the inferior field as would be expected from a rotated eye, but that it is completely muddled.

EXPT XRE 5

Figure 15

Visual projections to the left and right tecta from both eyes, mapped with the left eye centred. The left eye was rotated approximately 180°, and the right eye was normal.

Figure 15


LEFT TECTUM
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6
5
ROSTRAL
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8
6
7
5
FIELD THROUGH RIGHT EYE
S
N
T
I
6
7
8
FIELD THROUGH LEFT EYE (CENTRED)
RIGHT TECTUM
3
4
1
2
ROSTRAL
S
T
N
I
1
3
4
FIELD THROUGH RIGHT EYE
S
N
T
I
3
4
1
2
FIELD THROUGH LEFT EYE
(CENTRED)

The ipsilateral projection from the non-centred left eye was abnormal also, consisting of 2 groups of visual responses. One group which projected to the more medial tectum was located in the far nasal superior field of the left eye. The other, which was recorded from the more lateral parts of the tectum, occupied the inferior field. Tectal locus 17, near the middle of the lobe, received visual input from both these areas of the visual field.

The ipsilateral visual responses in the inferior field of the left eye arose from approximately the same part of the visual field as did responses via the right eye to the same points on the left tectum. The one exception to this is point 12, the responses through the 2 eyes being about 45° apart.

The contralateral projection from the normal left eye to the right tectum is shown in Fig. 14. It is normally arranged in the superior visual field. However the ipsilateral projection from the rotated right eye to the right tectum consists of only 4 points, in the infero-nasal field, which do not allow any organised visual map to be constructed. The results from this animal will be discussed later.

The histological appearance of this animal was normal.

The other animal in this series which did not give a clear result was XRE5. In this animal the left eye was rotated by 180° at stage 40, and it was this eye which was centred when the visual projections of this animal were mapped.

The visual projection from the rotated left eye to the right tectum (Fig. 15) was rotated by 180° so that the projection was restricted to the inferior field. The rostral tectal loci 1-2 were receiving stimuli from the temporal field, instead of the nasal field as in the normal situation; the field positions 3-4 in the nasal field projected abnormally to the caudal tectum. The lateral part of the tectum received an input from the most superior areas of the visual field and the medial tectal positions from the more inferior parts of the visual space - this is the reverse of the situation in the normal animal (see Figs. 3 and 4).

The ipsilateral projection from the non-centred normal right eye consisted of only three points (Fig. 15). However these show clearly that the map was abnormal. Point 3 recorded from the caudo-lateral tectum was more superiorly placed than point 4 which projected to the caudo-medial tectum. The input to the rostrolateral edge of the tectum, tectal locus 1, was from the superior visual quadrant and from a slightly more nasal part of the visual field of the right eye than positions 3 and 4.

Fig. 15 shows clearly that field positions 3 and 4 were congruent through the two eyes whereas positions 1 were about 90° apart.

The contralateral projections from the normal right eye to the left tectum was normal (Fig. 15), whereas the ipsilateral projection from the rotated left eye was not. The three points 6-7-8, on the ipsilateral map were all found in the temporal field. These results will be considered more fully in the discussion.

Contralateral eye grafts

I found that when a specified eye was rotated through 180° thereby reversing both its naso-temporal and the dorso-ventral axes, the ipsilateral projection from the normal eye was rotated in these axes. This supported the hypothesis of binocular interaction. As another test of the theory it was decided to investigate the visual projections in animals with one normal eye and one eye in which only one axis was reversed. One way of reversing only one axis is to remove an eye from the host animal and replace it by an eye from the opposite side of a donor. In this way if the orientation of the eye is not altered, the naso-temporal axis is reversed and the dorso-ventral axis is normal.

The two animals successfully recorded in this series had been operated upon at stage 38; the left eye had been removed and replaced by a right eye from a donor at the same stage, keeping as far as was possible the dorso-ventral axis unaltered. Eye transplants carried out between tadpoles at stage 45 proved unsuccessful, as the transplanted eye completely degenerated.

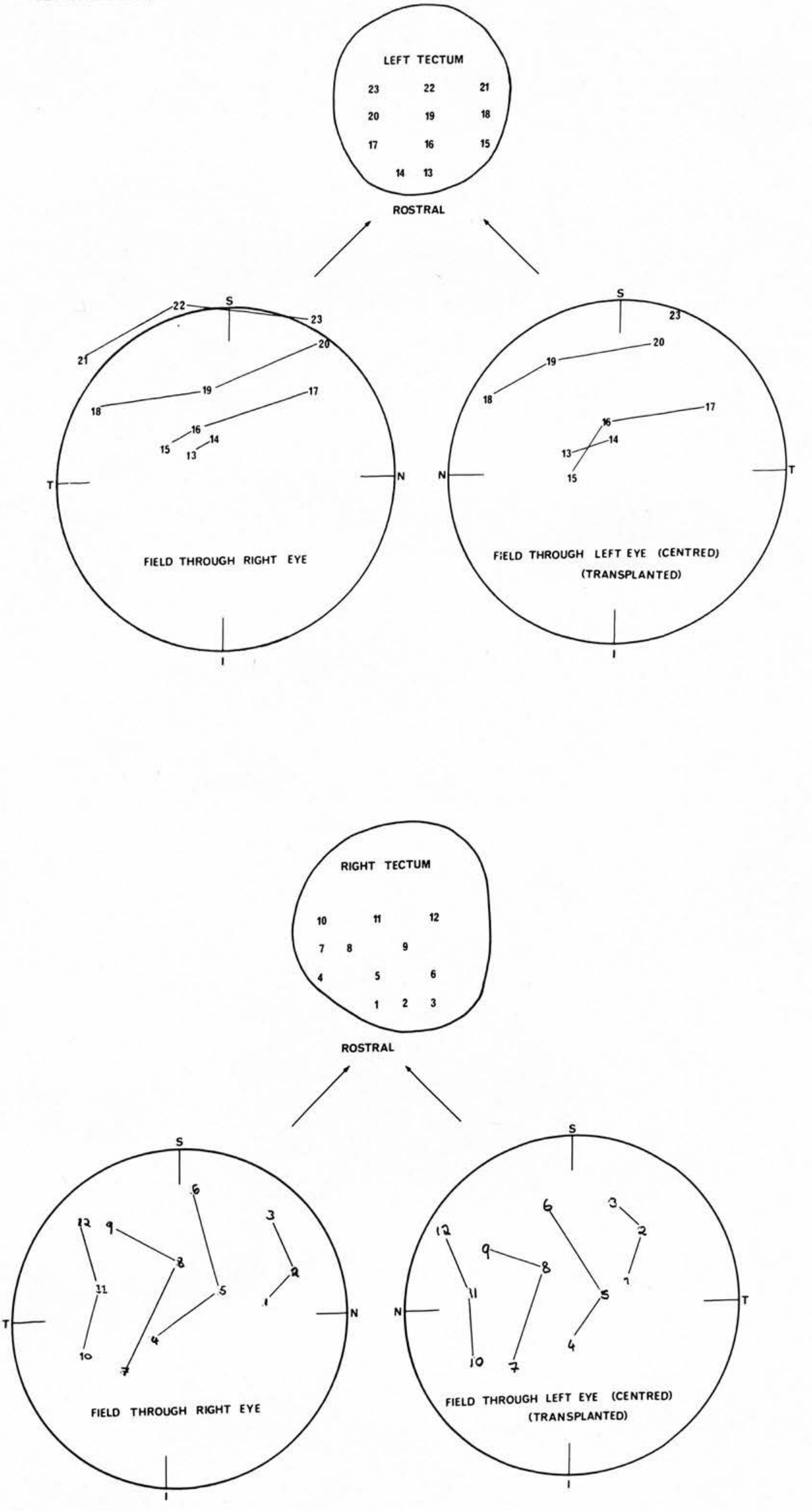
The animals were set up with the transplanted left eye centred on the perimeter. In Contrans 3 the contralateral projection from the transplanted left eye (Fig. 16) was normal in the dorso-ventral axis, but reversed in the naso-temporal one. Thus points at the rostral pole of the tectum, row 1-2-3 were receiving stimuli from the temporal visual field, instead of from the nasal field as in the case of a normal animal (Fig. 3.). The most superior points in

EXPT CONTRANS 3

Figure 16

Visual projections to the left and right tecta from both eyes, mapped with the left eye centred. The left eye was a transplanted right eye, and the right eye was normal.

Figure 16


LEFT TECTUM
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ROSTRAL
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13
T
N
FIELD THROUGH RIGHT EYE
I
S
23
20
19
18
17
16
14
13
15
N
T
FIELD THROUGH LEFT EYE (CENTRED)
(TRANSPLANTED)
I
RIGHT TECTUM
10
11
12
7
8
9
4
5
6
1
2
3
ROSTRAL
S
6
12
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3
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T
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4
10
7
FIELD THROUGH RIGHT EYE
I
S
6
3
12
2
9
8
11
5
1
N
T
4
10
7
FIELD THROUGH LEFT EYE (CENTRED)
(TRANSPLANTED)
I

the visual field, 3, 6, 9,12, projected to the medial tectum, as they do in a normal animal.

Fig. 16 shows clearly that the ipsilateral projection from the normal right eye reflected almost exactly the abnormalities of the contralateral projection from the transplanted left eye. The naso-temporal axis of the ipsilateral projection from the normal eye was reversed as compared to that in a normal animal, but the dorso-ventral axis was normal. Furthermore, the rows of field positions through the two eyes to the same tectal points were very similar in their position in visual space.

By comparison with the visual maps recorded from a normal animal (Fig. 3), it can be seen that visual map recorded through the non-centred normal right eye to the contralateral left tectum was normal (Fig. 16). The ipsilateral projection from the abnormal left eye was also normal (Fig. 16).

Histological examination of this animal showed no abnormalities.

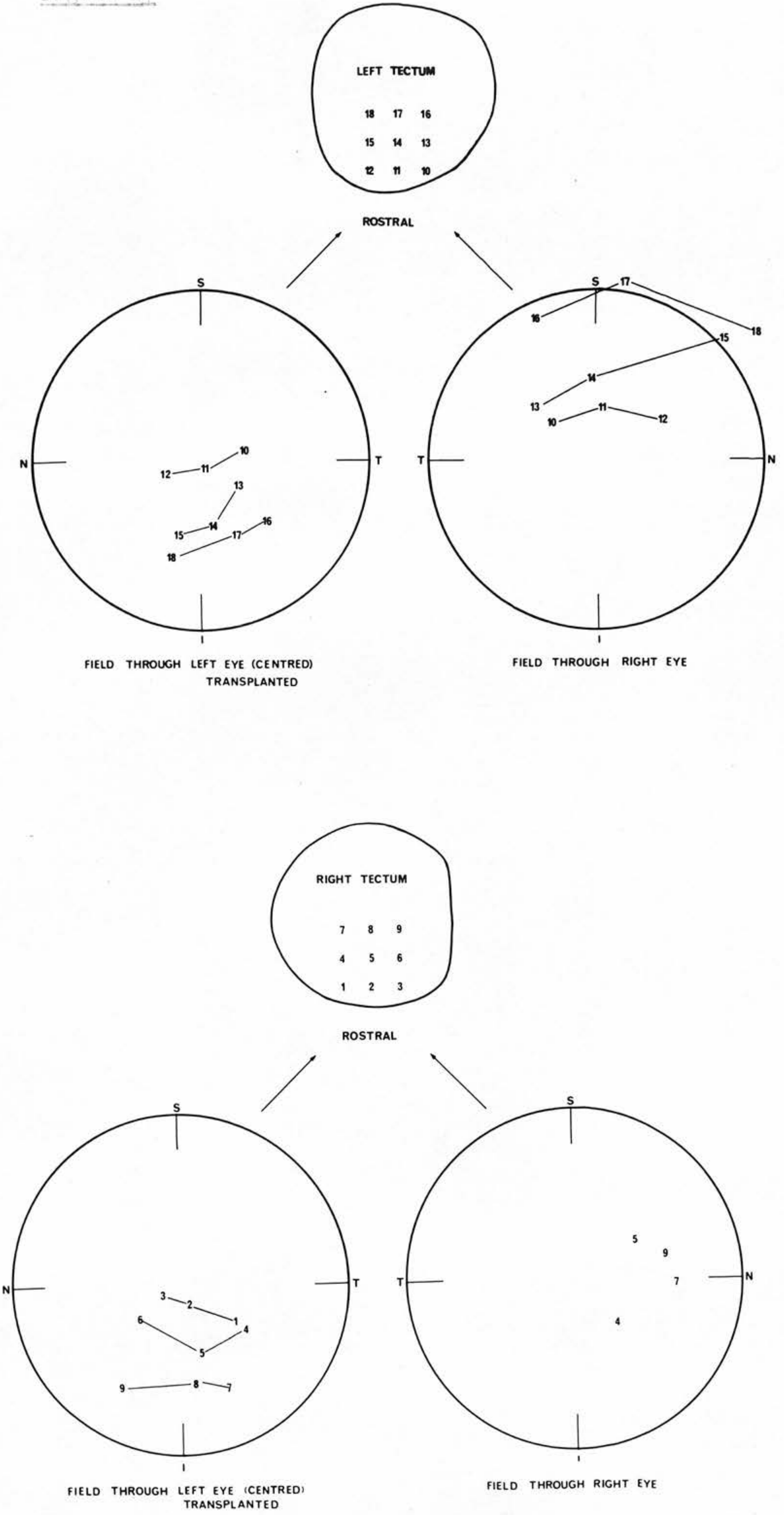
In Contrans 4 the left eye had been replaced by the right eye from another embryo, both host and donor being at stage 38. The transplanted eye was tilted by about 45° anti-clockwise. This could be seen clearly since the iridial notch of the left eye occupied a more temporal position in the orbit. This rotation of the eye was apparent in the visual map from the centred left eye to the right tectum (Fig. 17). The rows of points instead of running in a supero-inferior direction as in the normal animal (Fig. 3)

CONTRANS 4

Figure 17

Visual projections to the left and right tecta from both eyes, mapped with the left eye centred. The left eye was a transplanted right eye, and the right eye was normal.

Figure 17


LEFT TECTUM
18 17 16
15 14 13
12 11 10
ROSTRAL
S
N
T
I
12 11 10
13
15 14
18 17 16
FIELD THROUGH LEFT EYE (CENTRED) TRANSPLANTED
S
T
N
I
17
16
18
15
14
13
10 11 12
FIELD THROUGH RIGHT EYE
RIGHT TECTUM
7 8 9
4 5 6
1 2 3
ROSTRAL
S
N
T
I
3 2 1
6 4
5
9 8 7
FIELD THROUGH LEFT EYE (CENTRED) TRANSPLANTED
S
T
N
I
5
9
7
4
FIELD THROUGH RIGHT EYE

ran from the supero-nasal quadrant to the infero-temporal one. Nevertheless, the reversal of the naso-temporal axis of the eye was reflected in the contralateral projection. Positions as the rostral pole of the tectum, 1-2-3, were receiving stimuli from the temporal field, instead of from the nasal field as in the normal animal. The dorso-ventral axis although rotated by about 45° was not inverted, the most superior points projecting to the medial tectum in the same way as in a normal animal.

Fig. 17 also shows the visual projection from the normal right eye to the right tectum. Only 5 visual positions were mapped and these did not seem to be arranged into rows, the projections being highly abnormal. This result will be further considered in the discussion.

The contralateral projection from the non-centred right eye to the left tectum was normal, (Fig. 17). The ipsilateral projection from the transplanted left eye to the left tectum was well ordered. The most superior points in the field projected to the most rostral tectum, and the most inferior to the more caudal tectum. The most nasal positions on the visual field to the medial part of the tectum, and the most temporal to the lateral tectum. Comparison of the contralateral and ipsilateral projections from the left eye shows the great similarity between these two visual maps. The significance of this similarity will be considered in the discussion.

Double-nasal compound eyes

The original observations which led Keating to put forward the hypothesis of binocular interaction had been made using a series of Xenopus with compound eyes. It was decided to record from another series of such animals to check the results. This extra series was also of value because the animals were set up with the compound eye centred on the perimeter throughout the recording. It would then be possible to find out whether one point in visual space did project via the two eyes to the same tectal locus.

In the previous series of compound-eyed Xenopus one eye was centred and the visual projections through that eye to both tecta were mapped before changing the orientation of the animal and centring the other eye, previous to recording the visual projections from this eye. Thus the contralateral and ipsilateral projections to one tectum were mapped with different eyes centred on the perimeter. It is not possible to calculate the position of points in the visual field of one eye in terms of the visual field of the other, this is because the exact angle through which the eye has been moved cannot be assessed accurately, due to the unavoidable inaccuracies in centering the eye. It was not possible therefore to be certain that in the first series of Xenopus that the visual maps through the two eyes to one tectum were congruent. By mapping an animal with one eye centred throughout this could readily be seen.

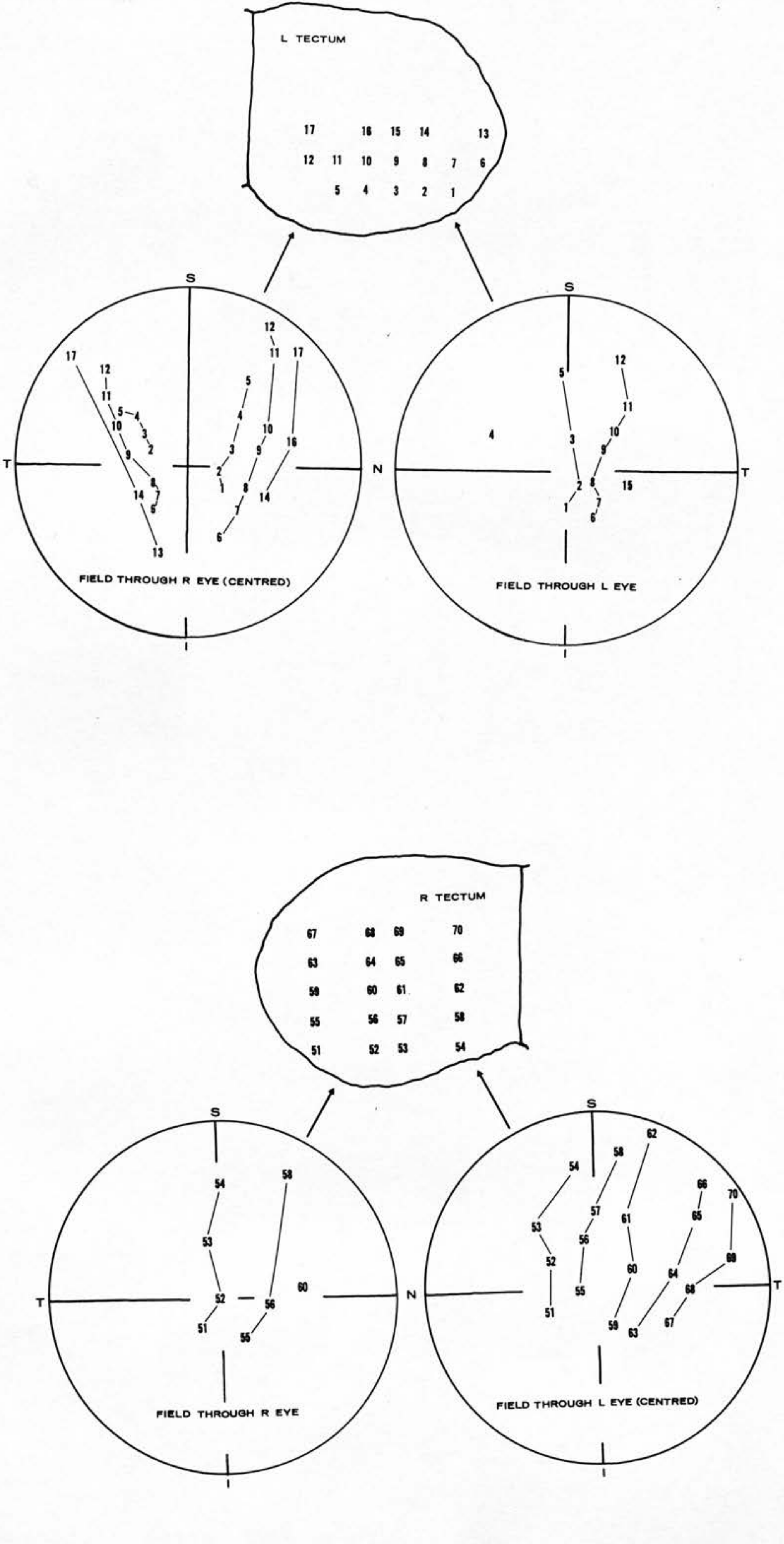
In this series four animals with a double-nasal compound right eye were recorded. In three animals XCN2,3 and 4, the right eye centred throughout the experiment; in the fourth

EXPT XCN 1

Figure 18

Visual projections to the left tectum from both eyes mapped with the right eye centred on the perimeter; visual projections to the right tectum from both eyes, mapped with the left eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

Figure 18


L TECTUM
17 16 15 14 13
12 11 10 9 8 7 6
5 4 3 2 1
S
T
N
I
FIELD THROUGH R EYE (CENTRED)
FIELD THROUGH L EYE
R TECTUM
67 68 69 70
63 64 65 66
59 60 61 62
55 56 57 58
51 52 53 54
FIELD THROUGH R EYE
FIELD THROUGH L EYE (CENTRED)

however, XCN1 the right eye was centred while mapping the projections through both eyes to the right tectum. The four animals gave similar results in terms of the binocular interaction hypothesis. Small variations in the contralateral projections, however, complicate the results and warrant a full description of the visual maps in each case.

In Xenopus XCN1, the contralateral projection from the centred compound eye to the left tectum (Fig. 18) was similar to the projections mapped by Gaze, Jacobson and Szekely (1963, 1965). Rows of field positions ran vertically downwards in the visual field as they do in a normal animal, and were normally organised in the dorso-ventral axis. Thus medial tectal points 5, 12, 17 received input from the most superior part of the visual field and lateral tectal points 1, 6, 14 from positions in the more inferior field. The rows of field positions were mirrored along a vertical line through the centre of the visual field. The rows in the temporal field were normally organised in the naso-temporal axis, with the most nasal points projecting to the rostral tectum. The naso-temporal axis of rows of field positions in the nasal field was reversed, the most nasal parts projecting to the more caudal tectal loci.

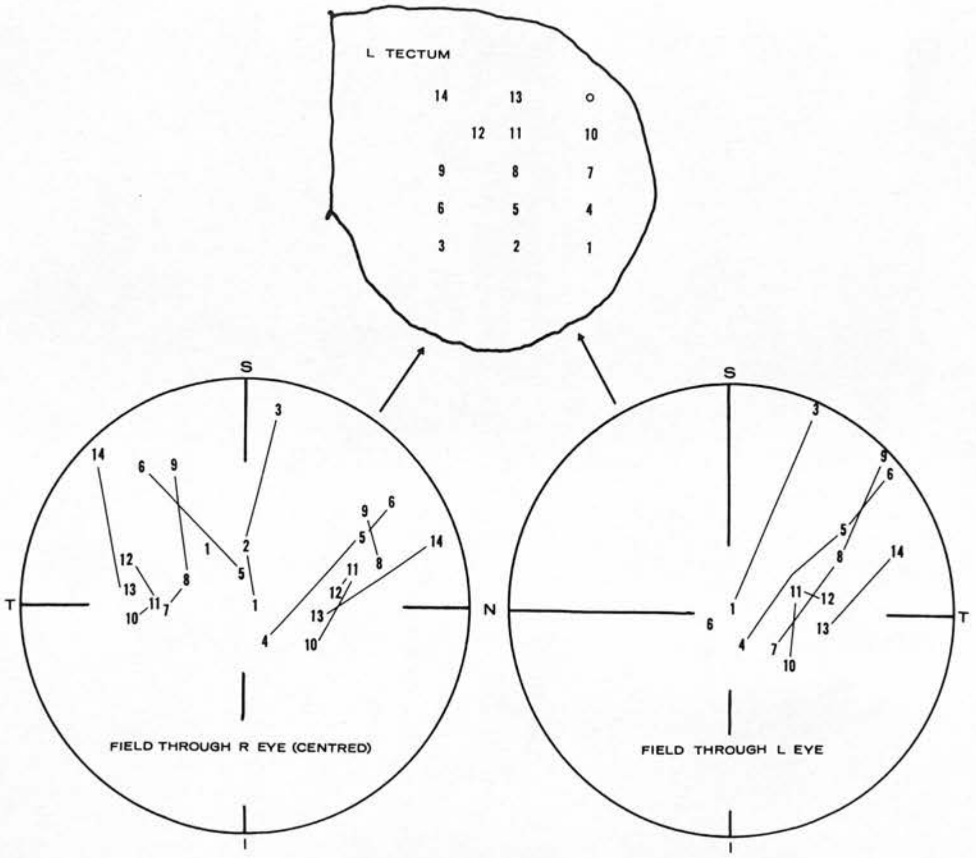
The ipsilateral projection through the normal left eye to the left tectum was itself abnormal and reflected the abnormality in the rows of field positions in the nasal field of the contralateral projection from the compound eye to the same tectum. In other words, the naso-temporal axis of the

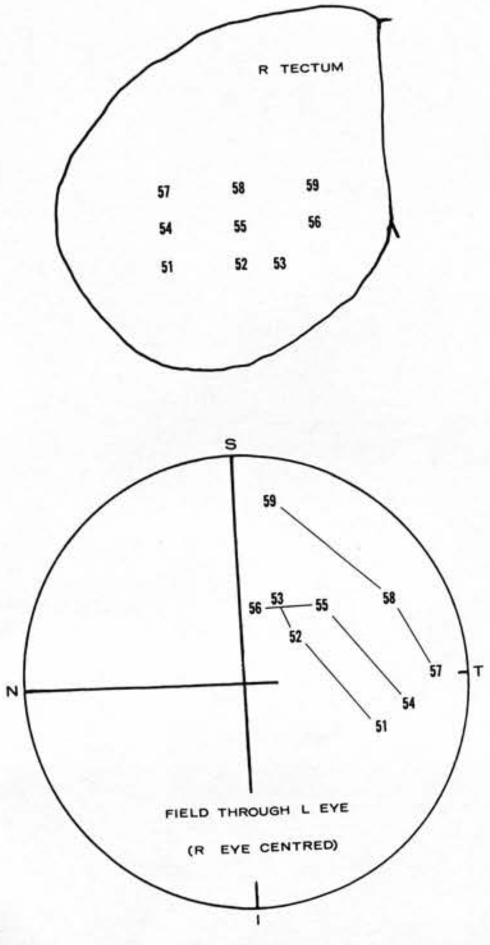
EXPT XCN2

Figure 19

Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter; visual projection from the left eye to the right tectum, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

Figure 19


L TECTUM
14 13 0
12 11 10
9 8 7
6 5 4
3 2 1
S
T
N
I
14 6 9 3 1 2 5 12 13 11 10 7 8 1 9 6 5 8 14 11 12 13 4 10
FIELD THROUGH R EYE (CENTRED)
S
N
T
I
3 9 6 5 14 8 1 11 12 6 4 13 7 10
FIELD THROUGH L EYE


R TECTUM
57 58 59
54 55 56
51 52 53
S
N
T
I
59 58 56 53 55 52 57 54 51
FIELD THROUGH L EYE
(R EYE CENTRED)

ipsilateral projection was reversed. There were no field positions corresponding to the temporal field positions of the contralateral projection from the right eye, with the exception of field positions 4.

The visual projections to the right tectum with the left eye centred are illustrated in Fig. 18. It can be seen that both the contralateral projection from the normal eye and the ipsilateral from the compound eye were normal. Further, the field positions mapped via the two eyes to the same tectal loci can be seen to arise from very similar areas of visual space.

Three visual projections were mapped in Xenopus XCN2 before the animal died. The right eye was centred throughout the experiment. Fig. 19 shows the contralateral and ipsilateral maps to the left tectum. The rows of field positions mapped via the compound eye did not run vertically but are rotated by about 45° clockwise and are somewhat mixed up. However, despite the slight misalignment of the rows, the map was similar to that recorded from Xenopus XCN1, in terms of the naso-temporal and dorso-ventral axes. The ipsilateral map from the normal left eye to the left tectum was reversed in the naso-temporal axis but not in the dorso-ventral one. The projection was rotated by approximately 45° clockwise and slightly mixed up. The nasal half of the contralateral projection to the left tectum is extremely similar to the ipsilateral projection to that tectum.

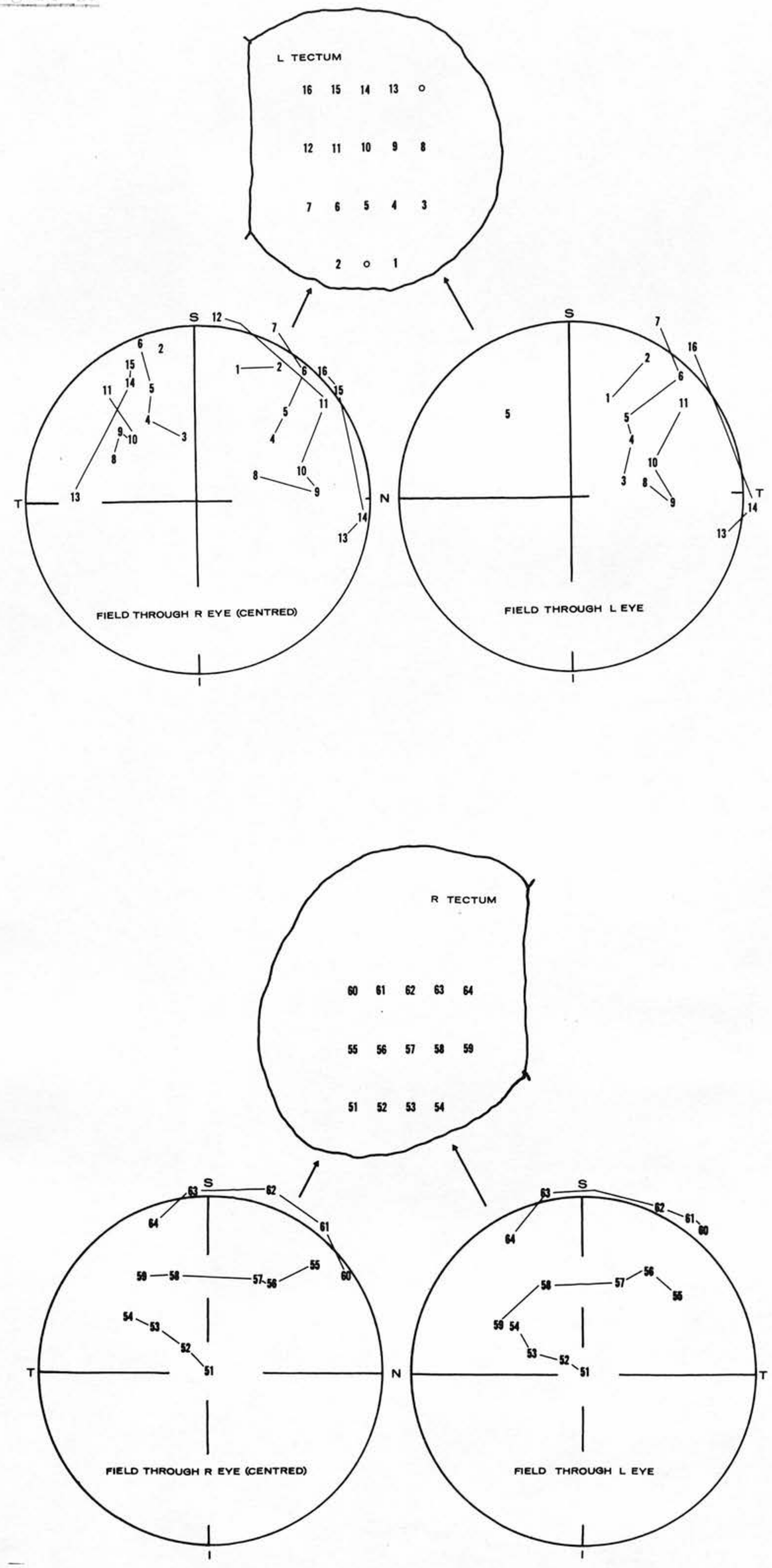
The contralateral projection from the left eye to the right tectum was normal (Fig. 19); the ipsilateral projection to this tectum was not mapped.

EXPT. XCN3

Figure 20

Visual projections to the left and right tecta from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

Figure 20

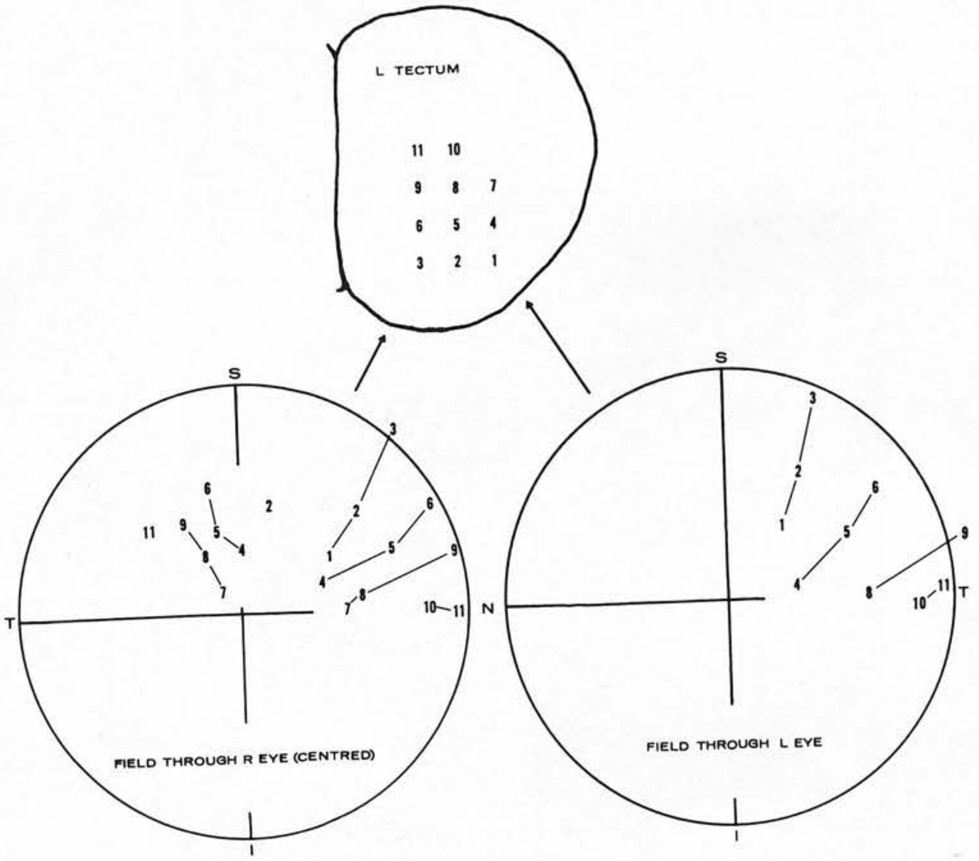

L TECTUM
16 15 14 13 o
12 11 10 9 8
7 6 5 4 3
2 o 1
S
T
N
I
FIELD THROUGH R EYE (CENTRED)
S
N
T
I
FIELD THROUGH L EYE
R TECTUM
60 61 62 63 64
55 56 57 58 59
51 52 53 54
S
T
N
I
FIELD THROUGH R EYE (CENTRED)
S
N
T
I
FIELD THROUGH L EYE

EXPT XCN4

Figure 21

Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

Figure 21


L TECTUM
11 10
9 8 7
6 5 4
3 2 1
S
T
N
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6
2
11
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5
4
8
7
3
2
1
6
5
9
4
8
7
10
11
FIELD THROUGH R EYE (CENTRED)
S
3
2
1
6
5
4
9
8
11
10
T
I
FIELD THROUGH L EYE

The contralateral map from the compound right eye to the left tectum in Xenopus XCN3, is shown in Fig. 20. The map displayed the usual characteristics of a double nasal compound eye. The ipsilateral projection from the normal left eye was abnormal being reversed in the naso-temporal axis. Slight irregularities in this projection matched almost exactly those in the nasal half of the contralateral projection from the compound eye to the left tectum.

The contralateral projection from the normal left eye (Fig. 20) appeared normal except that it was rotated anti-clockwise by 45°. It was noticeable that the relationship between the optic axes of this Xenopus were abnormal, the compound eye being abnormally positioned in the orbit. This abnormality was reflected in the orientation of the contralateral projection from the non-centred normal left eye. The ipsilateral projection from the compound right eye is also normal apart from a similar anti-clockwise rotation of 45°.

In Xenopus XCN4, the contralateral projection from the compound right eye (Fig. 21) was essentially similar to those mapped in the other Xenopus of this series, except that the rows of field positions in the nasal field were rotated by 45° clockwise and those in the temporal field by 45° anti-clockwise. The ipsilateral projection from the non-centred left eye was reversed in the naso-temporal axis and showed a rotation of 45° clockwise. This projection corresponded closely with the nasal field positions mapped via the right eye to the left tectum. The contralateral projection from the normal left eye was normal. The ipsilateral projection from the compound eye was not mapped.

The results of these four compound eye experiments is summarised in Table 4, shown below:-

TABLE 4

Xenopus	L eye to L tectum	R eye to R tectum	R eye to L tectum	L eye to R tectum
XCN1	Reversed in NT axis. Normal in DV axis. No rotation	Normal	Reversed in NT axis. Normal in DV axis. No rotation	Normal
XCN2	Reversed in NT axis. Normal in DV axis. Rotated by 45° clockwise	-	Reversed in NT axis. Normal in DV axis. Rotated by 45° clockwise	Normal
XCN3	Reversed in NT axis. Normal in DV axis. No rotation	Normal but rotated 45° anti-clockwise	Reversed in NT axis. Normal in DV axis. No rotation	Normal but rotated 45° anti-clockwise
XCN4	Reversed in NT axis. Normal in DV axis. Rotated 45° clockwise	-	Reversed in NT axis. Normal in DV axis. Rotated 45° clockwise	Normal

Eye rotation in stage 65/66 Xenopus juveniles

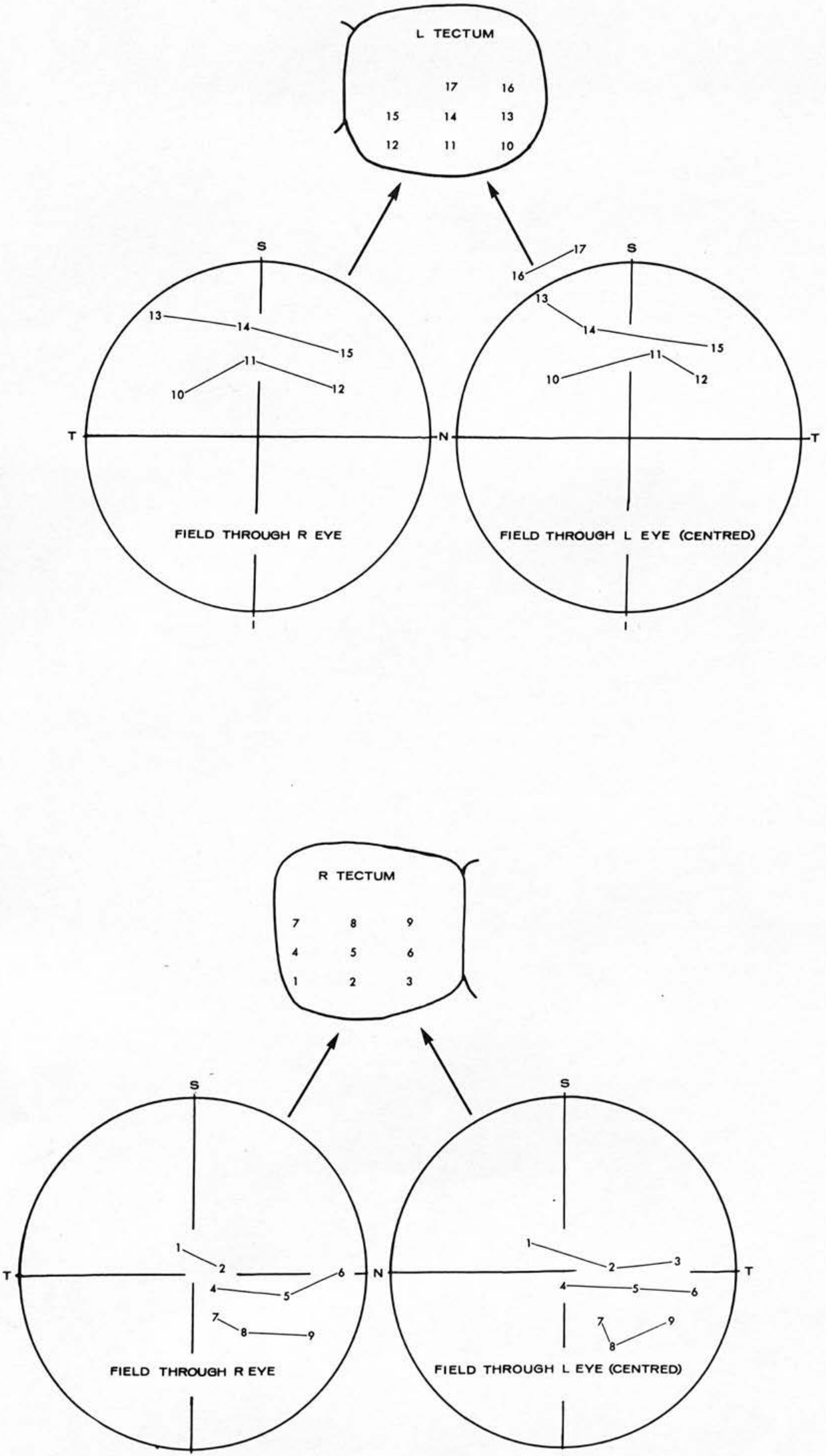
We knew that if an eye was rotated in a stage 58 Xenopus tadpole, then that animal grew up with the visual projections through the rotated and non-rotated eyes to one tectum being congruent. I wanted to pinpoint the latest stage at which this result would be obtained in Xenopus, to find out when interaction took place. In order to delimit

EXPT XYAR8

Figure 22

Visual projections to the left and right tecta from both eyes, mapped with the left eye centred on the perimeter. The left eye was rotated approximately 90° clockwise, and the right eye was normal.

Figure 22


L TECTUM
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15
11
10
12
T
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FIELD THROUGH R EYE
I
16
17
S
13
14
15
11
10
12
T
FIELD THROUGH L EYE (CENTRED)
I
R TECTUM
7
8
9
4
5
6
1
2
3
S
1
2
T
4
5
6
7
8
9
N
FIELD THROUGH R EYE
I
S
1
2
3
T
4
5
6
7
8
9
FIELD THROUGH L EYE (CENTRED)
I

the period involved, I decided to carry out one series of eye rotations in stage 65/66 Xenopus and then another in adult Xenopus. Stage 65/66 seemed a particularly interesting one since ipsilateral responses were developing at approximately this stage. In fact this assumption was true and eye rotations at this stage gave very relevant results.

The left eye was rotated in a series of stage 65/66 Xenopus juveniles. In a few instances the rotation was not successful and it was necessary to carry out an enucleation. These enucleate animals were kept along with the animals with a rotated eye. The animals were used for recording after several months; the enucleate animals were set up with the remaining eye centred on the perimeter; the other animals in this series were positioned so that the rotated left eye was centred on the perimeter throughout the recording.

Ten animals in which one eye had been successfully rotated were used for recording. The 10 animals fell into two groups, of 7 animals and of 3, in terms of the results they gave.

The results of visual mapping of Xenopus XYAR8 are typical of those recorded from the 7 animals which constituted the larger group. In these animals the left eye had been rotated by 90° clockwise.

The contralateral projection from the rotated left eye is shown in Fig. 22. The rows of field positions can be seen to run horizontally across the visual field and not vertically as they do in a normal Xenopus. The rostral tectum, loci 1-2-3, received an input from the superior visual field and the medial tectum 3,6,9 from the temporal field. The map is therefore rotated by 90° clockwise compared with the normal situation.

The ipsilateral projection from the normal right eye to the right tectum is also rotated clockwise by 90° (Fig. 22). There is a strong similarity between this ipsilateral projection and the contralateral projection from the left eye to the right tectum. It can be seen that the visual inputs to any one tectal position mapped through the two eyes were recorded as being less than 10° apart, despite the 90° rotation of the left eye.

The contralateral visual projection mapped from the right eye to the left tectum was normal (Fig. 22). The ipsilateral projection from the rotated left eye to the left tectum was also normal, and corresponded closely with the contralateral projection to this tectum.

The results from this Xenopus and the other 6 animals in the series which gave similar results are summarised in table 5 below, (p.67).

Xenopus XYAR 5 was one of the three Xenopus which gave results significantly different from those found in the Xenopus listed in table 5. In this animal, the left eye had been rotated 90° clockwise.

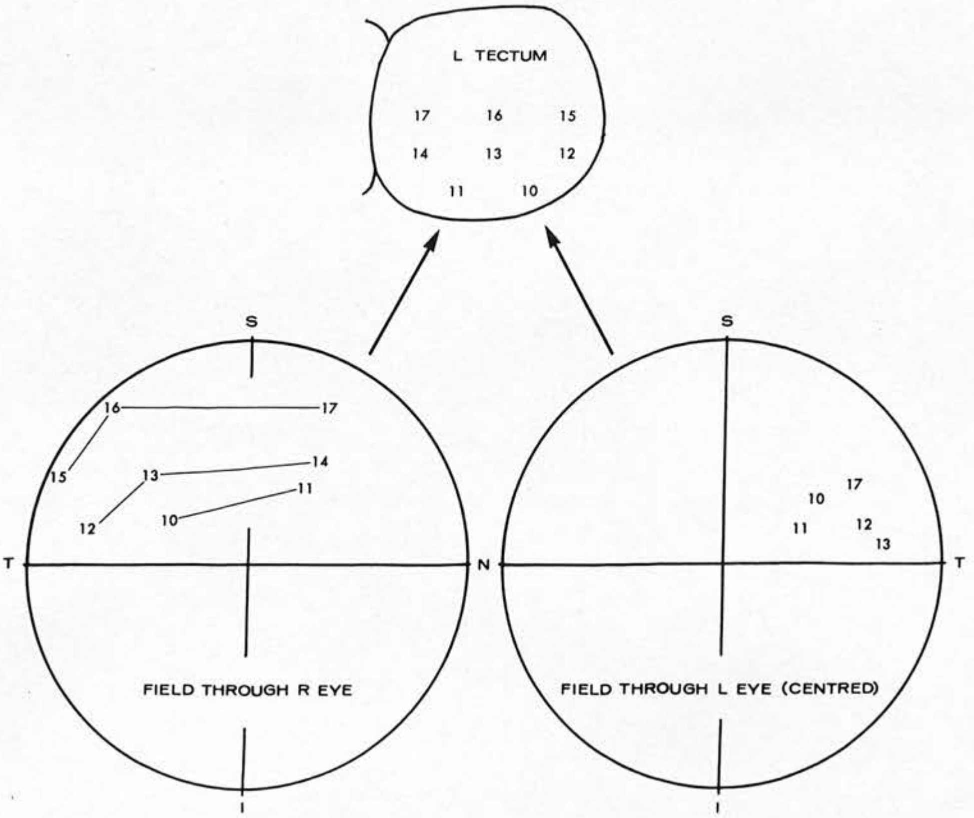
Fig. 23 shows the visual projections from the left and right eyes to the right tectum in XYAR5. The contralateral map from the rotated left eye was rotated by 90° clockwise. The rostral tectum, positions 1-2-3, received its input from the superior part of the field. This is in contrast to the

EXPT XYAR5

Figure 23

Visual projections to the left and right tecta from both eyes, mapped with the left eye centred. The left eye was rotated 90° clockwise.

Figure 23


L TECTUM
17 16 15
14 13 12
11 10
S
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16
17
15
13
14
11
12
10
FIELD THROUGH R EYE
I
S
T
10
17
11
12
13
FIELD THROUGH L EYE (CENTRED)
I

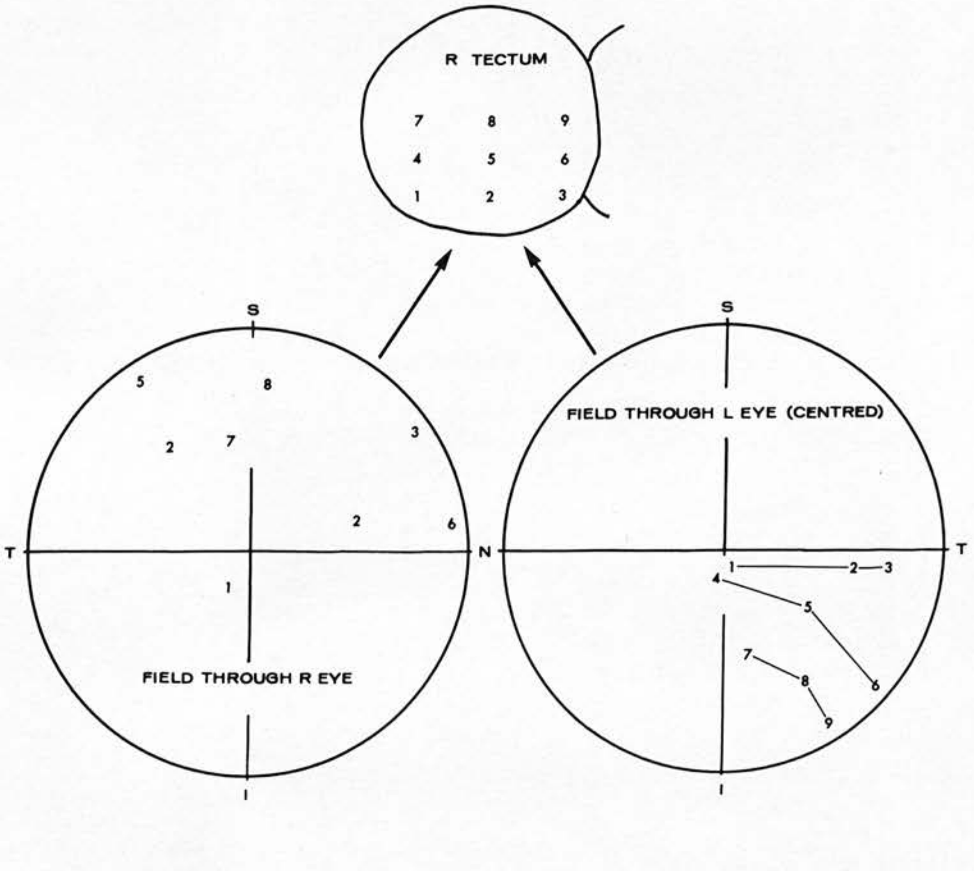

R TECTUM
7 8 9
4 5 6
1 2 3
S
5
8
2
7
3
2
6
T
N
1
FIELD THROUGH R EYE
I
S
FIELD THROUGH L EYE (CENTRED)
T
1
2
3
4
5
7
8
6
9
I

TABLE 5

Xenopus	Left Eye to Right Tectum	Right Eye to Right Tectum	Right Eye to Left Tectum	Left Eye to Left Tectum
XYAR8	Rotated 90° clockwise	Rotated 90° clockwise	Normal	Normal
XYAR2	Rotated 90° clockwise	Rotated 90° clockwise	"	"
XYAR3	Rotated 75° clockwise	Rotated 75° clockwise	"	"
XYAR4	Rotated 90° clockwise	Rotated 90° clockwise	"	"
XYAR5	Rotated 90° clockwise	Rotated 90° clockwise	"	"
XYAR7	Rotated 90° clockwise	Rotated 90° clockwise	"	"
XYAR11	Rotated 90° clockwise	Rotated 90° clockwise	"	"

normal situation, where the rostral tectum is innervated by fibres of the temporal retina (nasal visual field). The most nasal field positions 1,4,7 projected to the lateral edge of the tectum, the tectal area normally receiving an input from the more inferior part of the visual field.

The ipsilateral projection from the normal right eye to the right tectum was abnormal. The projection was disorganised but was restricted to the superior visual field. Each tectal locus received a single visual input except point 2, which received inputs from positions in both the nasal and temporal visual quadrants.

The visual projection from the normal right eye to the left tectum was normal (Fig. 23). The ipsilateral projection from the rotated left eye to the left tectum was abnormal. It was restricted to a small area of the visual field near the horizontal meridian and consisted of field positions which did not appear to be organised in rows. Thus in Xenopus XYAR5, both the ipsilateral projections from the normal eye and the rotated eye were disorganised and highly abnormal.

In Xenopus XYAR10, the left eye had been rotated by 90° clockwise. The contralateral projection from the left eye to the right tectum was rotated by 90° clockwise also. The ipsilateral projection from the normal right eye to the right tectum was rotated to the same extent. In other words, the ipsilateral and contralateral projections to this tectum were congruent.

The contralateral projection from the normal right eye to the left tectum was normal but ipsilateral projection from the rotated eye was abnormal. It was not possible to determine the nature of this abnormality since only two visual field positions were mapped. Nevertheless, it was quite clear that the tectal loci concerned received visual information from different areas of the visual field via the two eyes.

In the third Xenopus of this group, XYAR6, the left eye was rotated by 90° clockwise. This was reflected in the orientation of the visual map recorded from the left eye to the right tectum. The ipsilateral map from the normal right eye to the right tectum bore no resemblance to the map of the left eye onto the tectum.

The right eye projected to the left tectum in a normal fashion, but the ipsilateral projection from the rotated left eye to the left tectum was abnormal. The ipsilateral map was internally organised but projected to an abnormal part of the visual field. Its orientation corresponded to neither that of the contralateral map through the normal eye to that tectum nor to the rotated alignment of the left eye.

Table 6 summarises the results of the three Xenopus XYAR10, XYAR5 and XYAR6 in which it was found that different areas of this visual field project via the two eyes to the same tectal locus.

TABLE 6

Xenopus	Left Eye to Right Tectum	Right Eye to Right Tectum	Left Eye to Left Tectum	Right Eye to Left Tectum
XYAR10	Rotated by 90° clockwise	Disorganised	Disorganised	Normal
XYAR5	Rotated by 90° clockwise	Rotated by 90° clockwise	Abnormal	Normal
XYAR6	Rotated 90° clockwise	Disorganised Abnormal	Orderly abnormally positioned	Normal

Two animals which had been enucleated at stage 65/66 were later successfully recorded. In one animal XYAR12, the contralateral and ipsilateral maps from the remaining eye were normal.

The contralateral projection from the remaining right eye to the left tectum were found to be normal in XYAR11. Only five visual field positions were recorded from the right eye to the right tectum. All positions were in the nasal field as is the case in a normal animal. However, there was not a sufficient number of points mapped, to allow a meaningful map to be constructed.

When the animals in this series were originally operated on, a photograph of the head was taken, to show the relative positions of eyes. In all the animals which were later successfully recorded, it was found that the eyes at the time of operation had not reached their final position relative to each other.

Eye rotation in adults

The results of eye rotation at stage 65/66 in Xenopus indicated that interaction was taking place at that stage. To find out whether the phenomenon occurred at even later stages of development, it was decided to investigate the visual projections of young adults with one eye rotated. At least 50 days was allowed to elapse between eye rotation and the subsequent recording. Two Rana esculata and one Xenopus laevis were successfully recorded and gave essentially similar results. The results from Rana RER2 are described fully as being typical of the results obtained.

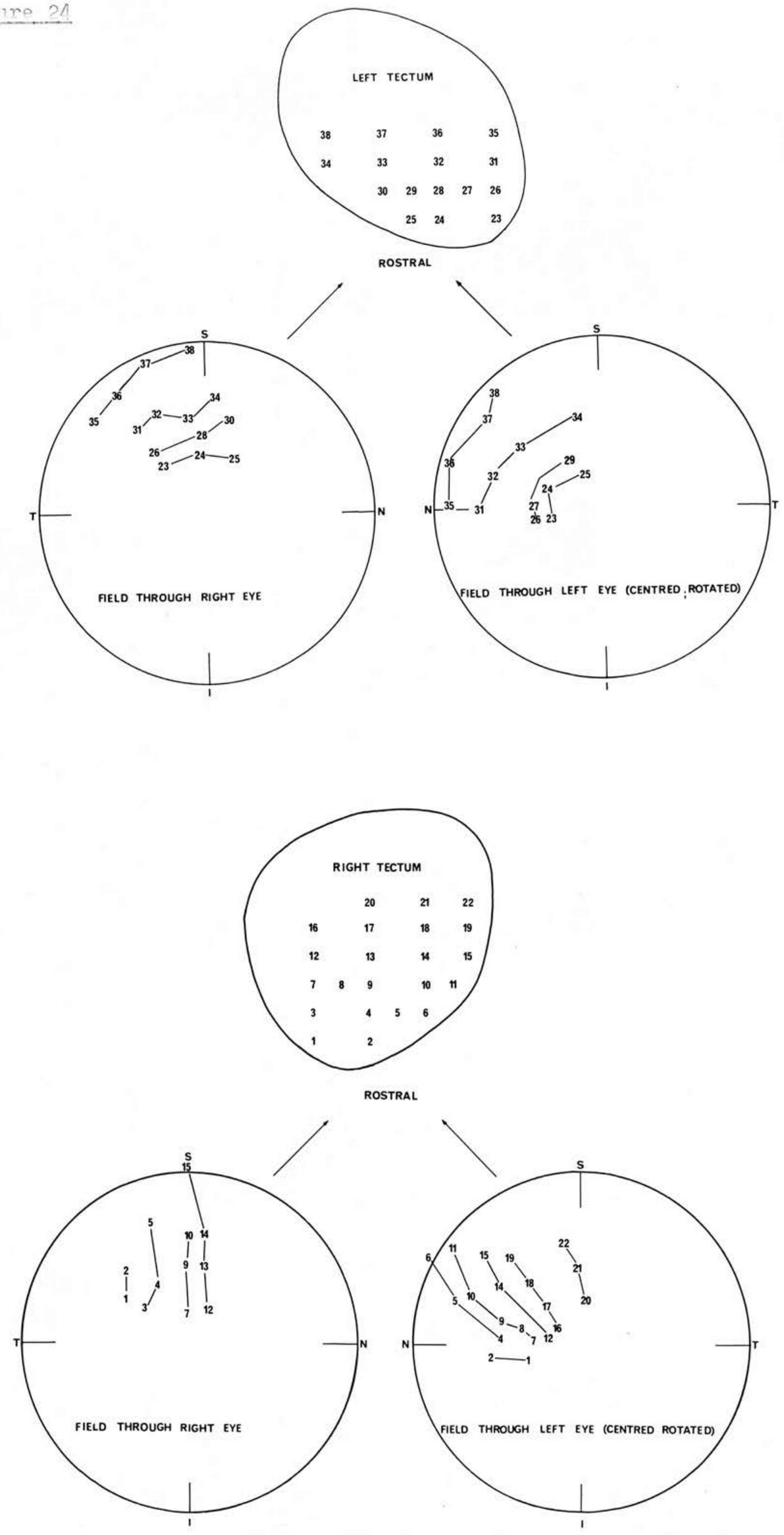
In this frog the left eye had been rotated by 45° in an anti-clockwise direction, 52 days previous to the recording. It was set up with the left eye centred on the perimeter. Fig. 24 shows the visual projections from the left and right eyes to the right tectum. Considering first the projection from the left eye in RER2, the most nasal field positions 1-2, 3-4-5, projected to the most rostral tectum, and temporal

EXPT RER2

Figure 24

Visual projections to the left and right tecta from both eyes, mapped with the left eye centred on the perimeter. The left eye was rotated by approximately 45° anti-clockwise, and the right eye was normal.

Figure 24


LEFT TECTUM
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
ROSTRAL
S
T
N
I
FIELD THROUGH RIGHT EYE
FIELD THROUGH LEFT EYE (CENTRED, ROTATED)
RIGHT TECTUM
20
21
22
16
17
18
19
12
13
14
15
7
8
9
10
11
3
4
5
6
1
2
ROSTRAL
S
T
N
I
FIELD THROUGH RIGHT EYE
FIELD THROUGH LEFT EYE (CENTRED ROTATED)

positions 20-21-22, to the caudal tectum. The most medial tectal area 11,15,19,22 received visual input from the most superior field. The map in these respects was normal (see Fig. 1). However the rows of visual points are arranged at an abnormal angle, being rotated anti-clockwise by 45°.

By comparison with the normal situation (Fig. 1) it can be seen that the ipsilateral projection from the non-centred right eye was normal. The slight discrepancy between the positioning of the rows of points in the ipsilateral projection of the normal animal and that in RER2 was due to difficulties involved in centring the eye reproducibly. However, the significant difference in RER2 is that between the contralateral and ipsilateral projections to the right tectum, in that they are arranged differently in visual space, being at a 45° angle to each other.

Fig. 24 shows the visual maps recorded, via the left and right eyes to the left tectum. The contralateral map was normal. However the ipsilateral map from the rotated eye was abnormal, being rotated by 45° anti-clockwise. Thus comparison of the visual projections through the two eyes to the left tectum shows that the maps were not congruent being aligned at a 45° angle to each other.

Table 7 summarises the findings for RER2 and the other two animals in this series. It can be seen that in no case were the two projections to one tectum congruent.

No histological examination of this animal was carried out, since no surgical technique beyond eye rotation had been carried out.

TABLE 7

Animal	Left Eye to Right Tectum	Right Eye to Right Tectum	Left Eye to Left Tectum	Right Eye to Left Tectum
RER1	Rotated 45° clockwise	Normal	Rotated 45° clockwise	Normal
RER2	Rotated 45° anti-clockwise	Normal	Rotated 45° anti-clockwise	Normal
XER1	Rotated 90° clockwise	Normal	Rotated 90° clockwise	Normal

The stage at which ipsilateral responses are first recorded

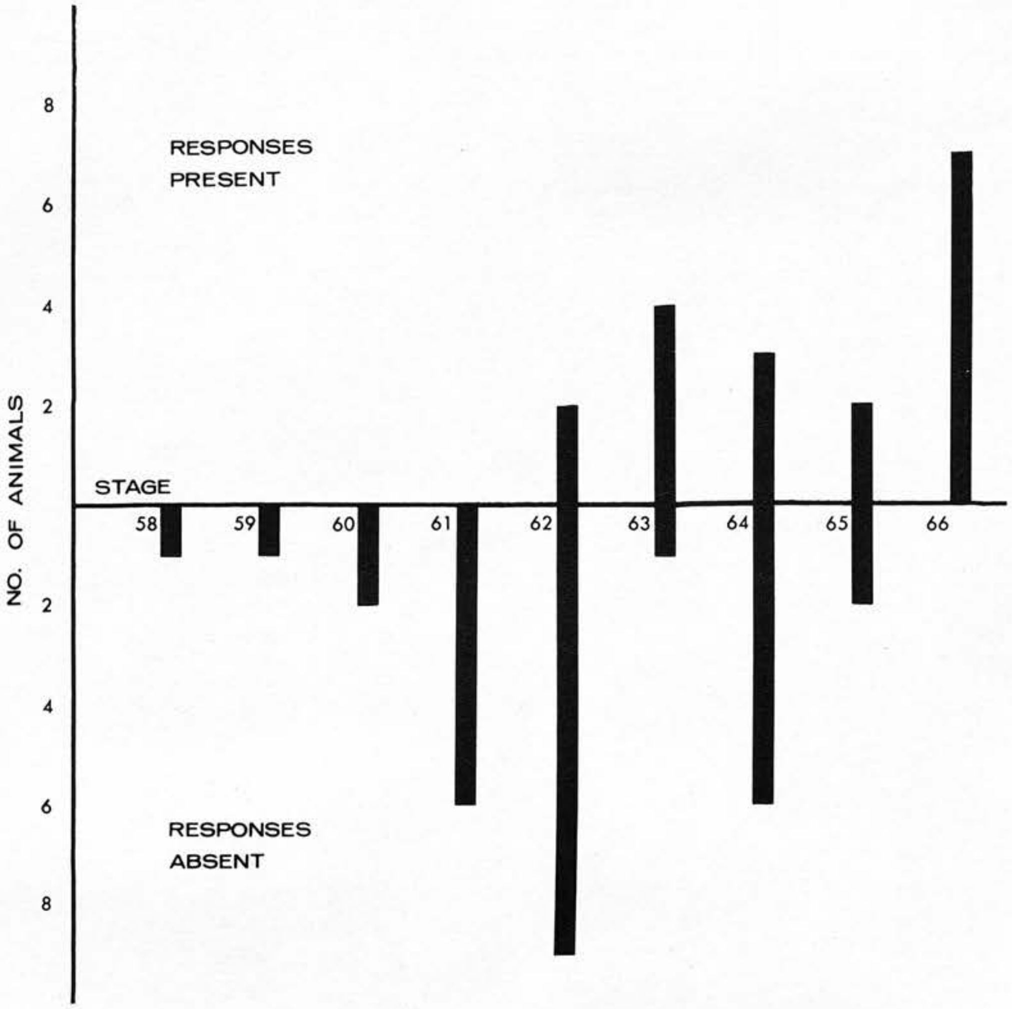
In this experimental series, the normal Xenopus tadpoles were staged by reference to the Normal Table (Nieuwkoop and Faber 1956) and then tested electrophysiologically for the presence of visual responses from each tectum, to stimulation of each eye. Only if the contralateral responses to visual stimulation were easily and reliably elicited, was the preparation considered to be in an adequate condition to warrant searching for ipsilateral responses. A positive result was recorded even if ipsilateral responses were recorded from only one tectal position during the investigation of that animal.

One stage 58 tadpole, another at stage 59 and two at stage 60, were investigated and no ipsilateral responses were found. In all the stage 66 juveniles examined there were clear ipsilateral responses. It was therefore decided to concentrate the search to animals between stage 60 and stage 66. The histogram in Fig. 25 shows the numbers of animals of

Figure 25

Histogram showing presence or absence of ipsilateral responses in Xenopus juveniles at different stages of development (stages 58-66, Nieuwkoop and Faber 1956).

Figure 25


NO. OF ANIMALS
8
6
4
2
2
4
6
8
RESPONSES
PRESENT
RESPONSES
ABSENT
STAGE
58
59
60
61
62
63
64
65
66

each stage in which ipsilateral responses were present and were absent. Thus it seemed that the earliest stage at which ipsilateral visual responses were recorded in Xenopus was stage 62. The reasons why some stage 62 tadpoles gave positive results whereas some at stage 65 were negative are considered in the discussion.

The ipsilateral pathway

Previous experiments by Gaze and Jacobson (1963) had shown that the ipsilateral pathway was an indirect one involving initial passage through the contralateral tectum. It was decided to repeat these lesion experiments to check these findings.

Keating (Personal Communication) had found that the ipsilateral pathway in the frog involved an intertectal linkage via the postoptic commissures. Lesions of the optic tract in this area were therefore carried out to find out if a similar situation existed in Xenopus. Investigation of the ipsilateral pathway thus consisted of two experimental series.

In the first series, two normal adult Xenopus were investigated and gave similar results. The contralateral projection from the centred left eye to the right tectum was mapped, as was the ipsilateral projection from the left eye to the left tectum. A small lesion was then made in the rostral part of the right tectum. On remapping the contralateral projection from the left eye to the right tectum, visual responses were absent from the destroyed area of the tectum, but were normal from the rest. The ipsilateral projection from the left eye to the left tectum was then mapped and it was found that one part of the tectum no longer received a visual input. This was the tectal area which had previously received its input from that part of the visual field now missing from the contralateral projection to the right tectum. It seemed therefore that by destroying part of one tectum the ipsilateral input to the other tectum was impaired.

Four normal adult Xenopus were used to investigate the intertectal linkage involved in the ipsilateral visual projection. The four visual projections were mapped to establish whether the preparation was functioning normally. In three animals, XIP1, XIP2, XIP4, the four visual projections were present. In the fourth animal, however, only the contralateral projection from the right eye to the left tectum and the ipsilateral projection from the right eye to the right tectum were present. It was concluded that the left optic nerve had been damaged during the operation to open the skull in this animal.

After mapping the visual projections the left optic tracts were cut in XIP2 and XIP4. Having done this, by remapping the tecta it was found that only the contralateral projection from the left eye to the right tectum remained in each case. The right optic tract was sectioned in Xenopus XIP1 and XIP3 and afterwards only the contralateral projection from the right eye to the left tectum remained. The results of Xenopus XIP1,2,3,4 are seen summarised in the table below.

TABLE 8

Xenopus	Tract Cut	Left Eye to Left tectum		Left Eye to Right tectum		Right Eye to Right tectum		Right Eye to Left tectum	
		Before Tran-Section	After	Before	After	Before	After	Before	After
XIP1	Right	✓	-	✓	-	✓	-	✓	✓
XIP2	Left	✓	-	✓	✓	✓	-	✓	-
XIP3	Right	-	-	-	-	✓	-	✓	✓
XIP4	Left	✓	-	✓	✓	✓	-	✓	-

Tract cuts in adults

When the optic nerve regenerates in Amphibia the fibres reform their previous connections. It seemed relevant therefore to investigate whether the intertectal fibres regenerated and whether an orderly ipsilateral visual projection resulted. In a series of adult Xenopus therefore the optic tract was cut and the visual projections were later mapped. One group of experimental animals was mapped approximately 3 months after tract section; the other group was left for 8 months or more before recording.

Five Xenopus were recorded from the short term group. In each case some ipsilateral regeneration had taken place, however regeneration was poor and only a few field positions were mapped ipsilaterally. These positions did not appear to be ordered systematically.

Three Xenopus of the long term group were successfully recorded. In each animal the intertectal linkages had regenerated to restore normal ipsilateral visual projections. In addition there was an anomalous retinotopically ordered visual projection from the right eye to the right tectum, in each animal.

The results of Xenopus MJCT1 will be described in detail; similar results were obtained from the two other Xenopus in the series. In Xenopus MJCT1 the right optic tract had been severed 292 days previous to the recording; the right eye was centred on the perimeter throughout the mapping experiment.(except left eye to right tectum).

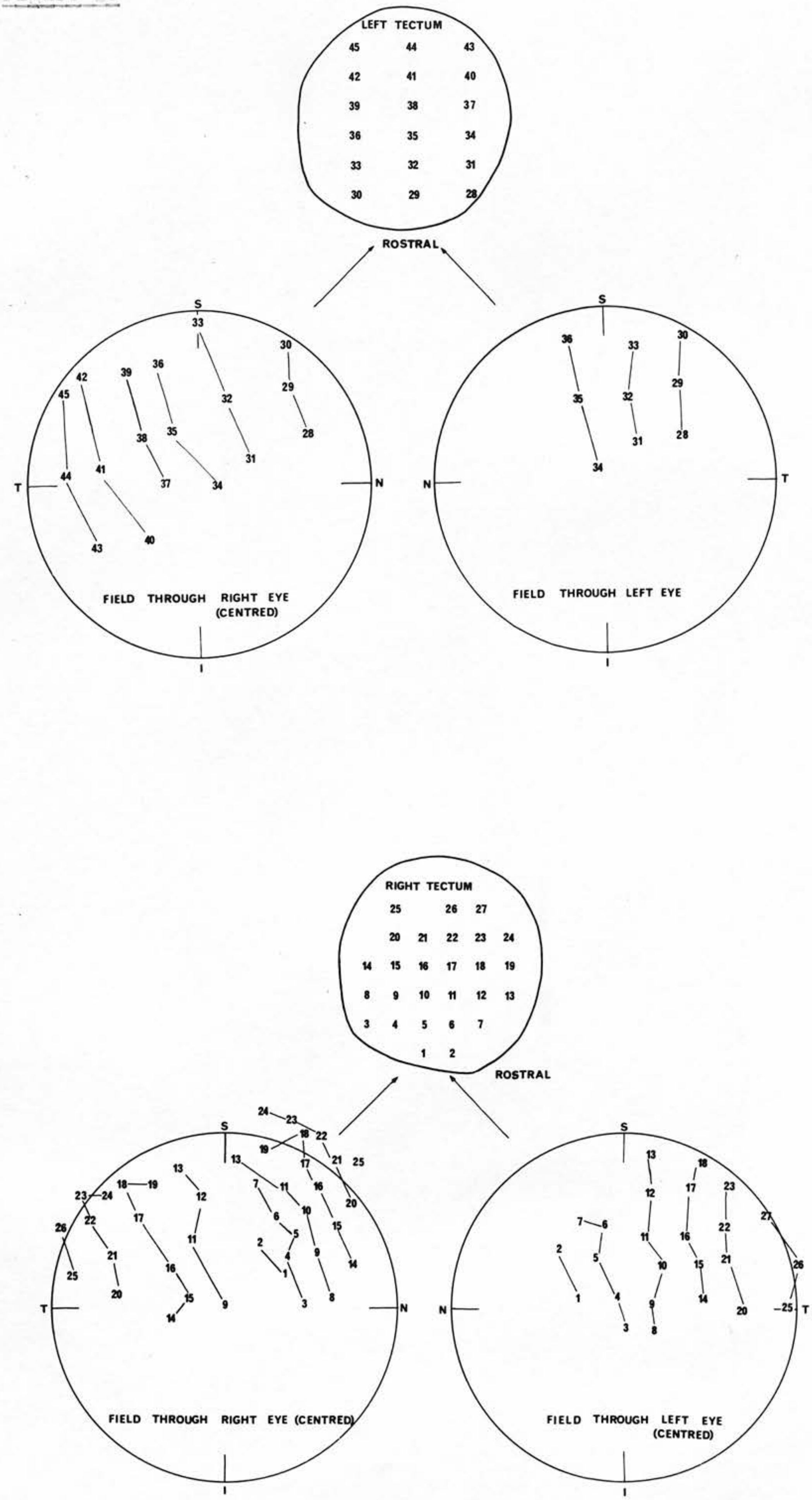
Fig. 26 shows the visual projection from the non-centred

EXPT MJCT1

Figure 26

Visual projections to the left tectum from both eyes mapped with the right eye centred on the perimeter. Visual projection from the right eye to the right tectum, mapped with the right eye centred; visual projection from the left eye to the right tectum, mapped with the left eye centred. The right optic tract was severed 292 days previous to the recording; both eyes were normal.

Figure 26


LEFT TECTUM
45 44 43
42 41 40
39 38 37
36 35 34
33 32 31
30 29 28
ROSTRAL
S
T
N
I
FIELD THROUGH RIGHT EYE (CENTRED)
S
N
T
I
FIELD THROUGH LEFT EYE
RIGHT TECTUM
25 26 27
20 21 22 23 24
14 15 16 17 18 19
8 9 10 11 12 13
3 4 5 6 7
1 2
ROSTRAL
S
T
N
I
FIELD THROUGH RIGHT EYE (CENTRED)
S
N
T
I
FIELD THROUGH LEFT EYE (CENTRED)

left eye to the right tectum. By comparison with Fig. 4 it can be seen that the map is normal. The ipsilateral projections from the right eye to the right tectum are shown in Fig. 26. One projection from the nasal part of the visual field was arranged similarly to a normal ipsilateral projection. The visual projection from the temporal part of the visual field of the right eye was itself retinotopically ordered. The most temporal field positions projected to the caudal tectal areas, and the most nasal field positions to the rostral tectum. The medial tectal areas received an input from the more superior positions in the visual field, and the lateral areas from the more inferior field positions. This anomalous projection is considered further in the discussion.

The visual projections to the left tectum are shown in Fig. 26. Both the contralateral projection from the right eye and the ipsilateral projection from the left eye were normal.

Adult eye rotation and nerve section

Since we knew that the fibres of the intertectal linkage probably run with fibres of the optic tract and that it regenerated when the optic tract was cut, I decided to carry out an experiment to investigate the mechanism by which intertectal fibres reform their connections during regeneration. The method was to cut the intertectal fibres and rotate an eye; later the visual maps were recorded. Unfortunately, by cutting the optic tract, it seemed impossible to cut all the intertectal fibres while leaving the retinal axons intact. As a control experiment, therefore, only the retinal axons were cut, by severing the optic nerve prechiasmally, and the corresponding eye rotated. Visual projections were later mapped.

Since it was known that pattern 4 regeneration may occur following nerve cut but does not occur if the nerve is crushed, it was decided to carry out two experimental series; one in which the nerve was crushed and another with nerve cut. The left eye was rotated and the left optic nerve was cut in a series of Xenopus of which five were successfully later recorded; the left eye was rotated and the left optic nerve was crushed in a series of Rana, three of which were mapped at a later date.

The optic nerves were found to have regenerated in every animal that was successfully recorded. No pattern 4 regeneration was evident in any of the preparations, and the results of the two series of experiments in this group can be considered as a whole.

A typical result of the visual mapping was that obtained from Xenopus XER.RON3. In this animal the left eye had been rotated by 90° clockwise and the optic nerve cut 116 days prior to recording. The left eye was centred throughout the recording.

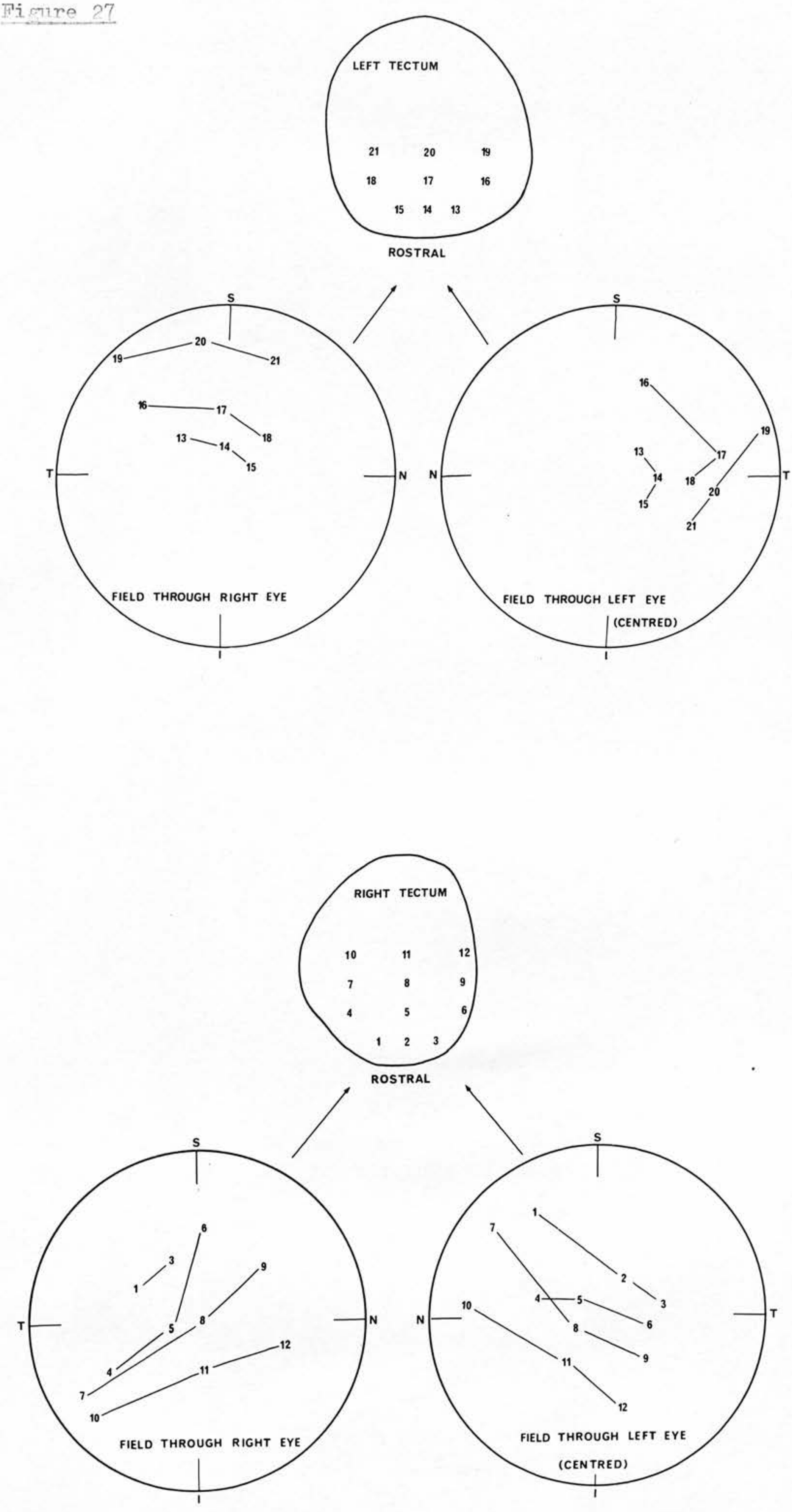
The contralateral projection from the centred left eye to the right tectum was rotated through 90° in a clockwise direction (Fig. 27). The rows of field positions did not run vertically as in a normal animal, but approximately in

EXPT XER.RON3

Figure 27

Visual projections to the left and right tecta from both eyes, mapped with the left eyes centred on the perimeter. The left eye was rotated approximately 90° clockwise and the left optic nerve cut 116 days previous to the recording; the right eye was normal.

Figure 27


LEFT TECTUM
21 20 19
18 17 16
15 14 13
ROSTRAL
S
T
N
I
19 20 21
16 17 18
13 14 15
FIELD THROUGH RIGHT EYE
S
N
T
I
16
19
13
17
14
18
20
15
21
FIELD THROUGH LEFT EYE
(CENTRED)
RIGHT TECTUM
10 11 12
7 8 9
4 5 6
1 2 3
ROSTRAL
S
T
N
I
6
3
1
9
5
8
4
7
11
12
10
FIELD THROUGH RIGHT EYE
S
N
T
I
1
7
2
3
4
5
6
10
8
9
11
12
FIELD THROUGH LEFT EYE
(CENTRED)

the naso-temporal axis. The most superior points in the visual field 1-2-3, projected to the rostral tectum instead of to the medial tectum as they do normally. The most temporal visual positions which were mapped 3,6,9,12 were represented on the medial part of the tectum whereas these positions in visual space usually project to the caudal tectum.

The ipsilateral projection from the normal right eye to the right tectum was normally organised; it was not rotated. Any one tectal locus in this animal was therefore receiving visual inputs from different parts of the visual field via the two eyes.

The contralateral projection from the normal right eye to the left tectum was normal (Fig. 27). The ipsilateral map from the rotated left eye to the left tectum was rotated by 90° clockwise. This meant that the ipsilateral and contralateral visual projections to the left tectum were not congruent.

The results from this animal and from the others in this series are summarised in table 9. Complete regeneration had taken place in every animal except XER.RON1 in which the ipsilateral projection from the normal right eye was somewhat muddled. It can be seen from the table (p.80) that in no instance were the visual maps via the normal and rotated eyes to one tectum congruent.

TABLE 9

Animal	Right Eye to L tectum	Left Eye to L tectum	Right Eye to R tectum	Left Eye to R tectum
RER NG1	Normal	Rotated 90° clockwise	Normal	Rotated 90° clockwise
RER NG2	Normal	Rotated 75° clockwise	Normal	Rotated 75° clockwise
RER NG3	Normall	Rotated 90° clockwise	Normal	Rotated 90° clockwise
XER RON1	Normal	Disorganised	Disorganised	Rotated 90° clockwise
XER RON2	Normal	Rotated 60° clockwise	Normal	Rotated 60° clockwise
XER RON3	Normal	Rotated 90° clockwise	Normal	Rotated 90° clockwise
XER RON4	Normal	Rotated 70° clockwise	Normal	Rotated 70° clockwise
XER RON5	Normal	Rotated 50° clockwise	Normal	Rotated 50° clockwise

Eye rotation and tract cuts in adult animals

Since we knew that the ipsilateral visual projection was restored in a normal orderly fashion following regeneration of the ipsilateral pathway, it became possible to find out whether interaction would take place as the ipsilateral fibres regenerated and reformed nervous connections.

Two groups of experimental animals were investigated in this section. One group consisted of young adult Xenopus, which had completed metamorphosis less than 8 weeks previously. In these animals the right eye was rotated and the left optic tract was sectioned. When mapped several months later it was found that the intertectal part of the ipsilateral pathway had not regenerated. However direct ipsilateral growth had taken place, giving rise to pattern 4 regeneration in each case.

The other experimental group consisted of Xenopus, at least 6 months post-metamorphosis. In these animals the left eye was rotated and the right optic tract was severed. If the eye rotation was not completed satisfactorily, the eye was removed, and enucleate animals with cut tracts, were also kept to be recorded later.

Eight animals of the second group of Xenopus were successfully recorded. In one Xenopus, XER CT1, recorded at the shortest period after tract section of these nine animals, ipsilateral regeneration was incomplete. The contralateral project from the rotated left eye was similarly rotated, the contralateral map from the normal right eye to the left tectum was normal. The ipsilateral maps however consisted of only a few visual field positions, which were completely disorganised.

In the other seven animals of this group however regeneration had taken place and organised visual projections were mapped. These Xenopus gave similar results, and Xenopus XER CT16 is described in detail as typical of the results obtained.

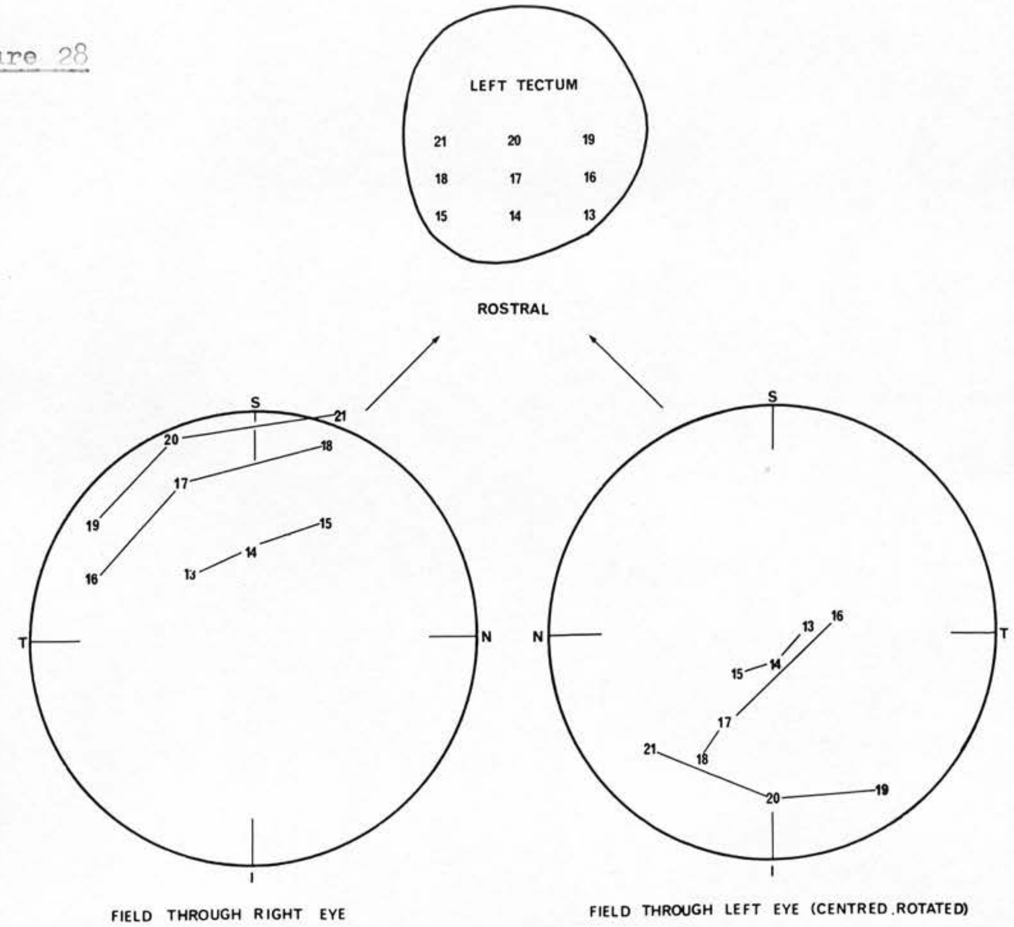
In Xenopus XER CT16, the left eye was rotated 90° clockwise and the right optic tract was cut 110 days previous to the recording. Throughout the electrophysiological mapping the left eye was centred on the perimeter.

EXPT XER CT16

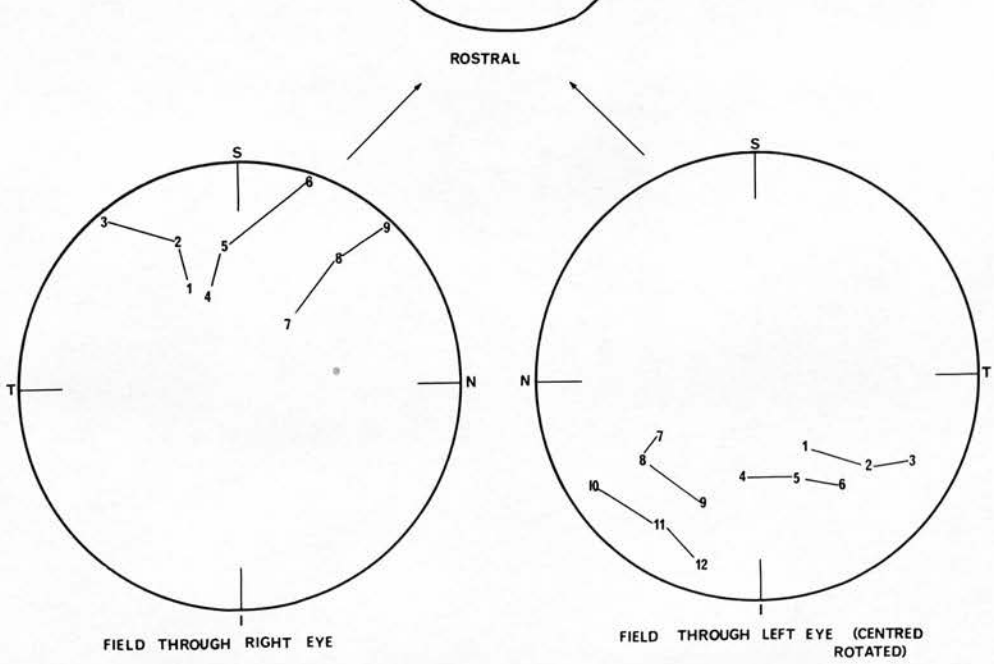
Figure 28

Visual projections to the right and left tecta through both eyes, mapped with the left eye centred on the perimeter. The left eye was rotated approximately 90° clockwise and the right optic tract cut 110 days previous to the recording.

Figure 28



LEFT TECTUM
21 20 19
18 17 16
15 14 13
ROSTRAL
S
T
N
I
21 20 18 17 19 16 15 14 13
FIELD THROUGH RIGHT EYE
S
N
T
I
16 13 14 15 17 21 18 20 19
FIELD THROUGH LEFT EYE (CENTRED.ROTATED)



RIGHT TECTUM
10 11 12
7 8 9
4 5 6
1 2 3
ROSTRAL
S
T
N
I
6 3 2 5 9 8 1 4 7
FIELD THROUGH RIGHT EYE
S
N
T
I
7 8 1 2 3 4 5 6 10 9 11 12
FIELD THROUGH LEFT EYE (CENTRED ROTATED)

The contralateral visual projection from the rotated left eye to the right tectum is shown in Fig. 28. The map was restricted to the inferior part of the visual field and the rows of field positions ran horizontally across it. The most nasal visual field positions, 1,4,7,10 projected to the lateral edge of the tectum, the most superior points 1-2-3 projected to the rostral tectal area. The map was therefore rotated by 90° clockwise compared with a normal animal.

The ipsilateral projection from the normal right eye was normal. Comparison of the two visual projections to the right tectum, show that the maps were not congruent with different parts of the visual field projecting through the two eyes to the same area of the tectum.

The contralateral projection from the normal right eye to the left tectum was normal (see Fig. 28). The ipsilateral projection to this tectum from the rotated left eye was, however, abnormal, being restricted to the inferior part of the visual field. This ipsilateral projection was internally organised but was rotated by 90° clockwise. The contralateral projection via the right eye and the ipsilateral projection from the left eye were therefore arranged in different parts of the visual field.

In table 10, the projections of this Xenopus XER CT16, are summarised along with those from the other 6 animals which gave similar results.

Figure 29

Transverse section through chiasma region in Xenopus XER CT15. The site of section of the optic tract can be seen; nerve fibres can be seen to have regenerated back across the lesioned area. Holmes' silver stain. Bar represents 0·1 mm.

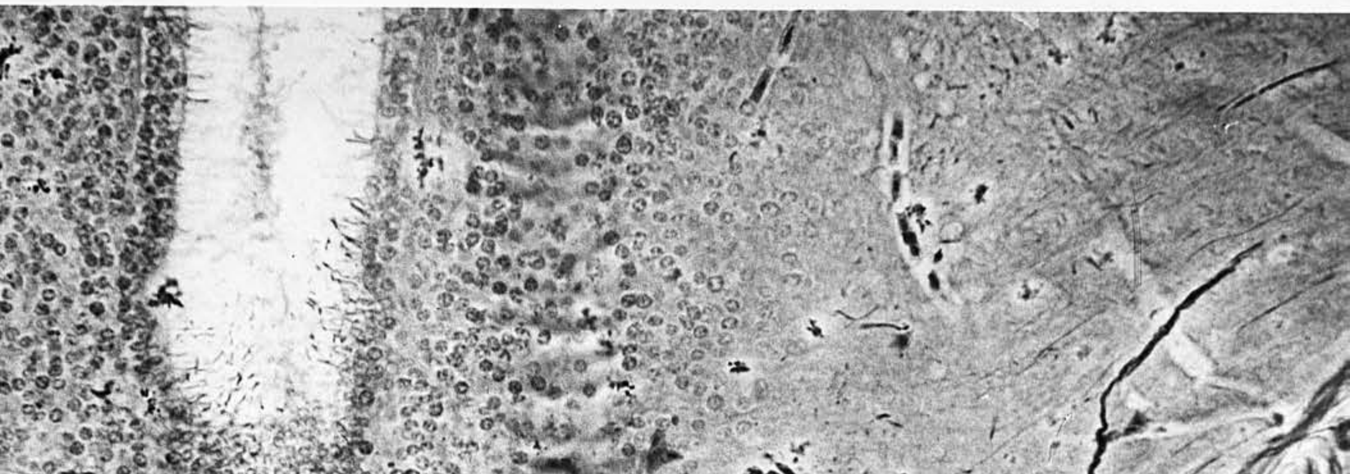


TABLE 10

Xenopus	Left Eye to L Tectum	Right Eye to L Tectum	Right Eye to R Tectum	Left Eye to R Tectum
XER CT6	Rotated 45° clockwise	Normal	Normal	Rotated 45° clockwise
XER CT7	Rotated 70° clockwise	Normal	Normal	Rotated 70° clockwise
XER CT8	Rotated 90° clockwise	Normal	Normal	Rotated 90° clockwise
XER CT11	Rotated 75° clockwise	Normal	Normal	Rotated 75° clockwise
XER CT13	Rotated 60° clockwise	Normal	Normal	Rotated 60° clockwise
XER CT15	Rotated 90° clockwise	Normal	Normal	Rotated 90° clockwise
XER CT16	Rotated 90° clockwise	Normal	Normal	Rotated 90° clockwise

Histological examination showed that the tracts had been cut in the Xenopus of this series (see Fig. 29).

Of the adult Xenopus, in which the right optic tract had been cut and the left eye removed, two were later successfully recorded. These were Xenopus XER CT4 and XER CT5. In both animals the contralateral projections and the regenerated ipsilateral projections were normal. The visual projections mapped from Xenopus XER CT4 are shown in Fig. 30.

Summary of Results

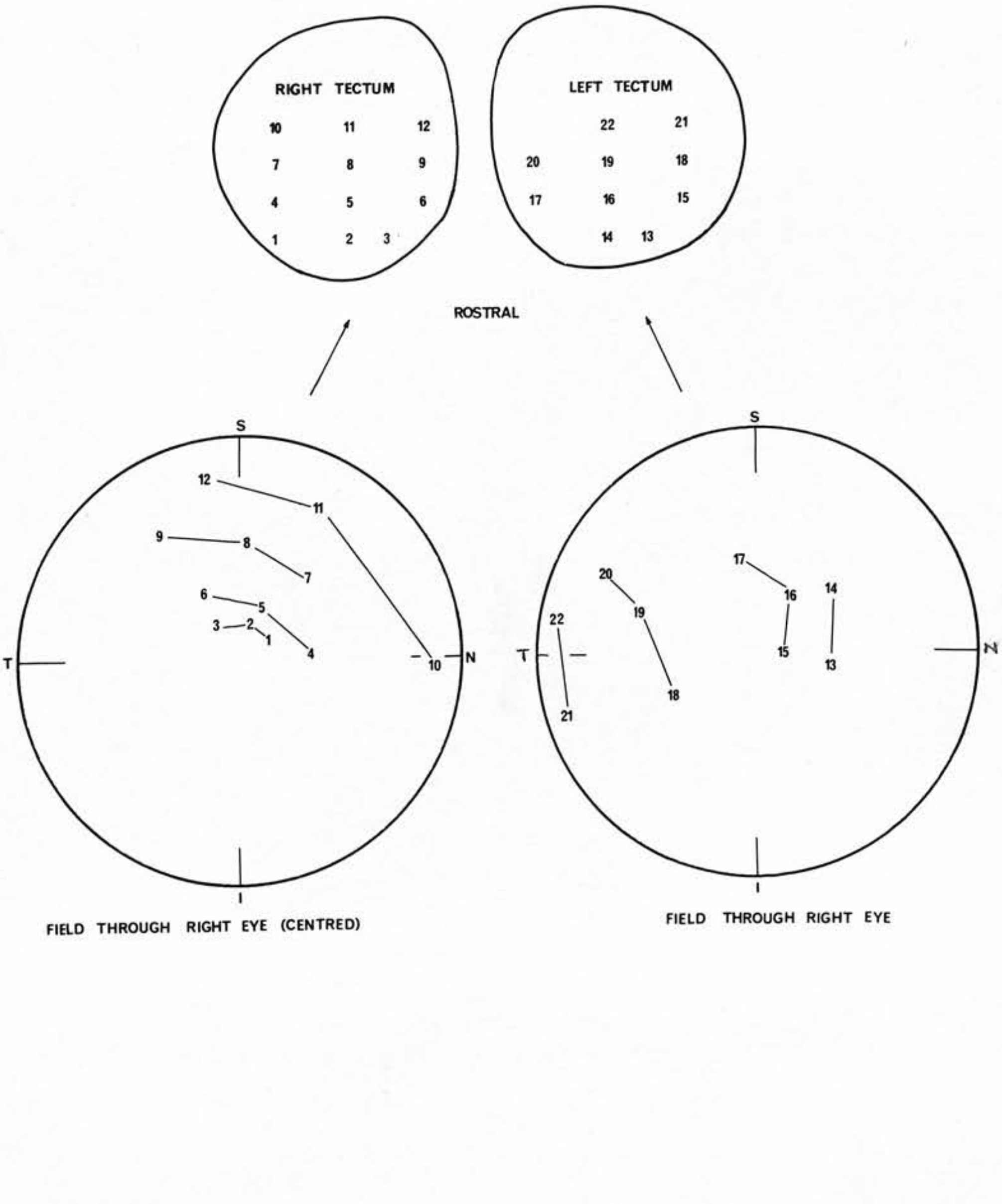
In table 11, shown below (P. 84) are listed the results obtained from the majority of animals in various experimental series carried out in this thesis; if approximately equal numbers of animals gave differing results, both types of result are listed. Although the visual maps are considered

EXPT XER CT4

Figure 30

Visual projections to the right and left tecta from the right eye, mapped with the right eye centred on the perimeter. The left eye of this animal had been removed 125 days previously, and the right optic tract cut. The right eye was normal.

Figure 30


RIGHT TECTUM
10 11 12
7 8 9
4 5 6
1 2 3
LEFT TECTUM
22 21
20 19 18
17 16 15
14 13
ROSTRAL
S
T
N
I
12 11 9 8 7 6 5 3 2 1 4 10
FIELD THROUGH RIGHT EYE (CENTRED)
S
T
N
I
17 16 14 20 19 22 15 13 18 21
FIELD THROUGH RIGHT EYE

in detail in the discussion, a major indication of the processes of specification of nervous connections is given by whether the visual projections through the 2 eyes to one tectum are congruent or non-congruent. The results are summarised in this form.

TABLE 11

Type of Experiment	Stage at which operation carried out	Normal eye → contralateral tectum (tectum A) Abnormal eye → ipsilateral tectum (tectum A)	Abnormal eye → contralateral tectum (tectum B) Normal eye → ipsilateral tectum (tectum B)
Compound eye	32	C	C
eye rotation	32-58	C	C
contralateral eye grafts	38	C NC	C NC
eye rotation	65-66	C	C
eye rotation	adult	NC	NC
eye rotation and nerve cut	adult	NC	NC
eye rotation and tract cut	adult	NC	NC

Key: C = congruent NC = non-congruent

DISCUSSION

Throughout this thesis, the development and regeneration of neural connections has been assessed by the technique of mapping electrophysiologically the visual projections from the eye to the optic tectum. By this means it is hoped to trace the terminations of axons to find out the part of the tectum with which they have chosen to make synaptic contacts. It is therefore of the greatest importance to be sure that the recording is being made from axonal terminations, and not for example from post-synaptic tectal cells or from pre-terminal parts of axons passing across the tectum.

There are several arguments to support the idea that recordings are in fact made from axonal terminations in the tectum. It is known that in the lower vertebrates the retinal ganglion cell axons travel across the tectum for certain distances before turning to enter the synaptic tectal layer. An electrode lowered onto the surface of the tectum therefore must be surrounded by many axons passing across to other tectal areas. If the electrode picked up electrical impulses travelling along these axons of passage, then no orderly visual projection could be mapped. Since orderly visual projections can be mapped, this rules out the possibility that the electrophysiological recordings are made from passing axons.

This left two possible sites from which recordings could be made - the pre-synaptic terminations of the optic axons and the post-synaptic tectal cells. When recordings are made from the superficial tectum the responses are almost always triphasic spikes. The visual field units that exhibit these triphasic spikes have the same receptive field properties as the units recorded from the retina or in the optic nerve. It seems extremely unlikely that many of the optic nerve fibres are efferents from the brain to the eye (Maturana 1959). This being so, the similarity between the units recorded in the retina or the optic nerve and those recorded in the superficial tectum probably means that the triphasic spikes cannot be of tectal origin. At each electrode penetration, 20 or so units of this sort are recorded and at the most four or five units of a quite different type,which have different retinal receptive field properties.

It might be argued that instead of recording visual units from axonal terminations the responses might be picked up from tectal cells which exactly match the retinal ganglion cells in their activity. This would seem to be an unwarranted waste of cells. Estimates of the number of tectal cells in the frog have recently been increased considerably; the estimate of 250,000 made by Maturana now seems too low, Lazar and Szekely consider 876,000 a more realistic assessment (1967). There are probably about 450,000 ganglion cells in the retina, the majority of which send their axons to the tectum. Nevertheless, although it may be physically possible for every retinal axon to synapse with a tectal cell of identical receptive field properties, it seems extremely unlikely that it would do so. It would seem more likely that the cells with which retinal axons make contact would not merely repeat this signal but in some way integrate the input from many ganglion cells. The functional properties of the tectal cells in the neuropil and below it seem to be identical with the properties to be expected of an integrator cell.

Assuming, therefore, that the recordings are made from presynaptic fibres, which part of the fibre is detected? A point-to-point visual projection from the eye to the tectum can be mapped precisely. The electrode must, therefore, be picking up signals from only a small discrete part of the axon with certain unique electrical properties. It would seem likely that at branching points it would be easier to record signals, the number of signals increasing in proportion to the number of branches. Maturana (unpublished observations quoted 1960) has found that optic axons do not branch except at their end region, where they branch repeatedly before making synaptic contacts in the tectum. One is led to conclude that the tectal responses recorded when the representation of the visual field on the tectum is being mapped, must originate from the pre-terminal branches of optic axons.

The hypothesis being tested in this thesis may be stated briefly as:- one point in visual space projects through both eyes to both tecta by direct contralateral pathways; these points on the two tecta are receiving similar excitation patterns and because of this the loci on the two tecta become linked. It is therefore essential to know that the ipsilateral pathway to one tectum involves an initial projection to the other tectum and then an intertectal linkage back to the ipsilateral tectum. Gaze and Jacobson (1963) found that selective lesions in the contralateral tectum of the frog, to destroy a part of the contralateral visual map, resulted in the absence of ipsilateral responses from the same part of the visual field. The finding along with an analysis of the latencies of contralateral and ipsilateral responses led these authors to conclude that the ipsilateral projection involved an initial passage through the contralateral tectum.

Since the hypothesis of binocular interaction is based on the two stage nature of the ipsilateral visual projection, I decided to repeat the lesion experiments in normal Xenopus. The results were in accord with those of Gaze and Jacobson, and therefore throughout this thesis the assumption is made that the ipsilateral pathway involves an initial projection to the contralateral tectum. Also,I have assumed that a similar pathway has developed in the experimental animals which I have recorded, to study the development of the ipsilateral projection. There is no reason to doubt this since in all the experimental series concerned with development of the ipsilateral projection, rather than with its regeneration, no surgical interference with the visual system has been undertaken beyond manipulations of the eye itself.

The hypothesis that binocular interaction determines the pattern of the intertectal linkage in Xenopus was originally put forward (Keating 1968) to explain the following observations made on Xenopus with one double nasal compound eye (NN) and one normal eye (Gaze, Jacobson and Szekely 1965):

1. The ipsilateral projection from the normal eye is abnormal being reversed in the naso-temporal axis.
2. The ipsilateral projection from the compound eye is normal.

In this discussion I propose to outline the arguments which led Keating to discard the mechanisms of specificity and modulation as inadequate explanations of this result, and instead to suggest the significance of functional interaction between information coming through the two eyes. I shall then discuss possible explanations of the experiments carried out in this thesis designed to test the interaction hypothesis further.

Before considering the results of the compound eye Xenopus in detail, it is relevant to examine the situation in the normal animal. It was found by Gaze and Jacobson (1962) and further verified in this thesis that the ipsilateral projection arises from temporal retina (nasal visual field) and projects to the rostral part of the ipsilateral tectum. This situation is shown experimentally in Figs. 3 and 4 and can be represented diagrammatically as in Fig. 31. Fig. 31 shows clearly that the naso-temporal axis of the ipsilateral projection is reversed as compared with the contralateral projection to the other tectum. Thus in the case of the contralateral projection, the most nasal field (temporal retina) point 1, projects to the extreme rostral

Figure 31

The normal visual projection to the ipsilateral tectum.

The ipsilateral projection involves an initial passage of information from the retina to the contralateral tectum; there is then passage back to the ipsilateral tectum via an intertectal linkage.

In figures 31 to 36 and figure 40 the diagrams of the retinotopic projection are idealised and simplified. Particularly, in figures 31 to 35 the ipsilateral projection is shown to arise from only the temporal retina (nasal field) and extending to the rostral part of the tectum; in fact the ipsilateral projections involve more than half of the visual field of each eye.

These simplifications do not invalidate the principles illustrated in the diagrams.

Figure 31

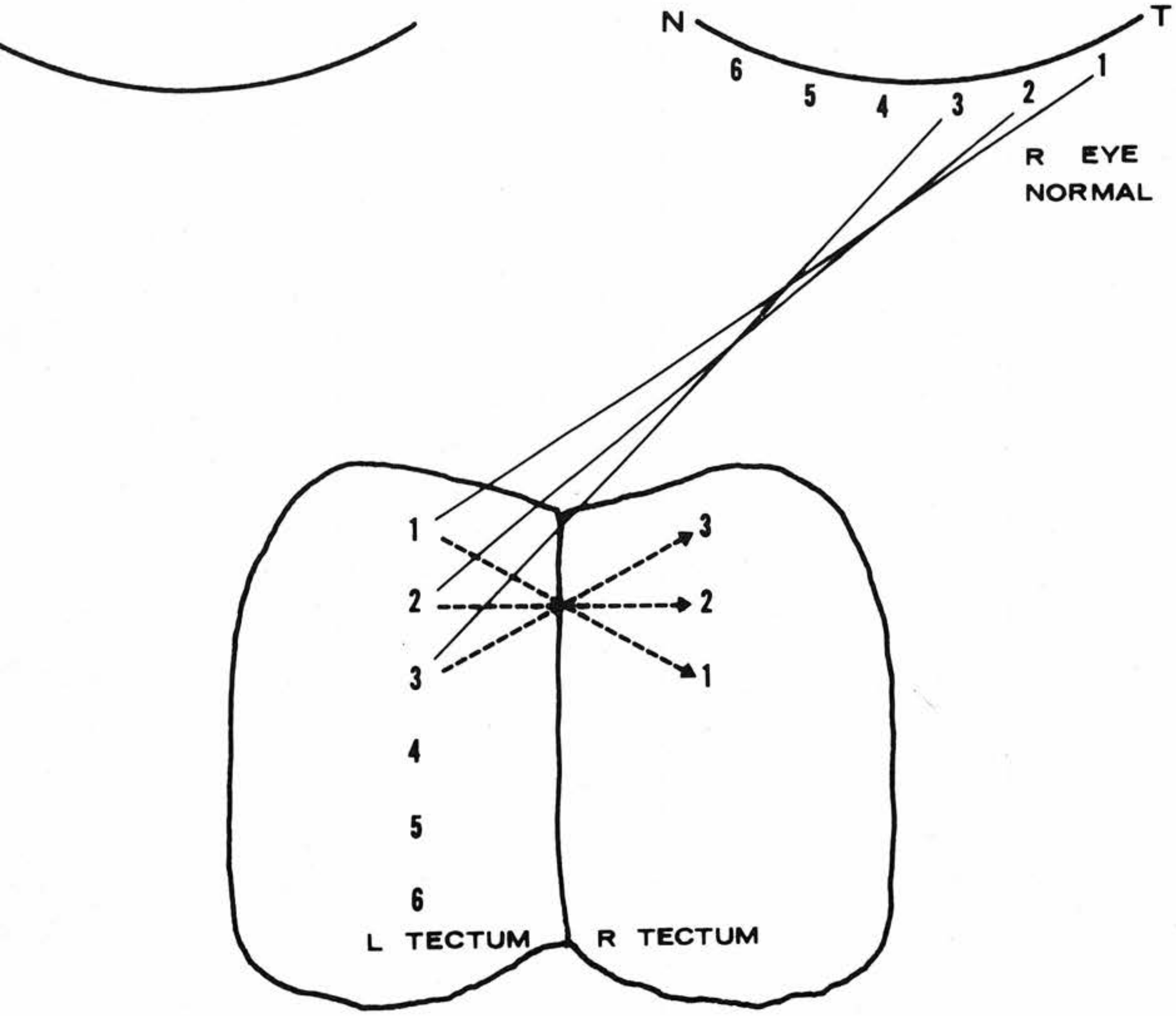
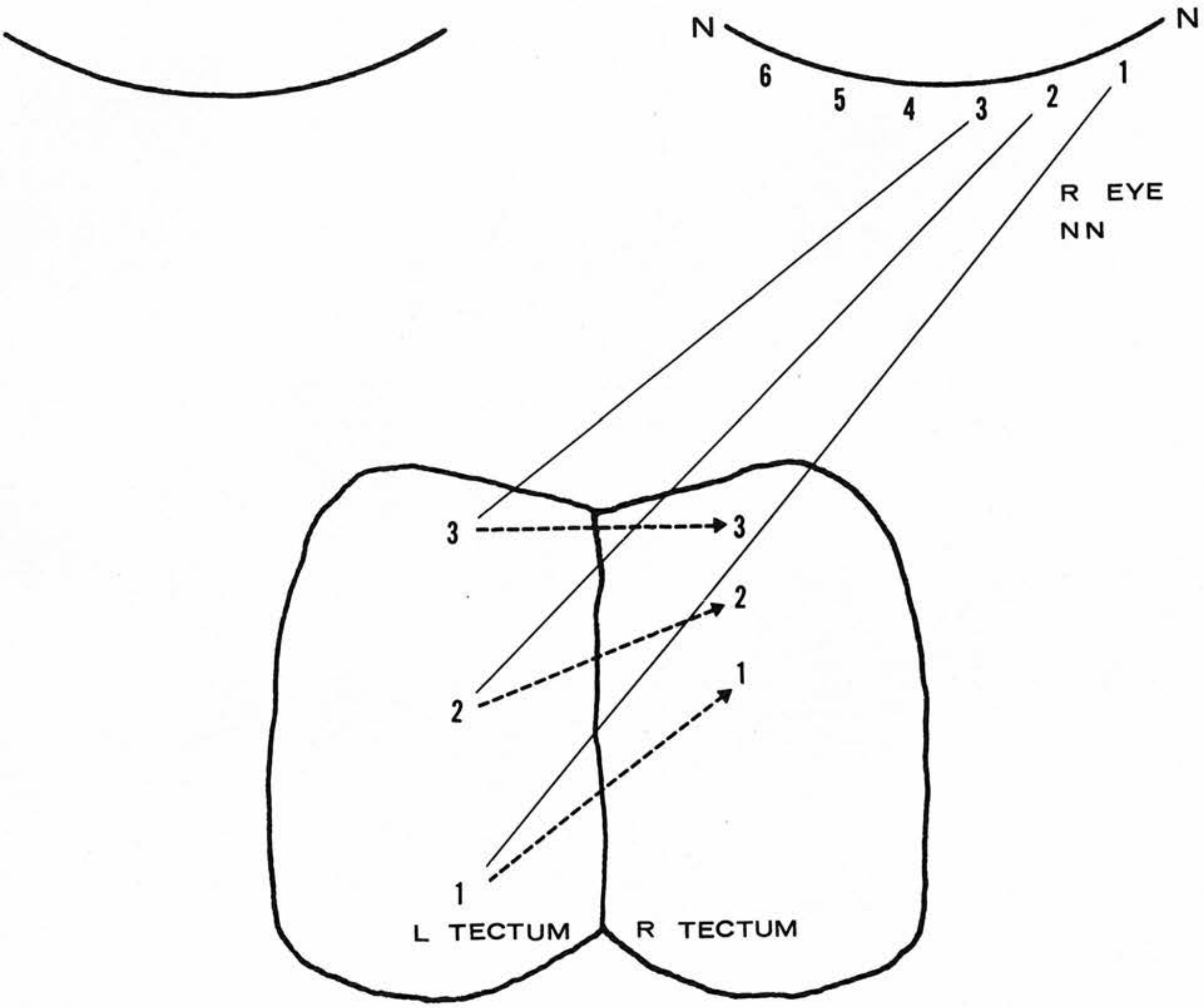


Figure 32

The ipsilateral projection from a double-nasal compound eye.

Figure 32



pole of the tectum; the ipsilateral map is arranged so that the most nasal field positions, retinal locus 1 projects to the more caudal tectal areas.

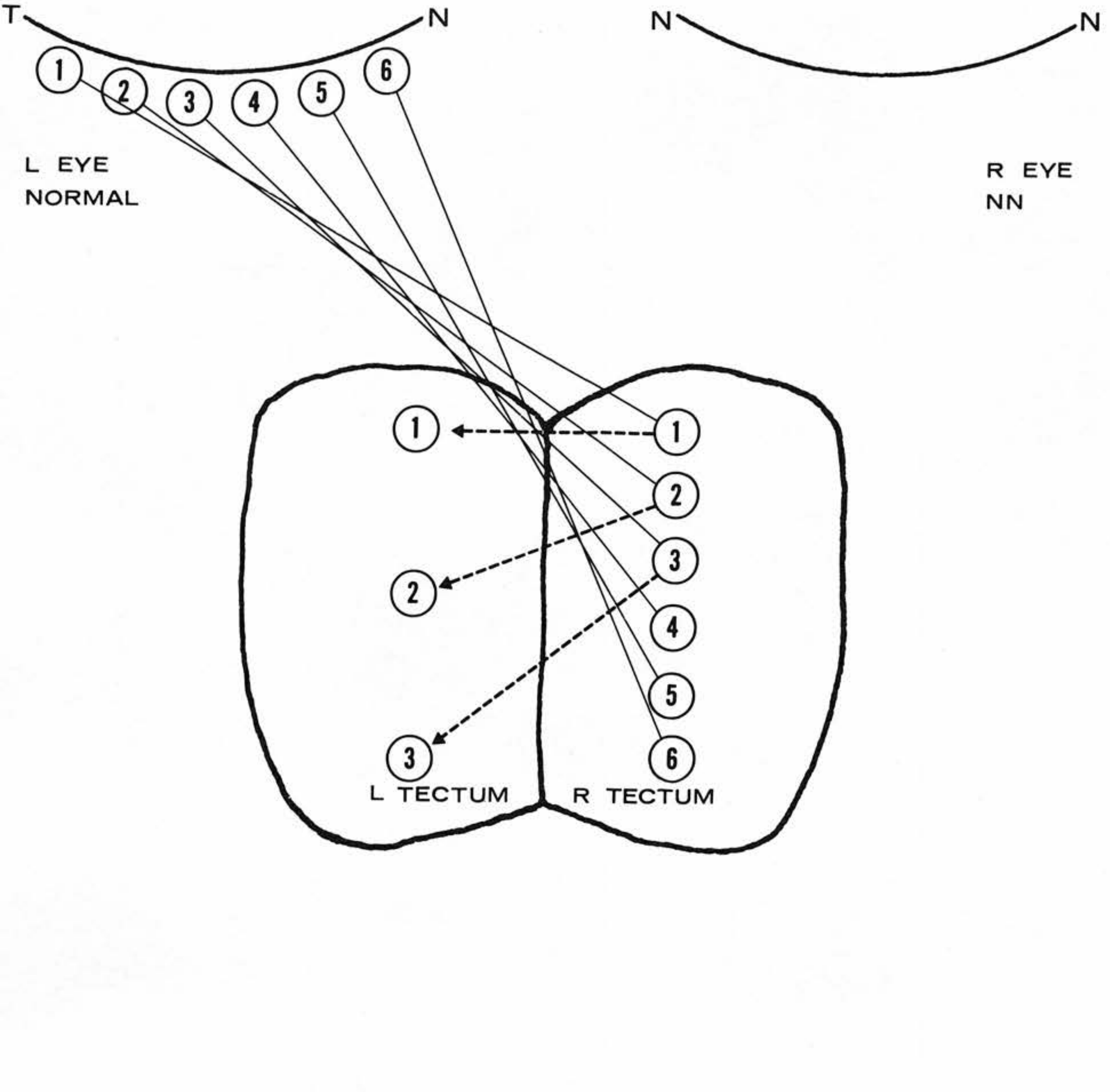
The original compound eye experiments of Gaze et al. (1965) and further experiments of this type reported in this thesis showed that the ipsilateral map from a NN eye was normal. The neural pathways which must exist are shown diagrammatically in Fig. 32. The contralateral projection is abnormally organised in the rostro-caudal axis. The reason for this is that the naso-temporal axis of the embryologically nasal retina which now occupies a temporal position in the orbit, has been reversed. However despite the abnormality in this contralateral projection, the ipsilateral projection from a compound eye is normal.

The retina which gives rise to an ipsilateral projection in this situation is of nasal origin. In the normal animal only temporal retina gives rise to an ipsilateral projection. Thus at the stage when the compound eyes were constructed, the retina cannot have been specified in terms of the ipsilateral visual connections. Neither can it be said that the contralateral projection is specified by embryological specificity mechanisms and then the intertectal linkage is separately specified so that the rostral part of one tectum connects with the caudal part of the other. If this were the case, then, in an NN animal such as is shown diagrammatically in Fig. 33, points 3 and 2 on the left tectum would connect with points 1 and 3 respectively on the right tectum.

Figure 33

The ipsilateral projection from the normal eye in an animal with one double-nasal compound eye.

Figure 33



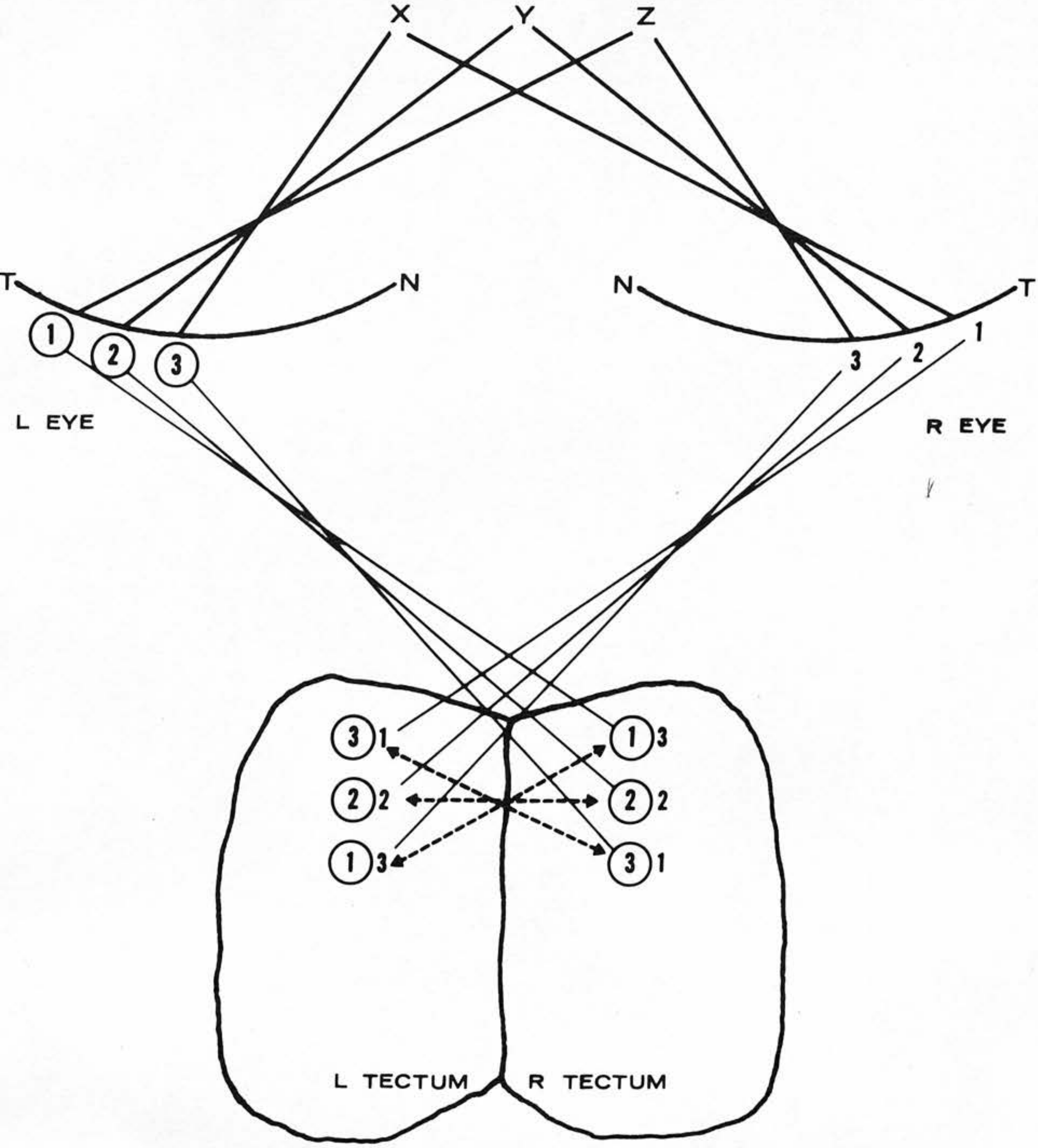
Another feature of the visual projections from an animal with one NN and one normal eye is that the ipsilateral projection from the normal eye is abnormal being reversed in the naso-temporal axis. Since the initial contralateral projection from the normal eye is normal, the intertectal connections must be abnormal. The situation is shown diagrammatically in Fig. 33. Had the intertectal linkages been governed by specificity mechanisms since the left eye is normal, the ipsilateral projection to the left tectum should be normal also. Since it is not, further proof is added to the argument that the intertectal linkage cannot be embryologically specified.

Another mechanism which might be considered to give rise to these ipsilateral maps in NN Xenopus is respecification or modulation. The ingrowing optic axons from a compound eye might influence the tectal cells with which they make contact and change the cell in such a way that it accepts a different ipsilateral input. The NN eye would if this were true, respecify the cells of the contralateral tectum and "stamp" them as nasal neurons, rendering them receptive only to intertectal fibres from nasal cells in the other tectum. In a normal animal the ipsilateral projection arises from temporal retina (nasal visual field) and projects to the "temporal" tectal cells. Considering an NN eye again, the intertectal fibres from the contralateral tectum would grow into the normal ipsilateral tectum all bearing a "nasal" stamp. No ipsilateral projection could therefore develop. But it is known that a normal ipsilateral projection arises from a NN eye, showing

Figure 34

The binocular representation of the visual field.

Figure 34



that the "modulation" hypothesis must be discarded.

It is possible to say therefore, for reasons described above, that pre-functional mechanisms of specificity and modulation cannot explain the results from NN animals. Keating hypothesised the importance of function in the determination of intertectal linkages. The functional inputs through the two eyes to the optic tecta are shown diagrammatically in Fig. 34, the ringed numbers referring to the left eye and the unringed to the right. This diagram is based on observations made by Gaze and Jacobson (1962) of the ipsilateral projection in the normal animal. These authors found that within the binocular field any point projected through the two eyes to the same tectal locus. By reference to Fig. 34 it can be seen that light from point X in the visual field falls on the retina of the left eye at point ③, and on that of the right eye at point 1. Position ③ of the left eye then projects contralaterally to point ③ on the right tectum; position 1 of the right eye projects to locus 1 on the left tectum. Tectal locus ③ on the left tectum and 1 on the right tectum are known to be joined in the normal animal, so that point X projects contralaterally through the left eye to position ③ and ipsilaterally through the right eye to position 1. Positions 1 and ③ are identical, for both tecta.

It had been assumed that this arrangement was a result of innate growth processes which determined the neuronal connections which developed, although detailed mechanisms had not been drawn up.

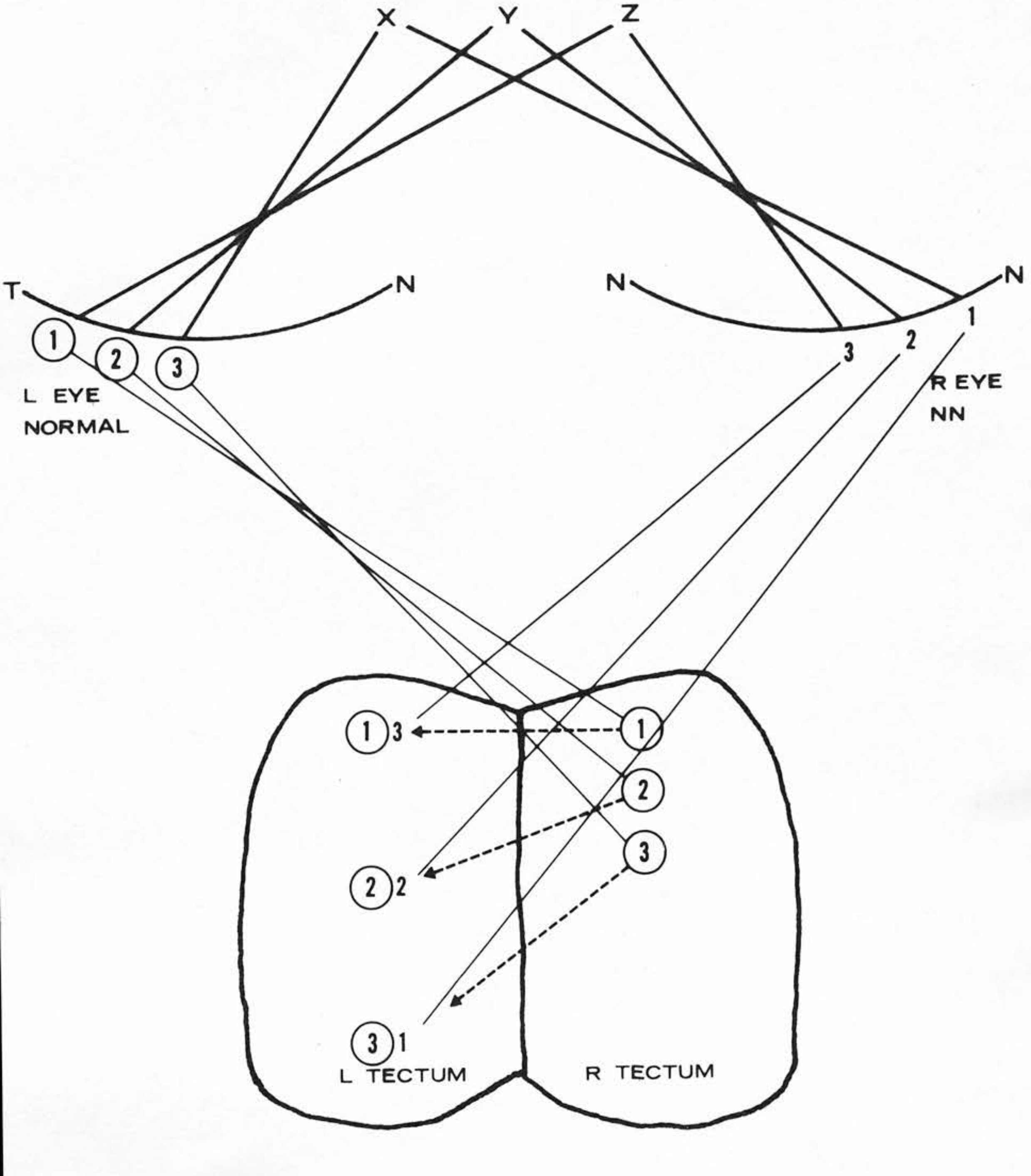
Keating (1968) suggested that the development of the intertectal linkages, shown diagrammatically in Fig. 34, might rely on the fact that the same point in visual space projects through the two eyes to one tectal locus in the normal animal, as is shown in Fig. 34; Point X in the visual field stimulates point ③ on the left retina and by the contralateral pathway, also point ③ on the right tectum; similarly field position X stimulates point 1 on the right eye and locus 1 on the left tectum. Points 1 on the left tectum and ③ on the right tectum receive similar visual input and therefore the same patterns of excitation. The hypothesis of binocular interaction is that at a certain developmental stage, points on the two tecta receiving similar patterns of excitation become joined by an intertectal linkage.

The hypothesis will explain the development of intertectal connections in a normal animal, but it must be dismissed if it cannot explain the development of the visual projections in animals with an NN eye, or with other abnormalities in the polarity of the eye. In Fig. 35 the field projection of a double nasal compound eye is shown. It can be seen that the point X stimulates point ③ on the left retina and point 1 on the right retina. Point ③ projects from the left eye to the normal position on the right tectum. Point 1 on the retina of the NN eye projects abnormally to the caudal area of the left tectum, due to the abnormality of the right eye. The binocular interaction hypothesis would predict that point 1 on the left tectum and point ③ on the right tectum, since they are receiving

Figure 35

The ipsilateral projections from a double-nasal compound eye and from a normal eye. The field projection.

Figure 35


X
Y
Z
T
N
N
N
①
②
③
3
2
1
L EYE
NORMAL
R EYE
NN
① 3
② 2
③ 1
①
②
③
L TECTUM
R TECTUM

visual information from point X in the visual field, become functionally linked. By similar considerations of field positions Y and Z it can be predicted that point 2 of the left tectum will join with point ② on the right tectum, and that point 3 on the left tectum will become connected to point ① on the right tectum. The hypothesis would therefore predict that the ipsilateral projection from the normal eye would reflect the abnormality of the NN right eye, and that the NN eye would give rise to a normal ipsilateral projection by interaction with the contralateral projection from the normal eye. The ipsilateral projections mapped in animals with a double nasal compound eye show precisely these features. It therefore seems that at a gross level considering only the extent of the representation of the ipsilateral projection on the tectum, and the organisation of the projection in the naso-temporal axis, that the binocular interaction hypothesis will explain the results obtained from compound eye animals.

In this study abnormalities in the polarity of the retina have been produced and the ipsilateral projections in the adult animal have been analysed to find out if they can be explained by any of the mechanisms - specificity, modulation and interaction, discussed in connection with the compound eye animals. Since one eye has been centred throughout the recording of each animal, it is possible to compare accurately the way in which one visual field position projects through the two eyes to one tectum, to test in detail the hypothesis that functional connections between two contralateral projections develop.

It was decided to investigate the visual projections in another series of double nasal compound eye Xenopus both to check the results of Gaze, Jacobson and Szekely (1965) and to extend the information of animals with one NN eye by mapping the visual projections with one eye centred throughout the experiment. Table 4 summarises the results of these animals; the observations agree well with those found previously. The ipsilateral projection from the normal eye in each case reflects the abnormality in the contralateral projection from the NN eye; the ipsilateral projection from the NN eye is, in each instance, normal. The explanation of the 45° rotation of the contralateral projection from the normal left eye in Xenopus XCN3 is given on page 63. It is significant that this rotation is apparent also in the ipsilateral projection from the compound right eye which was itself not rotated, for it means that one position in visual space projects through the two eyes to the same point in the right tectum, despite the abnormal alignment of the eyes.

The results of this series of Xenopus with a NN eye are similar to those of the group mapped previously by Gaze <u>et al.</u> Since Keating formulated the binocular interaction hypothesis to explain the results of this original series, all the arguments applied to those animals apply equally to the present series. This series of animals is particularly valuable since they demonstrate clearly that any small abnormalities in the contralateral projection are reflected in the ipsilateral projection to that tectum.

Thus the 45° clockwise rotations of the contralateral projections from the NN eyes in Xenopus XCN 2 and 4 are reflected in the ipsilateral projections from the normal eye.

The mapping technique employed in this study does not usually allow the mapping of a centre of a retinal receptive field, more accurately than to within a 5-10° radius of its marked position. If one allows for the margin of error, then the visual maps to one tectum are virtually identical in every one of the four compound eye Xenopus recorded. Xenopus XCN2 illustrates this point well. The visual maps, Fig. 19, of the representations of the visual field onto the left tectum are extremely similar and even show a slight mixing up of the rows of field positions mapped through each eye.

The results of Xenopus XCN1,2,3 and 4, therefore are evidence that one point in visual space projects through the two eyes to one tectal locus, despite various abnormalities of the eyes themselves. These findings are explicable by a hypothesis of binocular interaction. If interaction is taking place why does the ipsilateral projection from the normal eye not consist of two mirror image parts, like the contralateral projection with which it is interacting, but is a replica only of the nasal part of the contralateral map? In fact in two of the Xenopus recorded, there is definite evidence of a representation of the temporal half of the visual field in the ipsilateral projection from the normal eye; in both cases the temporal half of the visual field is represented by only one visual field position (point 4 in

Xenopus XCN1, Fig. 18; point 5, XCN2, Fig. 20) and this is situated in the most nasal part of the contralateral hemiprojection, from the temporal field. Since only part of the contralateral projection can have interacted with the ingrowing intertectal fibres, it follows that the two inputs from mirror image retinal positions cannot excite the same neuron. In a NN eye visual information from two points in visual space project to one tectal locus; one of these points is in the nasal binocular visual field but the other is in the temporal (non-binocular) field. The field positions in the binocular field project through both eyes to both tecta and therefore these tectal cells are receiving similar patterns of excitation and become linked; the positions in the non-binocular field project through only one eye to the contralateral tectum and since there are no cells on the other tectum receiving information from the same part of the visual field, there are no common patterns of excitation and no intertectal linkage is formed.

Seven Xenopus, in which one eye had been rotated at stages varying from 32 to 58 were successfully recorded as adults. Five of the seven animals recorded gave essentially similar results, shown in table 3. The results from these animals will be considered first.

Table 3 shows clearly that in each of these animals the rotation of an eye has resulted in the development not only of a rotated projection to the contralateral tectum but also a rotated ipsilateral projection from the normal eye. The ipsilateral projection from the rotated eye was found to be normal.

Figure 36

The ipsilateral projection from the normal eye in an animal with one eye rotated by 180°.

Figure 36

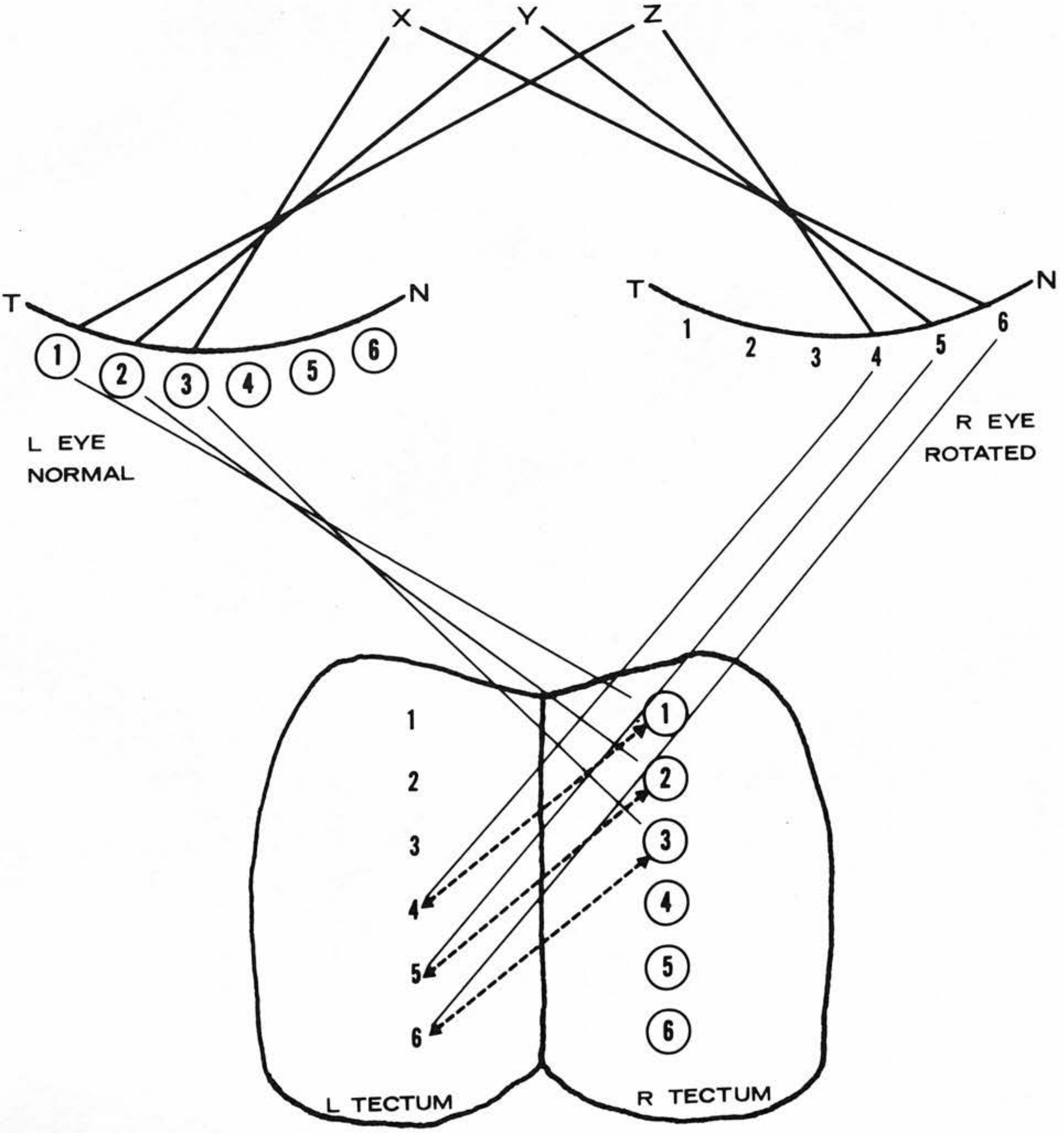

X
Y
Z
T
N
①
②
③
④
⑤
⑥
L EYE
NORMAL
T
N
1
2
3
4
5
6
R EYE
ROTATED
1
2
3
4
5
6
①
②
③
④
⑤
⑥
L TECTUM
R TECTUM

Fig. 36 shows, in a diagrammatic form, the visual mapping of a Xenopus in which one eye had been rotated by 180°. If specificity determined the intertectal linkage, the ipsilateral projection from the rotated eye would be rotated, and that from the normal eye would be normal. This is not found.

Since the intertectal linkage is not determined by the specification of the retinal ganglion cells, can these results be explained by a two stage process of specification in which the contralateral projection is specified in the usual way and the intertectal linkages develop to join points 1,2,3 on one tectum with points 3 2 1 respectively on the other tectum? Such an explanation is inadequate since the ipsilateral projection from the normal eye is recorded from the caudal tectum, which in a normal animal does not receive an ipsilateral input.

Another possible explanation of these results is modulation, or re-specification. This mechanism would suggest that the retinal axons growing into the tectum would specify the contralateral tectal cells. In normal animals the intertectal linkage forms between cells bearing a "temporal retina" stamp in the rostral part of the tectum. The rostral part of the tectum contralateral to a rotated eye, still receives "temporal" retinal axons, although they are receiving information from the temporal visual fields. It would therefore be predicted that intertectal fibres would grow into the rostral tectum and form connections as they do in a normal animal. Since, in these Xenopus with a rotated eye, it is the caudal tectum which receives an ipsilateral input, modulation cannot explain these results.

By reference to Fig. 36 , it can be seen that in Xenopus with a rotated eye a point X in the binocular visual field stimulates point ③ on the left retina and point 6 on the right retina. Point ③ then projects contralaterally to point ③ on the right tectum, and similarly point 6 projects to point 6 on the left tectum. Since point 6 on the left tectum, is receiving the same visual information as point ③ on the right tectum, the binocular interaction hypothesis would predict that these points become functionally joined. Similar arguments apply to field positions Y and Z. On the basis of the interaction hypothesis therefore, one would expect the ipsilateral projection from the normal eye to be rotated and that from the rotated eye to be normal. This is exactly what is found and therefore provides strong evidence that binocular interaction is taking place at an intertectal level between visual inputs through the two eyes.

However, two of the seven Xenopus recorded in this series gave results which did not agree with those from the five animals discussed above. In one, Xenopus XRE5, the left eye had been rotated by 180° at stage 40 (Neiuwkoop and Faber). The contralateral projection from the rotated eye was found to be rotated by 180°; the contralateral projection from the normal eye was normal.

Fig. 15 shows that the contralateral projection from the left eye to the right tectum and the ipsilateral map from the right eye to this tectum are somewhat similar. In fact tectal loci 3 and 4 receive visual information via identical parts of the visual field through both eyes. It is only tectal locus 1 which receives visual information from areas of the visual space 90° apart via the two eyes. Thus it might seem that the binocular interaction hypothesis could explain the visual inputs to tectal loci 3 and 4, but not that to locus 1. Can any other mechanism explain this result? A specificity mechanism would predict that the ipsilateral projection from a normal eye would be normal; the ipsilateral projection is abnormal and therefore invalidates this explanation. It might be thought that the ipsilateral projection might not be the result of the development of the normal ipsilateral pathway, but might represent direct ipsilateral growth of displaced retinal axons. If this were so the projection would be the same as the contralateral projection to the other tectum from this eye. Comparison of the maps in Fig. 37 shows that this is not the case. It is therefore necessary to say that this map from the right eye to the right tectum cannot be explained satisfactorily, but since so few points have been mapped, it is difficult to envisage the completed map and attempt to make a diminutive explanation of the mechanisms at work.

The ipsilateral projection from the rotated left eye to the left tectum is also abnormal, but again consists of only three visual field positions. Fig. 38 shows diagrammatically the arrangement of the visual maps to be predicted if interaction, specificity or pattern 4 mechanisms were at work. Again it can be seen that none of these explanations fit the experimental result.

Xenopus XRE5.

Figure 37

Visual projections from the right eye to the right tectum, mapped with the left eye centred on the perimeter, as predicted by various hypotheses of specific nerve connection.

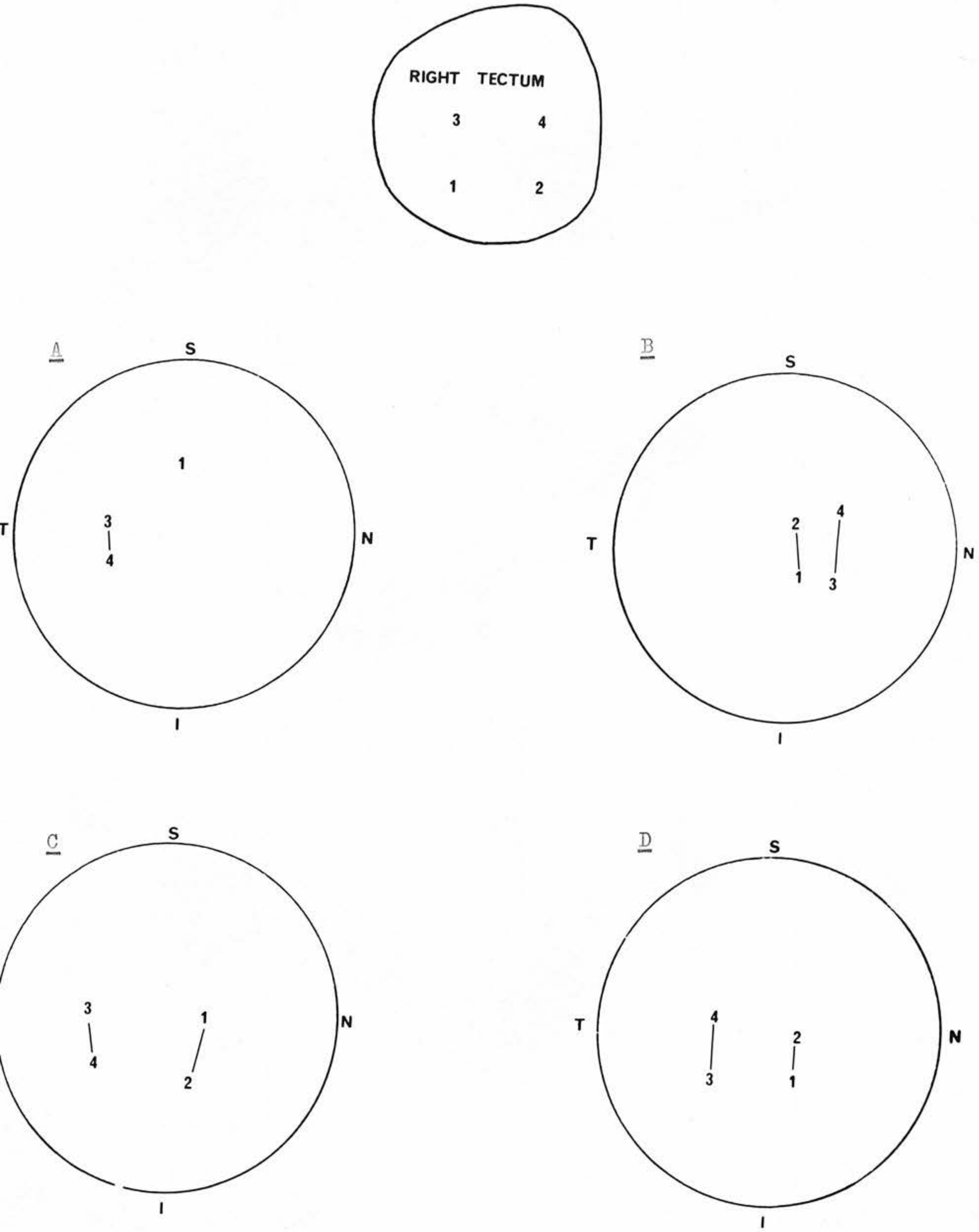
KEY:- A = Projection actually mapped.

B = Projection as predicted by a hypothesis of biochemical neuronal specificity.

C = Projection as predicted by the hypothesis of binocular interaction.

D = Projection as predicted if direct ipsilateral growth of optic axons has taken place (pattern 4).

Figure 37



Xenopus CONTRANS4

Figure 39

Visual projections from the left eye to the left tectum, mapped with the left eye centred on the perimeter, as predicted by various hypotheses of specific nerve connection.

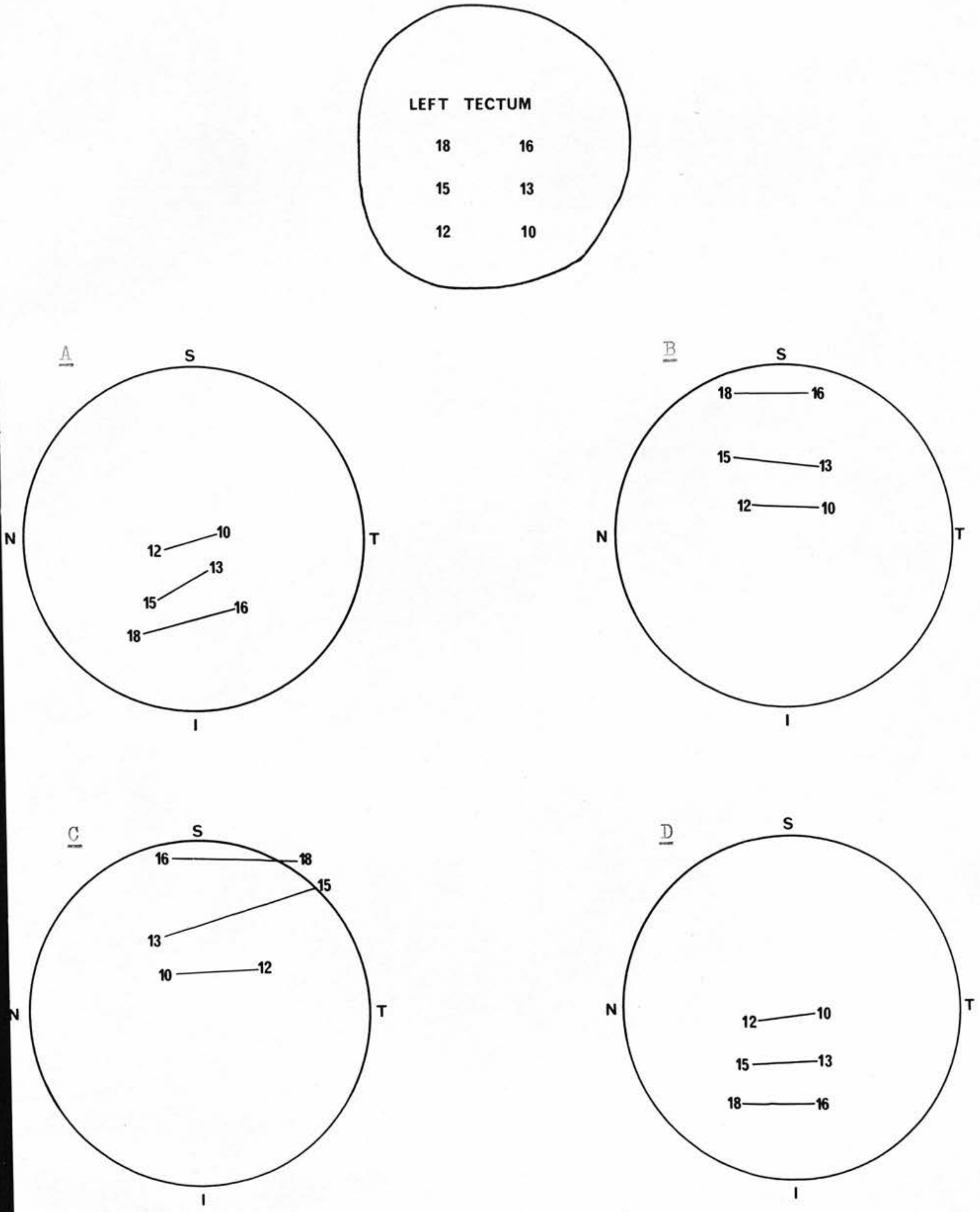
KEY:- A = Projection actually mapped.

B = Projection as predicted by a hypothesis of biochemical neuronal specificity.

C = Projection as predicted by the hypothesis of binocular interaction.

D = Projection as predicted if direct ipsilateral growth of optic axons has taken place (pattern 4).

Figure 38


LEFT TECTUM
18
16
15
13
12
10
A
S
N
T
I
10
12
13
15
16
18
B
S
18
16
15
13
12
10
N
T
I
C
S
16
18
15
13
10
12
N
T
I
D
S
N
T
12
10
15
13
18
16
I

The other Xenopus which gave aberrant results in this series was XRE9, in which the right eye had been rotated at stage 37/38. In this animal the only normal visual projection was that from the normal left eye to the right tectum. The contralateral projection from the rotated right eye was completely abnormal and seemed to consist of two groups of visual field positions; within these groups no order was apparent. Normal development of the primary visual pathways cannot therefore have taken place. The nature of this abnormality is not apparent by reference to the contralateral map from the rotated eye. Since no explanation of the contralateral map can be given for Xenopus XRE9, no interpretation of the ipsilateral projections can be undertaken. However, it may possibly be significant that despite the abnormality in the contralateral projection to the left tectum, several visual field positions project through both eyes to identical tectal loci. Perhaps interaction has taken place in the left tectum, between cells stimulated by the contralateral input and the ingrowing intertectal fibres; nevertheless this is obviously not the complete explanation of the result.

Of the seven Xenopus recorded in this series the five animals giving results which fitted the interaction hypothesis had been operated upon at the following stages (Nieuwkoop and Faber):- 32, 32, 58, 44, 45. The two aberrant results were found in Xenopus operated upon at stages 38 and 40. It is clear that interaction takes place both before stage 38 and after stage 40; furthermore evidence from the contralateral eye graft series of Xenopus indicates that interaction takes place at stage 38. Is it therefore merely a coincidence that both the animals which gave results inexplicable by any hypothesis were operated on at similar stages, 38 and 40? Without carrying out a far greater number of experiments it is not possible to say for certain. However in a stage 38 Xenopus tadpole the ingrowing optic nerve fibres have grown across the chiasma and have reached the tectum. If an eye is rotated in a later stage tadpole the optic nerve is visible and it is possible to rotate the eye without severing the optic nerve. At stages 38 and 40 it is impossible to avoid cutting the optic nerve.

Two Xenopus were successfully recorded in which the left eye had been removed at stage 37/38 and replaced by a right eye from a donor tadpole at the same stage of development, keeping the dorso-ventral axis of the transplanted eye approximately normal. In this way, the naso-temporal axis of the eye is inverted. These animals each displayed orderly visual projections, but the maps were differently arranged in the two cases. The results of each animal will therefore be discussed separately.

In Contrans 3 the naso-temporal axis of the left eye was reversed and this was reflected in the visual projection from the left eye. The ipsilateral projection from the normal eye is also reversed in the naso-temporal axis but normal in the dorso-ventral axis. The contralateral projection from the normal eye is normal, as is the ipsilateral projection from the transplanted eye. A specificity mechanism cannot explain the development of an abnormal ipsilateral projection from a normal eye and must therefore be dismissed as an explanation of this result.

Fig. 36 represents diagrammatically the visual projections of a Xenopus with an eye rotated 180° at the tadpole stage. Only the naso-temporal axis of the eye is represented on this diagram, for simplicity, this diagram therefore can also be taken as representing the visual projections in a contralateral eye graft Xenopus. A modulation hypothesis cannot explain the results of Contrans 3. Such a hypothesis predicts that the rostral part of the tectum would be specified appropriately by the ingrowing retinal ganglion cell axons. Since in the normal animals the intertectal fibres connect up with "temporal" tectal cells at the rostral pole of the tectum, if modulation were taking place then the ipsilateral projection from an eye reversed in the naso-temporal axis should occupy the rostral tectal areas. The ipsilateral projection arises from more caudal tectum, proving the interpretation of the results to be incorrect.

The binocular interaction hypothesis predicts that since the contralateral projection from the transplanted eye is reversed in the naso-temporal axis, the ipsilateral projection from the normal eye will also be reversed in this axis. Similarly the contralateral projection from the normal eye and the ipsilateral from the transplanted eye should be normal. Both these predictions fit the experimental results and provide further evidence that binocular interaction is taking place to specify the intertectal linkage.

Xenopus Contrans 4 does not show a similar congruence between the visual projections from the left and right eyes to one tectum. The contralateral projection from the transplanted eye is reversed in the naso-temporal axis and rotated by approximately 45° anti-clockwise. The ipsilateral projection from the normal eye is disorganised and random. The contralateral projection from the right eye is normal; the visual projection from the left eye to the left tectum is well ordered, but is abnormal in that it arises from the inferior part of the visual field.

Considering first the projections to the left tectum, since the ipsilateral and contralateral projections to this tectum are non-congruent, binocular interaction cannot be governing the development of the ipsilateral projection. If the pattern of the projection was a result of specificity mechanisms, it should reflect the abnormalities of the left eye, in other words, it should arise from the superior field, be reversed in the naso-temporal axis and rotated by 45° anti-clockwise. This is not found.

A modulation hypothesis requires that in animals with contralateral eye grafts, the ipsilateral projection should be normal, for reasons similar to those discussed on p. 98. In Contrans 4 both ipsilateral projections are however abnormal.

It would seem, therefore, that a well ordered projection had developed from the grafted left eye to the left tectum, which cannot be explained by processes of specificity, modulation or functional interaction. However, if one

Xenopus XRE 5

Figure 38

Visual projections from the left eye to the left tectum, mapped with the left eye centred on the perimeter, as predicted by various hypotheses of specific nerve connection.

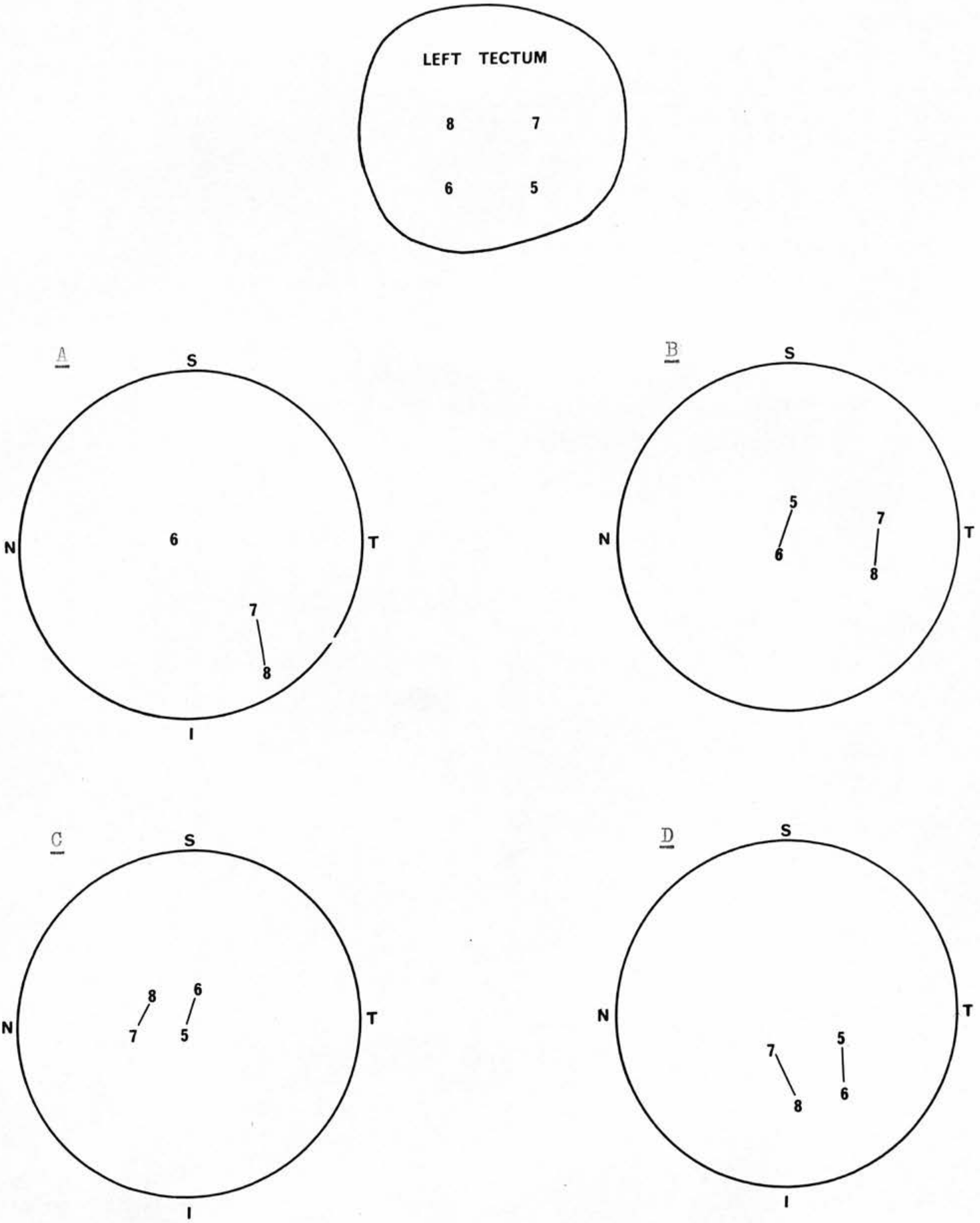
KEY:- A = Projection actually mapped.

B = Projection as predicted by a hypothesis of biochemical neuronal specificity.

C = Projection as predicted by the hypothesis of binocular interaction.

D = Projection as predicted if direct ipsilateral growth of optic axons has taken place (pattern 4).

Figure 39



compares the visual projections from the left eye to the left and right tecta they are seen to be very similar. The ipsilateral projection is, thus, explained if the assumption is made that direct ipsilateral growth of the retinal ganglion cell axons had taken place from the left eye to the left tectum (a pattern 4 ipsilateral growth), and that no normal intertectal ipsilateral projection has developed (Fig. 39).

The ipsilateral projection from the normal right eye is disorganised. This cannot be explained by the binocular interaction hypothesis, but neither can it be by the mechanisms of specificity, modulation or pattern 4 ipsilateral growth, all of which predict that orderly maps will develop.

The series of Xenopus enucleated before stage 29 (Nieuwkoop and Faber 1966) were recorded as a further check of the binocular interaction hypothesis, since in them no form of visual interaction could take place. It might be expected, therefore, that no ipsilateral projection would develop. In fact there is an ipsilateral projection from the remaining eye but it is diffuse and not retinotopically organised. One explanation of this result would be that the un-innervated tectum lacks the modulating influence of the ingrowing retinal axons, remains unspecified and cannot form intertectal linkages. However the results of the enucleates taken along with those from animals with rotated eyes, compound eyes and contralaterally grafted eyes (summarised in table 11) leads one to the conclusion that the intertectal fibres grow into the tectum in a diffuse or random fashion and only form precise specific connections by functional interaction with the contralateral projection from the other eye.

The experiments discussed up till now were all designed to test the binocular interaction hypothesis by altering the visual inputs to the optic tecta in various ways. The abnormalities introduced into the system were replacement of a temporal hemi-retina by a nasal one (NN eye), eye rotation, contralateral eye graft and enucleation. The table 12, summarising the results shows clearly that of the eighteen animals recorded the results of fifteen are completely explicable in terms of the binocular interaction hypothesis.

Although the majority of the results are explicable in terms of binocular interaction there is another hypothesis which must be considered as a possible explanation. This is the mechanism of delayed re-specification of the retinal ganglion cells. The original specification of the eye takes place between stages 29-32 (Nieuwkoop and Faber 1956) in Xenopus and the tectum may be independently specified, at a later stage; the re-specification hypothesis predicts, that the retinal ganglion cells become respecified by structures surrounding the eye. This specification would be such that retinal ganglion cells in the positionally temporal part of the eye becomes labelled so that the cells on which the retinal axons terminate, send out and accept intertectal connections. The process of respecification is shown diagrammatically in Fig. 40. It can be seen that to explain the experimental findings of an animal with a rotated eye such as Xenopus XRE7, p. it is necessary to assume that cells at position A on the left tectum carry instructions to connect

TABLE 12

Xenopus	Projection	Explained by specificity	Explained by modulation	Explained by B.I.	Other Explanations	No Explanations
XCN1 Right eye NN	R eye - R tectum L eye - L tectum			X X		
XCN2 Right eye NN	R eye - R tectum L eye - L tectum			X X		
XCN3 Right eye NN	R eye - R tectum L eye - L tectum			X X		
XCN4 Right eye NN	R eye - R tectum L eye - L tectum			X X		
XRE1 Right eye rotated 90° clockwise	R eye - R tectum L eye - L tectum			X X		
XRE2 Right eye rotated 180°	R eye - R tectum L eye - L tectum			X X		
XRE6 Right eye rotated 180°	R eye - R tectum L eye - L tectum			X X		
XRE7 Right eye rotated 180°	R eye - R tectum L eye - L tectum			X X		

[Contd.

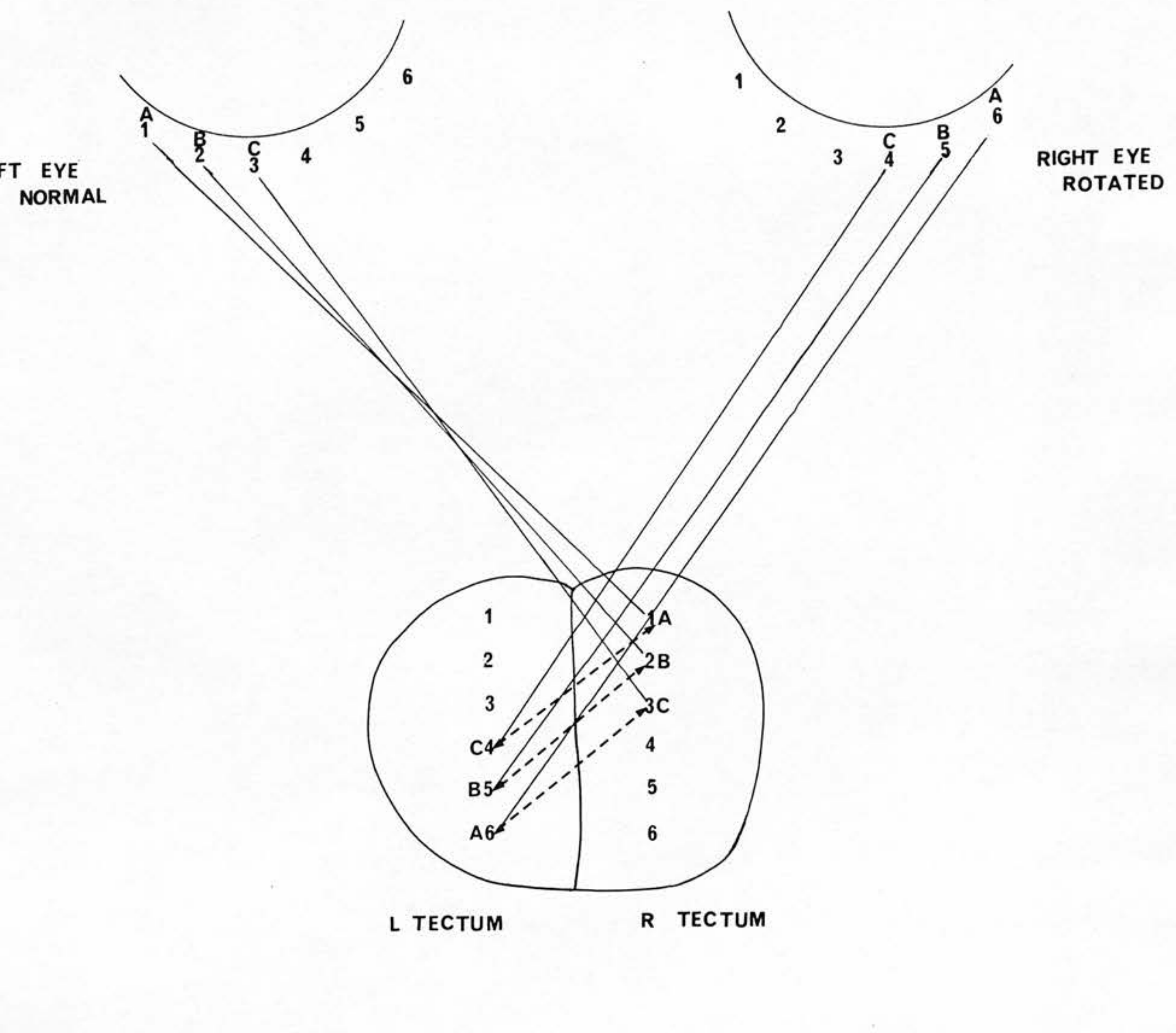
TABLE 12 (Contd.)

Xenopus	Projection	Explained by specificity	Explained by modulation	Explained by B.I.	Other Explanations	No Explanations
XRE8 Right eye rotated 180°	R eye - R tectum			X		
	L eye - L tectum			X		
XRE5 Left eye rotated 180°	L eye - L tectum					X
	R eye - R tectum					X
XRE9 Right eye rotated 180°	R eye - R tectum					X
	L eye - L tectum					X
Contrans 3 Left eye replaced by right eye	R eye - R tectum			X		
	L eye - L tectum			X		
Contrans 4 Left eye replaced by right eye	R eye - R tectum					
	L eye - L tectum				X (patt.4)	
Enucleates (5 animals with right eye removed)	Remaining eye-ipsilateral tectum		5	5		

Figure 40

Hypothesis of delayed respecification.

Figure 40



FT EYE
NORMAL
RIGHT EYE
ROTATED
A
1
B
2
C
3
4
5
6
1
2
3
C
4
B
5
A
6
1
2
3
C4
B5
A6
1A
2B
3C
4
5
6
L TECTUM
R TECTUM

only with cells at position C on the right tectum. Similarly C would have to be connected to A and B to B.

There are however reasons for doubting whether this is the correct interpretation of the results. One is that interaction can take place at stage 65/66 (Nieuwkoop and Faber 1956) in Xenopus, when the eye is free in the orbit apart from its attachments by eye muscles, the optic nerve and blood vessels. It would seem highly unlikely that eye muscles or other structures within the orbit could specify adequately an eye capable of considerable movement.

A compelling argument against respecification is that although it can explain the overall orientation of the visual maps, in experimental animals, it cannot explain the detailed variations in the ipsilateral projection which are found to follow closely the contralateral projections. In Xenopus XCN2, p. 62 the contralateral projection from the NN eye arises from only a narrow strip of retina and the rows of points in the visual map are somewhat muddled. At the time this animal was set up for recording it was noticed that a double lens had developed in the NN eye; this may explain the abnormalities in the contralateral projection. The ipsilateral projection from the normal eye reflects these abnormalities in detail. The process of specification would not predict this finding. The angle between the optic axes of Xenopus XCN3 was abnormal, despite this abnormality the ipsilateral and contralateral projections to one tectum arose from identical parts of visual space, this again cannot be explained by a process of specification.

One must therefore say that both the hypothesis of binocular interaction and of delayed respecification explain these results. Although there are reasons to think that the latter is in all probability not the correct explanation, final proof is not yet available. To settle the issue it would be necessary to alter the functional inputs to one eye without interfering with the orientation of the eye, for example by growing animals in the dark or fixing inverting prisms or lenses over the eye.

The remainder of this study is concerned with the stage at which intertectal linkages develop, and interaction or respecification takes place; it also investigates the regeneration of the intertectal fibres. For simplicity, and since delayed respecification is probably not the correct interpretation of the findings, the results are discussed in terms of binocular interaction, although it must be borne in mind that the results are also interpretable in terms of the alternative hypothesis.

Four experimental series were carried out to investigate the stages at which the binocular interaction is taking place to specify the intertectal neuronal connections. These were:

1. Eye rotation at stage 65/66 and later mapping the visual projection.
2. Enucleation at stage 65/66 and later mapping the visual projection.
3. Eye rotation in adults, and later mapping the visual projection.

4. Investigation to find out the stage at which ipsilateral responses at first detected.

These series will be described and discussed individually, I shall then discuss the results in relation to each other.

The results of the series of Xenopus in which one eye had been rotated at stage 65/66 are shown in tables 5 and 6. It can be seen that seven of the ten animals recorded gave similar results. These will be considered first.

In these seven animals (table 5) the visual maps recorded through both eyes to one tectum are congruent; one point in visual space projects through both eyes to one tectal locus. For the same reasons as those discussed in connection with the results of embryonic eye rotation these results can be explained by the hypothesis of binocular interaction; hypothesis of specificity or modulation are inadequate explanations. It is clear that in these animals binocular interaction at stage 65/66 or later has taken place.

However in the three other Xenopus of this series abnormal ipsilateral projections were mapped. In Xenopus XYAR10, the contralateral projection from the rotated left eye is itself rotated, and the ipsilateral projection from the normal eye is similarly rotated. Therefore, it would seem that this ipsilateral projection was determined by a process of binocular interaction. The ipsilateral projection from the rotated eye was abnormal; unfortunately only two visual field positions were mapped, and so no clear picture of the visual projection was obtained. However the two field positions recorded to the ipsilateral tectum both arose in different parts of the visual space from the contralateral field positions to those tectal loci. Binocular interaction, therefore, cannot explain this result; since the visual map was so fragmentary no interpretation of its determination can be attempted.

The ipsilateral projections from both eyes in Xenopus XYAR5 were disorganised, despite the presence of well ordered contralateral projections. In Xenopus XYAR6 the ipsilateral projection from the normal eye was disorganised, the ipsilateral projection from the rotated left eye was internally ordered but arose from a part of the visual field which would not be predicted by any of the hypotheses (binocular interaction, specificity, modulation, direct ipsilateral growth) put forward in this thesis.

From the results of these two groups of Xenopus in which one eye was rotated at stage 65/66, it might be argued that binocular interaction was taking place in some Xenopus at or after stage 65/66, but that in others, XYAR10,6,5, the eye rotation was carried out at a critical stage in terms of binocular interaction and disorganised ipsilateral projections resulted. However it is not possible to be certain of this; in the series of Xenopus with eye rotations at earlier developmental stages 32-58, although the majority of experimental animals gave results which fitted the binocular interaction hypothesis, abnormal results were found in a minority. Perhaps it is necessary to accept that some animals in which the eye has been rotated before the adult is adult, abnormal results are found. Nevertheless, it is possible to say that in most Xenopus of stage 65/66 binocular interaction is not completed.

Of the two Xenopus enucleated at stage 65/66 a normal ipsilateral projection was recorded from XYAR12. In Xenopus XYAR11, the ipsilateral projection seemed to be normal, but since only five visual field positions were mapped, no conclusive decision can be made about this.

Three Xenopus were mapped in which one eye had been rotated when the animal was adult. The youngest of the animals was two months post-metamorphosis when the eye rotation was performed. Similar results were found in these three Xenopus; in each case the contralateral and ipsilateral projections from the rotated eye were rotated and the projections from the normal eye were both normal. Visual projections to one tectum were therefore not congruent, showing that no binocular interaction had taken place after the rotation of the eye.

The earliest stage at which ipsilateral responses were recorded was 62. This result, however, does not necessarily indicate that ipsilateral fibres first develop at this stage; the fibres may have been present earlier but only at this stage acquire the property of electrical conduction. Nevertheless, since the binocular interaction hypothesis predicts that functional connections develop between points on the two tecta receiving similar patterns of excitation, it would seem that the stage at which ipsilateral fibres become electrically conducting is most relevant in this context.

The histogram in Fig. 25 shows clearly that ipsilateral responses were not recorded from all tadpoles at stage 62; of the 11 stage 62 tadpoles investigated, ipsilateral responses were present in only two. It was not until stage 66 that ipsilateral responses were reliably present. This result could mean that the stage at which ipsilateral fibres develop varies from animal to animal being stage 62 in some and stage 66 in others. However another explanation of this variation is that ipsilateral responses are present from stage 62 onwards, but they are not always detected at first, being very faint. The tectum at these stages is approximately 300μ across, and might easily be damaged by the electrode, to give a falsely negative result.

Unfortunately, it was not possible to map accurately the ipsilateral projections in these juvenile Xenopus, to find out if the ipsilateral projection developed immediately in the final form or whether there were certain different intermediary stages. There were two reasons why this could not be done. One was that the tectum was too small to allow many tectal loci to be investigated; the other reason was that the optics of the eye at this stage are such that to elicit a response it is necessary to move the stimulus object close to the eye, and not at a sufficient distance away from the animal, to map accurately the visual field position which projected to that tectal area.

The ipsilateral visual projection of an animal enucleated at stage 29, is abnormal. If enucleation is carried out at stage 65/66 the ipsilateral projection is normal. This result could be explained by saying that the process of binocular interaction takes place before stage 65/66. However if an eye rotation, rather than an enucleation is carried out at this stage, the resulting visual projections are as would be predicted by the binocular interaction hypothesis, in most animals. The conclusion would seem to be that in most Xenopus the process of binocular interaction starts to specify the intertectal linkage before stage 65/66 but that the system is still labile at the time the eye rotations were carried out. To establish the exact sequence of events, it would be necessary to carry out an extensive series of eye rotations at various stages from 62 onwards. The present study was a pilot one to establish the approximate time at which binocular interaction takes place.

In tadpoles up to stage 58, the eyes are situated on the side of the head and there is no nasal binocular field. As metamorphosis becomes more advanced the shape of the head changes so that the eyes are brought closer together and are placed more rostrally than before. This process starts at stage 60 and is not complete until 3-4 weeks post metamorphosis; this change can be seen clearly in Fig. 41, if the ipsilateral connections are determined by binocular interaction, as is suggested in this work, then it seems reasonable to expect that the interaction between the visual inputs through both eyes will not be complete until the eyes have reached their adult position.

Since I wished to investigate the regeneration of the ipsilateral visual system it was necessary to discover the path taken by the ipsilateral fibres. I had previously checked that the ipsilateral pathway involved an initial projection to the contralateral tectum, it therefore remained to establish the path taken by the intertectal nerve connections.

Figure 41

Photographs of Xenopus at various stages of development, taken from in front of the animal.

KEY:- a = stage 46.

b = stage 58.

c = stage 63-64.

d = young adult Xenopus, overall body length 20 mm.

e = young adult Xenopus, overall body length 70 mm.

The magnification of all the photographs is X 7 .


a
b
c
d
e

Keating (personal communication) had found that in the frog the intertectal stage of the ipsilateral visual projection traversed the postoptic commissure. Hence it seemed appropriate to start the investigation by testing for the presence of ipsilateral responses after section of the optic tract in Xenopus. It was found that section of one optic tract destroyed not only the one contralateral visual projection, but also both ipsilateral projections. The ipsilateral fibres presumably, therefore, as in the frog, travel in the optic tract.

Since it was not possible to cut the intertectal fibres while leaving all the optic axons intact, any experiment to investigate regeneration of intertectal fibres involved also cutting the fibres which constituted the contralateral visual projection. However it is known that when an optic nerve is cut and allowed to regenerate normal contralateral and ipsilateral maps result (Gaze and Jacobson 1963); this is therefore a control experiment for those involving section of the optic tract.

The deep fibres probably including those crossing the postoptic commissure were cut, by sectioning one optic tract, in a series of normal adult Xenopus; several months later the visual projections of these animals were mapped. In some animals recorded within three months of cutting the optic tract, incomplete regeneration of the ipsilateral projections was found to have taken place. In Xenopus which were kept for eight months or more after operation before recording the visual maps, normal ipsilateral projections had regenerated.

In these long term animals, in which the right optic tract had been cut, there was however an extra anomalous retinotectal projection from the right eye to the right tectum. This projection was retinotopically organised and was similar to the contralateral projection from the left eye to the right tectum. It seemed therefore that some optic axons from the temporal part of the right eye had grown into the right tectum. This is a puzzling result, since these nerve fibres from the right eye had not been sectioned. There seems to be two possible explanations of this result; the one explanation is that the presence of degenerating myelin, or other factors present in the cut optic tract, has stimulated the optic axons to develop side branches in the region of the chiasma which have then grown ipsilaterally. Alternatively there may have been a continual growth of new optic axons from the eye to the tectum; some of these may have grown ipsilaterally to give this anomalous projection. No similar anomalous ipsilateral growth took place in those Xenopus in which one eye had been rotated and an optic tract cut; this may be because there was a shorter delay between the sectioning of the tract and recording in this group than in the group with simple tract section.

Since the ipsilateral pathway was found to regenerate in an orderly fashion to give normal visual maps, it seemed highly relevant to investigate the mechanism by which intertectal connections are specified when they regenerate. Since for instance, ipsilateral connections are specified by a process of binocular interaction during development, is a similar mechanism responsible for the selectivity of nervous connections during regeneration? This question was investigated by rotating an eye and cutting the opposite optic tract in a series of Xenopus. As a control experiment eye rotation and section of the corresponding optic nerve were carried out in another series of Xenopus and Rana which was later mapped electrophysiologically.

The results of this control experiment are shown in table 9, it can be seen that the regeneration of the contralateral visual projection did not affect the pattern of ipsilateral connections, these remained unchanged. Thus any variations from this result in animals with eye rotation and section of the opposite optic tract could be assumed to depend on the regeneration of the intertectal fibres.

Section of the optic tract and eye rotation in three newly metamorphosed Xenopus resulted in direct ipsilateral fibre growth, pattern 4 regeneration. However no regeneration of the intertectal connections had taken place. Since ipsilateral responses are present at stage 66 in Xenopus, this was an unexpected result. However it was noted at the time of operation that difficulty had been experienced in cutting the optic tract; the small size of the Xenopus meant that any cut disrupted the chiasmal region considerably. Perhaps the path along which the intertectal fibres normally grow had been destroyed.

The intertectal linkage had regenerated in the older animals used in this study. The first animal to be recorded had been operated on 97 days previously; the ipsilateral responses were poor and the map disorganised. The other animals in this series were left for between 147 and 222 days between tract section and final recording, in each one complete restoration of the ipsilateral projections had taken place.

Seven animals recorded in this series all showed similar results (table 10). The contralateral projection from the rotated left eye was rotated. Despite section of the right optic tract both ipsilateral projections were normal. Thus any one locus on the right tectum received visual information from different parts of the visual space through the two eyes.

Two other animals in this series were enucleates, the left eye had been damaged at the time of operation and had therefore been removed. Both the contralateral and ipsilateral projections from the remaining eye were normal.

These results show clearly that when regeneration of the intertectal nerve fibres takes place, they reform their original connections. This happens despite rotation of the one eye. Binocular interaction, therefore, cannot be responsible for the selection of connections by regenerating intertectal fibres. Latency studies suggest that the intertectal pathway is almost certainly a poly-synaptic one. It is therefore possible that the fibres being severed when the optic tract is cut are different from those which actually "search out" the appropriate tectal cell during development. The development and regeneration of all those fibres in the intertectal linkage apart from those synapsing on the tectal cell with an appropriate input, may be governed by the mechanism biochemical specificity. In fact, it would be logical to expect them to do so since functional interaction is required only at the level of the tectal cells to produce a viable mechanism.

The enucleate animals also support the hypothesis that functional binocular interaction is not of consequence during regeneration of the ipsilateral pathway, following optic tract section. Since the detailed anatomy of the ipsilateral pathway is not known, at this stage it is not possible to selectively destroy the axons which are this final link and presumably carry out a search during development and study their regeneration.

As a general conclusion to the experiments carried out in this study, it may be said that a hypothesis to explain the development of certain intertectal connections in Anurans has been put forward and tested. The hypothesis of binocular interaction is that points on the two tecta receiving similar spatio-temporal patterns of excitation become linked. The hypothesis was tested by altering the positional relations between the eye and brain in larval Xenopus and later recording the visual projections to the optic tecta. The majority of the results can be explained by a mechanism of binocular interaction, but not by any other known mechanism of neuronal selectivity, such as modulation or specificity. A small number of results are inexplicable by any hypothesis of the determination of nerve connections.

The determination of the intertectal connection takes place at or after stage 65/66 in Xenopus (Nieuwkoop and Faber

1956) and before two months of post metamorphic life has taken place. The intertectal fibres were found to regenerate and reform their original connections, no binocular interaction takes place when intertectal fibres regenerate.

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Binocular interaction in the formation of specific intertectal neuronal connexions

By R. M. Gaze, M. J. Keating, G. Székely and Lynda Beazley

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Binocular interaction in the formation of specific intertectal neuronal connexions

BY R. M. GAZE, M. J. KEATING,* G. SZÉKELY AND LYNDA BEAZLEY†

Neurobiology Research Unit, Physiology Department, Edinburgh University and the Anatomy Department, University Medical School, Pécs, Hungary

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The mechanisms concerned in the formation of the ipsilateral visual projection in *Xenopus* have been investigated by examining the results in the adult of various surgical manoeuvres on the embryonic eye:

1. In animals with one double-nasal compound eye, the ipsilateral projection from the compound eye was normal while that from the normal eye was abnormal but internally organized.
2. In animals with one double-temporal compound eye the ipsilateral projection from both operated and normal eyes was normal.
3. In animals with one rotated eye, the ipsilateral projection from the operated eye was normal while that from the normal eye was rotated.
4. In animals reared with only one eye the ipsilateral projection was abnormal in that it was diffuse.

The observations are interpreted as indicating that the formation of the retinotopically organized intertectal projection which forms part of the ipsilateral projection is dependent upon a process by which regions of the two tecta that generally receive similar spatiotemporal patterns of excitation become neurally interconnected.

INTRODUCTION

Connexions between neurons in many, if not all, parts of the nervous system appear to demonstrate a high degree of selectivity. The details of the developmental processes that produce these highly selective connexions are far from clear but studies on lower vertebrates emphasize the role of prefunctional mechanisms in neural specification (Sperry 1951*a*, 1963; Gaze 1960, 1967).

The hypothesis of neuronal specificity derives largely from work on the visual systems of lower vertebrates. The retinotopic projection of the optic nerve fibres to the contralateral optic tectum is restored following section of the optic nerve (Sperry 1943; Gaze 1959; Maturana, Lettvin, McCulloch & Pitts 1959). During regeneration the optic axons reach their correct tectal sites despite considerable scrambling of fibres at the site of section, which suggests that mechanical guidance of the fibres is not an essential factor in the formation of selective connexions.

In adult frogs and in late pre-metamorphic tadpoles rotation of the eye through 180°, accompanied by section of the optic nerve, resulted, after regeneration of the nerve, in a rotation of the visual field projection on the tectum (Sperry 1944).

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* Barry Stevens Memorial Fellow, Mental Health Research Fund.

† S.R.C. Student.

Similar results were obtained if the rotation was done in early embryonic stages, after retinal specification had occurred, but before any neuronal connexions had been established between the eye and the brain (Stone 1944; Jacobson 1968). Despite the rotation of the eye or embryonic eye-cup, retinal ganglion cells still made 'correct' connexions with their appropriate tectal loci; that is, connexions appropriate to their original retinal position rather than to their new, rotated position in the orbit. The resulting rotation of the visual field projection was functionally maladaptive since the animal consistently localized a visual stimulus to an erroneous position. Visual function thus seemed to play no part in the establishment of these specific connexions.

To explain these findings Sperry (1943, 1951*a*) put forward the hypothesis that during development, retinal ganglion cells come to differ cytochemically from each other, according to their retinal positions. A corresponding specification of tectal elements according to their positions in the tectum must also occur. Retinal neurons of a given cytochemical specificity selectively connect with tectal neurons of corresponding specificity. This concept of neuronal specificity has received much supporting evidence from studies on other neural systems and has been extended to include all aspects of neural ontogeny (Sperry 1963, 1965). The evidence for this hypothesis is compelling and we would tend to agree with Sperry & Hibbard (1968): 'the issue of growth versus function seems now to be quite settled and dead, at least in the case of retino-tectal connexions'.

Sperry & Hibbard were, of course, referring to the projection from the retina to the contralateral optic tectum; but there is another visual projection in these animals and that is the one from the retina to the ipsilateral tectum, described by Gaze & Jacobson in 1962. In frogs the binocular, nasal, part of the visual field (temporal retina), projects to the rostral part of the ipsilateral tectum. Gaze & Jacobson (1963) showed that the pathway from the retina to the ipsilateral tectum involves an initial passage from the eye to the contralateral tectum. The connexions in this stage of the pathway appear to be determined by the mechanisms of neuronal specificity already mentioned. The second stage of the pathway involves an intertectal linkage back from the contralateral to the ipsilateral tectum. Gaze & Jacobson showed that the ipsilateral projection survives extensive lesions separating the two tecta and recent work (Keating & Gaze, in preparation) indicates that this intertectal linkage traverses the post-optic commissures. The ipsilateral pathway in *Xenopus* appears to be similar to that in *Rana*.

This paper is concerned with the mechanisms that determine the formation of the specific neuronal connexions in this intertectal linkage. If neuronal specificity is at work in this more central system, as it is in the direct contralateral visual projection, then surgical procedures which alter the geometrical relationship between the eye and the body during embryonic life should produce corresponding alterations in the ipsilateral projection from that eye, as happens with the contralateral projection. This does not occur. Gaze, Jacobson & Székely (1965) found that the ipsilateral projection from double-nasal 'compound eyes' in *Xenopus* was normal;

whereas the contralateral projection from the same part of the compound retina was back-to-front on the tectum. Furthermore, and even more surprisingly, in these animals the ipsilateral projection from the *normal* eye was itself abnormal. This latter finding cannot be accounted for on the basis of innate neuronal specificity and an alternative mechanism must be sought.

Keating (1968) proposed an explanation for these peculiarities of the ipsilateral projections in operated *Xenopus*. The explanation gives rise to a new hypothesis concerning the mode of development of specific neuronal connexions in certain parts of the central nervous system. The hypothesis states that these connexions are formed, not by the action of the innate mechanism of neuronal specificity but by a functional interaction between the two eyes. Those positions on the two tecta that are simultaneously receiving a similar spatiotemporal pattern of impulses through the two eyes from a stimulus at one position in the binocular visual field, become neuronally linked together. This paper examines the hypothesis by investigating the retinotectal projections in *Xenopus* with a variety of surgically induced abnormalities of one eye.

METHODS

Operations

Compound eyes

Embryos of *Xenopus laevis* were used at stages 30–32 (Nieuwkoop & Faber 1956). At these stages the retina has not yet formed and there is no neuronal connexion with the developing brain but the developing retinal cells are already specified according to their position in the retinal field (Jacobson 1968). Under anaesthesia with MS 222 (tricain methanesulphonate) the eye-cup was cut in half down the vertical midline and the temporal half removed and replaced by a nasal half taken from the opposite eye of another animal. The transplanted half-eye was held in position with a glass bridge for a few minutes to allow the tissues to heal together. Animals with double-nasal compound eyes formed in this fashion were reared to maturity and used for electrophysiological mapping of the retinotectal projections when they had reached a post-metamorphic size of 4 to 6 cm body length. Some of the animals with compound eyes discussed in this paper (those labelled NN and TT) formed part of the original series described by Gaze, Jacobson & Székely (1963, 1965).

Rotated eyes

Xenopus embryos of various stages of development were immobilized in MS 222 and one eye anlage was freed by the use of tungsten needles, then rotated about the optic axis. Rotations of various extents were performed in different animals and two of the *Xenopus* were used later, after metamorphosis, for investigation of the retinotectal projections.

Enucleated eyes

One eye-cup was removed from *Xenopus* embryos before stage 29. These animals were allowed to develop to maturity with only the residual eye forming connexions with the brain. The retinotectal projections from this eye were then mapped electrophysiologically.

Electrophysiological mapping

Each animal was anaesthetized with ether and the cranium opened with a dental drill. After decerebration the surface of the optic tectum was covered with mineral oil and the animal was given 0.1 mg tubocurarine intramuscularly. The dura was removed from the optic tecta and these were photographed at a magnification of × 50 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 of radius 33 cm. The eye under investigation was centred on the fixation point of the perimeter arc (but see below). The eye not being used was covered temporarily with an opaque shield.

The visual projections were mapped by placing a recording microelectrode (a metal-filled glass pipette tipped with platinum) serially on positions indicated by the intersections of the grid on the tectal photograph, and for each tectal position determining the optimal position for a stimulus in the visual field. Recording was normally performed in ordinary room lighting and the stimulus usually employed was a black cardboard disk subtending 5° at the eye. Action potentials following the presentation of this stimulus were recorded on the surface or in the superficial layers of the optic tectum. These potentials were amplified by means of a Tektronix 122 preamplifier, using a time-constant of 2 ms. Potentials were then further amplified and displayed on an oscilloscope as well as being monitored on a loudspeaker.

Animal orientation

The animal was arranged at the centre of the perimeter arc, with the optic axis of one eye alined with the fixation point of the perimeter. The results of electrophysiological mapping are presented as a series of stimulus positions on a perimetric chart of the visual field. The convention adopted, as in previous papers (Gaze, Jacobson & Székely 1963, 1965) is that the observer is considered to be looking through the chart to the animal, which in turn is looking out through the chart to the observer. Each chart covers the visual field of one eye from the fixation point in the centre outwards for 100°.

In some animals we have used the same method of positioning that was used in the previous experiments (Gaze *et al.* 1963, 1965); that is, for mapping the projections from the left eye, this eye was centred on the perimeter, while for mapping the projections from the right eye, the right eye was centred. This method has two disadvantages; first, the animal has to be moved to permit the projections from the other eye to be mapped and this introduces a positioning error in the relationship between the maps for the two eyes; and secondly, the maps are then presented on two different coordinate systems, one for each eye. Since in the present paper we are particularly concerned with the position of the stimulus in visual space relative to the two eyes, we have in some animals kept the one eye centred while mapping all four projections (ipsilateral and contralateral from both eyes) on the one coordinate system. This is made possible by the very extensive overlap of the visual fields in these animals.

Histological examination

In all experiments the head was eventually fixed in Susa, serially sectioned at 15 μm and stained with Holmes's silver method.

RESULTS

The experimental results may be subdivided according to the type of abnormality produced in the embryo: (1) double-nasal compound eye (animals NN 1, 2, 3, 4; XCN 1, 2, 3, 4), (2) double-temporal compound eye (TT 9, 10, 12, 13, 15, 16, 19, 20), (3) rotated eye (XRE 1, 2), (4) enucleated eye (TT 5; XE 1, 2, 3).

(1) *Animals with a double-nasal compound eye*

The visual projections were mapped in eight animals in which the right eye was a double-nasal compound eye.

(*a*) Four of these animals (NN 1, 2, 3 and 4) formed part of a previous series of observations (Gaze *et al.* 1965). Since these results formed the basis of the hypothesis presently under examination, they are here presented more fully than in the previous paper.

Figure 1 shows, for reference, maps of the normal contralateral and ipsilateral visual projections from both eyes. Each of the four visual projections (i.e. contralateral and ipsilateral from each eye) is shown in two forms. In one the relevant eye is itself centred on the perimeter, in the other the opposite eye is centred.

In experimental animals NN 1, 2, 3 and 4 the contralateral projections from both eyes to the tecta are shown in figures 2 to 9. In all four cases the contralateral projection from the normal left eye to the right tectum was normal (figures 3, 5, 7, 9). The contralateral projections from the right (NN) eyes to the left tecta (figures 2, 4, 6, 8) showed the pattern of retinotectal connexions described for this type of eye by Gaze *et al.* (1963, 1965). The original nasal half-retina (temporal field) projects retinotopically to the contralateral tectum in appropriate fashion, namely the most nasal retina (most temporal field) projects to the caudal part of the tectum and the least nasal retina (least temporal field) projects to the rostral part of the tectum. Animal NN 1 (figure 2) is not typical in that the right eye, as well as being compound, was rotated 90° clockwise (see later). The projection from the original half-retina appears, however, to have spread over the whole available tectum rather than to have restricted itself to the caudal half of the tectum as would be the case in a

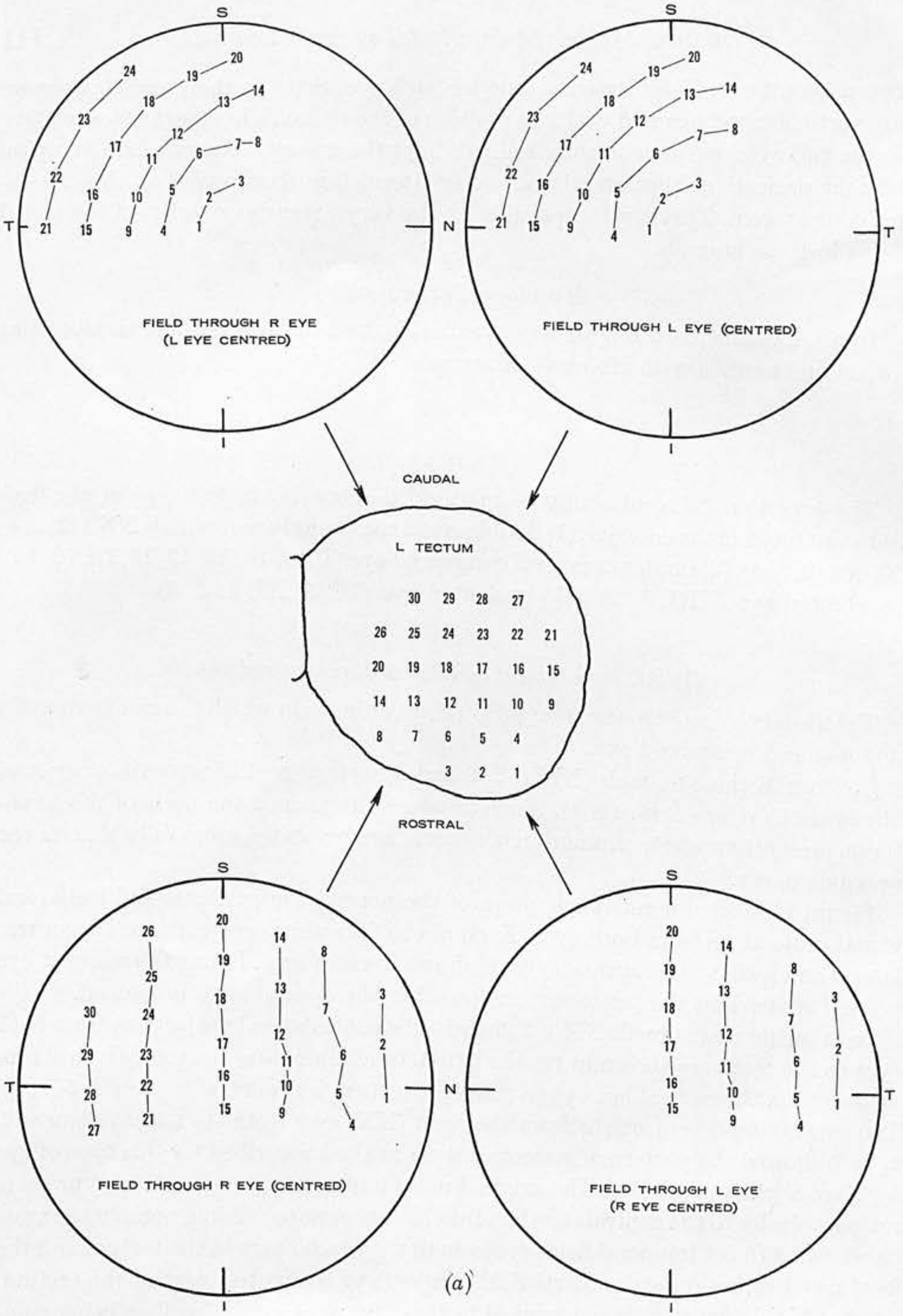
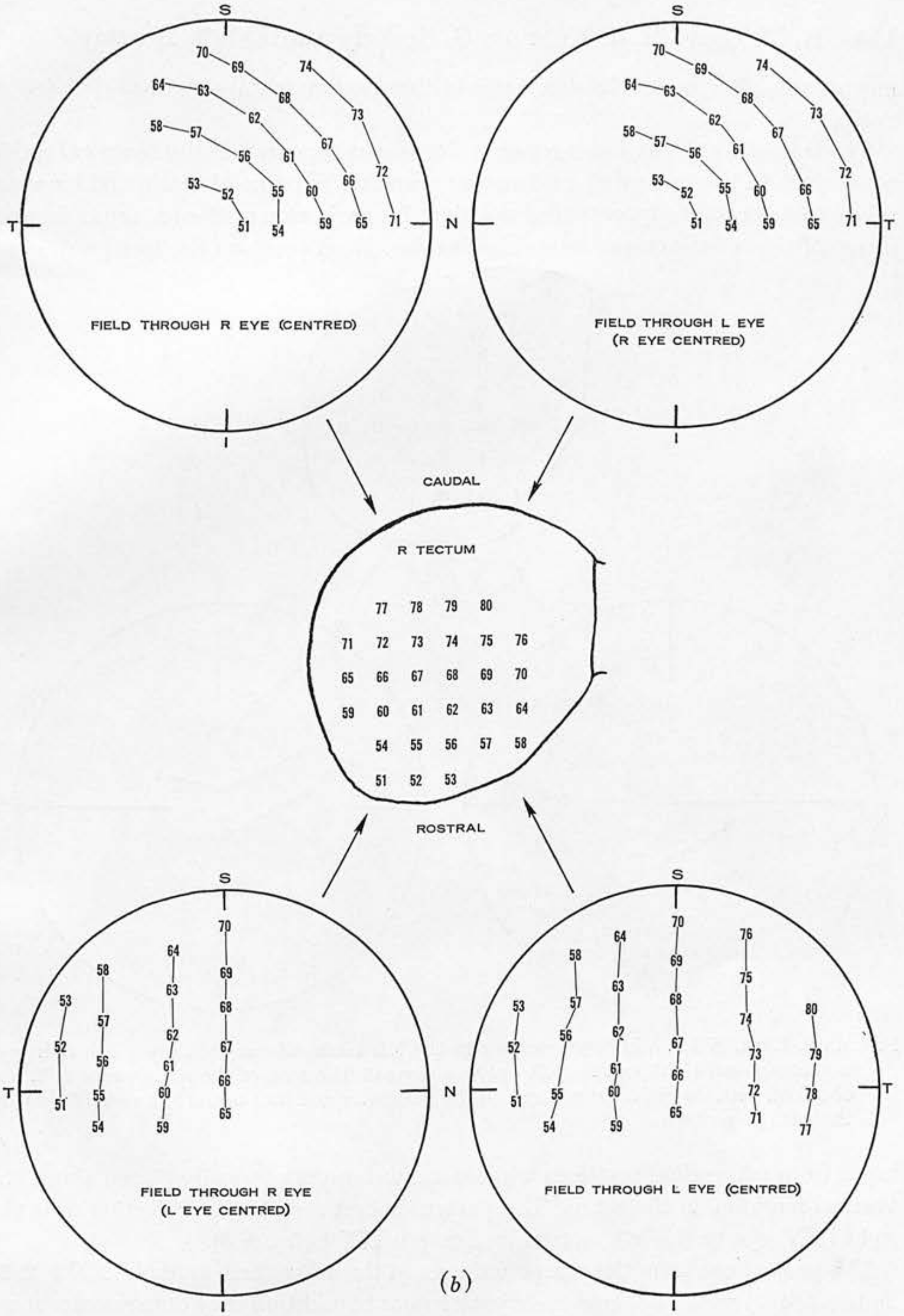


FIGURE 1. Normal visual projections.

(*a*) The projection of the visual field through both eyes to the left tectum. The numbers on the tectal diagram represent electrode positions. For each tectal position the corresponding field position is indicated by the appropriate number on the perimetric chart representation of the visual field. N, nasal pole; T, temporal pole; S, superior (dorsal) pole; I, inferior (ventral) pole. In all experiments the rows of tectal positions are numbered from lateral to medial with the lowest numbers rostrally and the highest numbers caudally. The field projections through both eyes are plotted with the left eye centred on the perimeter in the two upper charts in the figure and with the right eye centred on the



(*b*)

perimeter in the two lower charts. When both contralateral and ipsilateral projections to one tectum are mapped with the same eye centred it can be seen that any one position on the rostral tectum is excited via *both* eyes from only one field position. The two perimeter charts are superimposable. Such a double map is made by placing the electrode at one position on the tectum, covering one eye with an opaque shield and determining the field position via the other eye; then the shield is transferred to the other eye and the process repeated.

(*b*) The projection of the visual field through both eyes to the right tectum. In this case the two upper field plots represent the projections via both eyes with the right eye

(*Continued at foot of p.* 114)

normal animal. The significance of this is discussed more fully by Gaze *et al.* (1963, 1965).

The transplanted nasal half-retina which is now topographically temporal retina connects with the tectum in a manner appropriate to its nasal origin and inappropriate to its present topographical position. Its projection pattern is thus a mirror-image of that of the original nasal half-retina. Each point on the tectum receives input from two retinal positions which tend to be symmetrically placed about the vertical meridian of the retina. This pattern is best seen in the projection from the right (NN) eye to the left tectum in *Xenopus* NN 4 (figure 8).

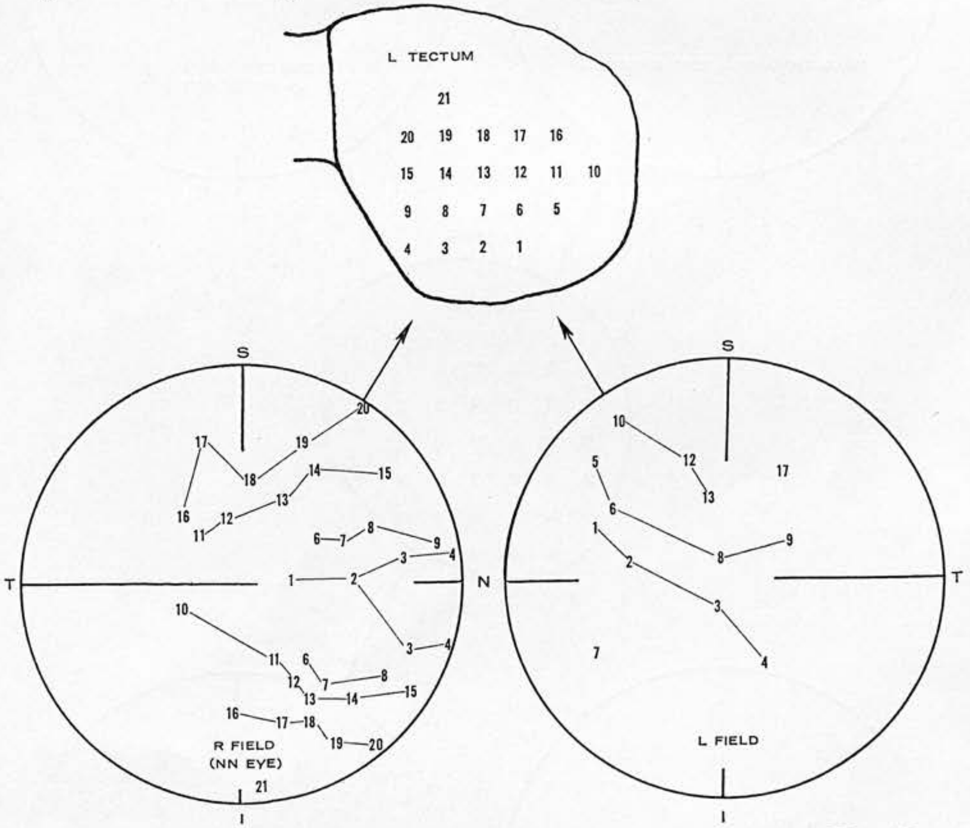


FIGURE 2. Expt. NN 1. Visual projections to the left tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The map of the right visual field was obtained with the right eye centred on the perimeter and that of the left visual field with the left eye centred.

The projections from the compound eyes in the other three animals (NN 1, 2, 3; figures 2, 4, 6) show the same basic pattern but in addition they demonstrate some deviations from it. Thus the projection from the right nasal field (i.e. transplanted nasal retina) in animals NN 2, 3 (figures 4, 6), as well as showing the abnormality already described, has undergone a 45° clockwise rotation. In the case of animal

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centred on the perimeter and the two lower field plots the projections via both eyes with the left eye centred on the perimeter. Since we have found it difficult to obtain all eight projections, in sufficient detail, from any one animal, the field charts in figure 1 are somewhat diagrammatic representations of data combined from several animals.

NN 1 (figure 2) an even more marked abnormality is seen. The right (compound) eye was noted during the recording to be rotated by 90° as shown by the position of the ventral iridial notch. This rotation was reflected in the projection pattern from this eye to the left tectum. Thus the whole projection is rotated clockwise through 90° and the mirror-image projections from each half-retina find themselves symmetrically arranged about the horizontal field meridian instead of the vertical.

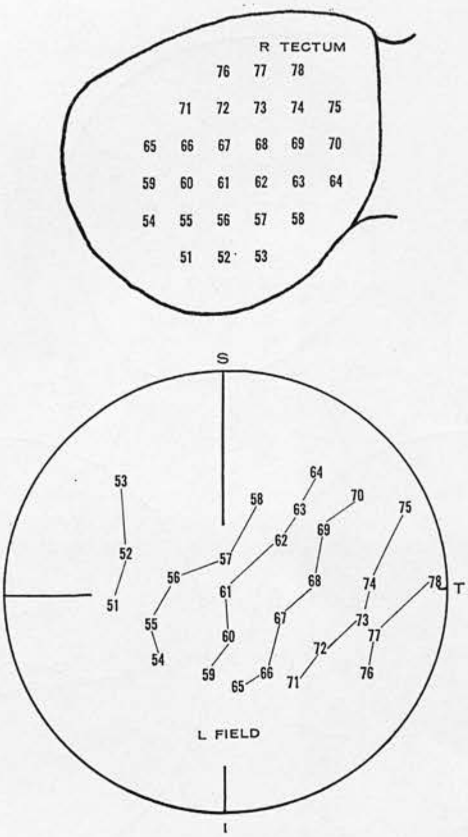

FIGURE 3. Expt. NN 1. Contralateral projection from the normal (left) eye to the right tectum with the left eye centred on the perimeter. In this animal the right eye was compound (NN).

The ipsilateral visual projections for these four animals are shown in figures 2 and 4 to 9. The ipsilateral projection from the compound right eye in animals NN 2, 3 and 4 (figures 5, 7, 9) may be seen to approximate closely to the normal (figure 1(*b*), top left). The map is not available for NN 1. The ipsilateral projection from the normal left eye, on the other hand, was in each case abnormal (figures 2, 4, 6, 8; compare figure 1(*a*), top right). It is immediately apparent, however, that these projections, though abnormal, are not random; in each case the ipsilateral projection from the normal eye is internally organized. The abnormality in the ipsilateral projection may be described by comparing the projection with a normal ipsilateral projection (see figure 1(*a*), top right). In two animals, NN 2 and 3 (figures 4, 6), the observed ipsilateral projection would be obtained if a normal ipsilateral map were inverted along an axis perpendicular to the row of field positions (i.e. along a NW–SE axis) and then the whole map rotated about 45° clockwise. In animals NN 1 (figure 2) and NN 4 (figure 8) the observed ipsilateral projections would be obtained if a normal ipsilateral map were rotated in a clockwise direction through about 90°. In these two latter cases no inversion along a field axis is involved.

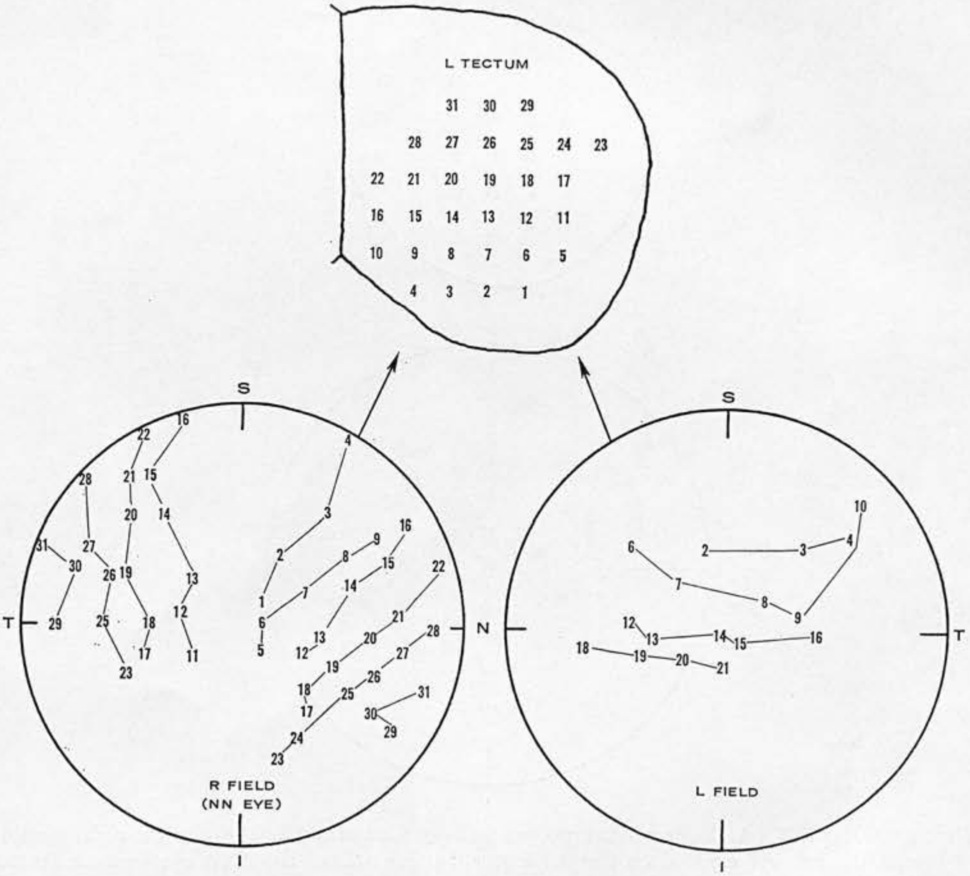

FIGURE 4. Expt. NN 2. Visual projections to the left tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

(*b*) The visual projections were mapped in a further four animals, XCN 1, 2, 3 and 4. In these animals the mapping procedure was slightly different. The usual practice involves centring the eye under investigation on the perimeter so that the centre of the visual field map corresponds to the optic axis of the eye. The visual fields of the right and left eyes obtained in this manner thus have different sets of coordinates and represent different aspects of visual space. If one does not know the exact relationship between the optic axes of the two eyes it is impossible to translate a point on one set of coordinates to a point on the other set.

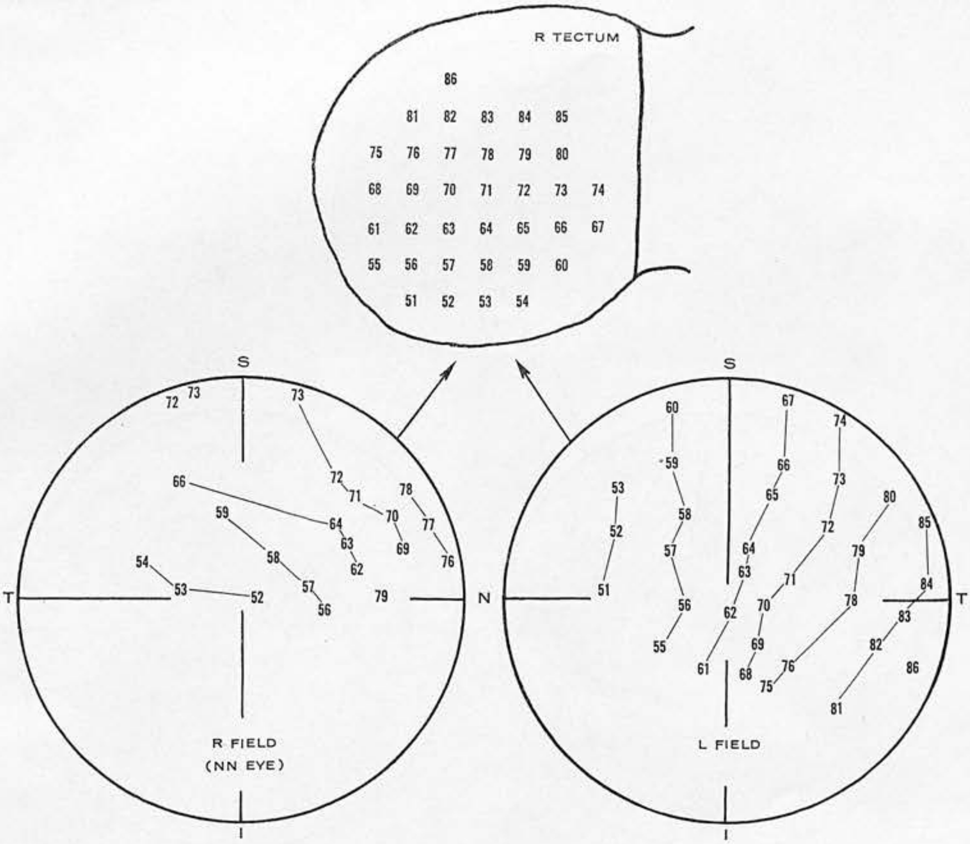

FIGURE 5. Expt. NN 2. Visual projections to the right tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

For a full examination of the hypothesis under investigation it is necessary to know the projections, through both eyes, of one point in visual space. It is therefore necessary to plot the projections through both eyes on one set of coordinates. It was thus decided to set up the animal with the right, operated, eye centred on the perimeter and to map the projections through both eyes to both tecta with the animal in this one position. The maps so obtained of the projections through the right eye to the two tecta are, of course, the same as those obtained by the previous method. The projections through the left eye, however, look quite different from normal because, although the points in visual space involved are the same, they are in this case being presented on a different set of coordinates. The visual projections from the left eye of a normal animal, when presented in this fashion, are illustrated in figure 1 (*a*), bottom right, and figure 1 (*b*), top right. The advantage of this method of presentation is that any point in visual space occupies the same coordinates in all the maps and it can be seen directly from the maps if indeed one point in visual space does project through both eyes to the same tectal point.

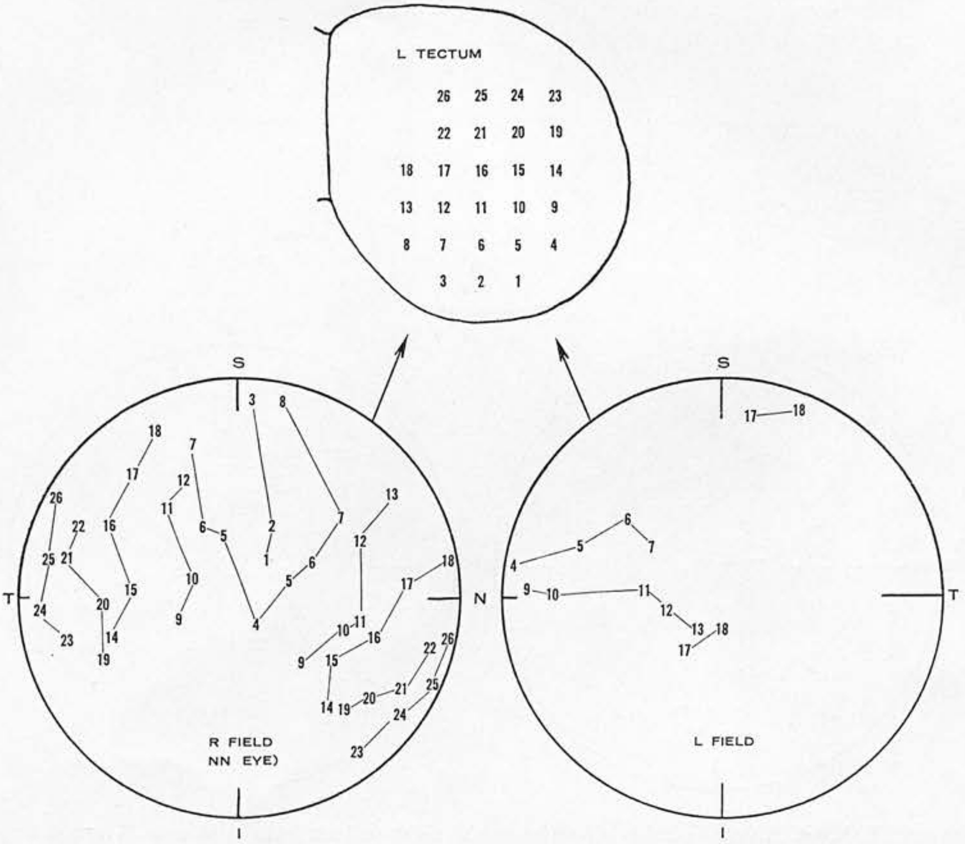

Figure 6. Expt. NN 3. Visual projections to the left tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

For three of the animals (XCN 2, 3, 4) the method described above was used so that all four maps in each animal refer to the same visual coordinates. In one animal (XCN 1) the method was slightly varied in that the projection from both eyes to the left tectum was described in terms of the optic axis of the right eye (for normal maps, see figure 1 (*a*), top left and right), while the projection from both eyes to the right tectum was plotted with the left eye centred on the perimeter (for normal maps, see figure 1 (*b*), bottom right and left).

The results from these four animals confirm the original observations of Gaze *et al.* (1965). The contralateral projections from the compound right eye in all four animals show the characteristic reduplication of visual field positions, although once again there are slight variations in the projection from the transplanted nasal retina (nasal field) from animal to animal. Thus while in *Xenopus* XCN 1 (figure 10) and XCN 3 (figure 14) the rows of field positions are approximately parallel to the vertical meridian as they should be, in XCN 2 (figure 12) they are slightly mixed up, rotated about 45° clockwise and arise mostly from a rather narrow strip of retina; and finally, in XCN 4 (figure 16) they are rotated about 45° clockwise.

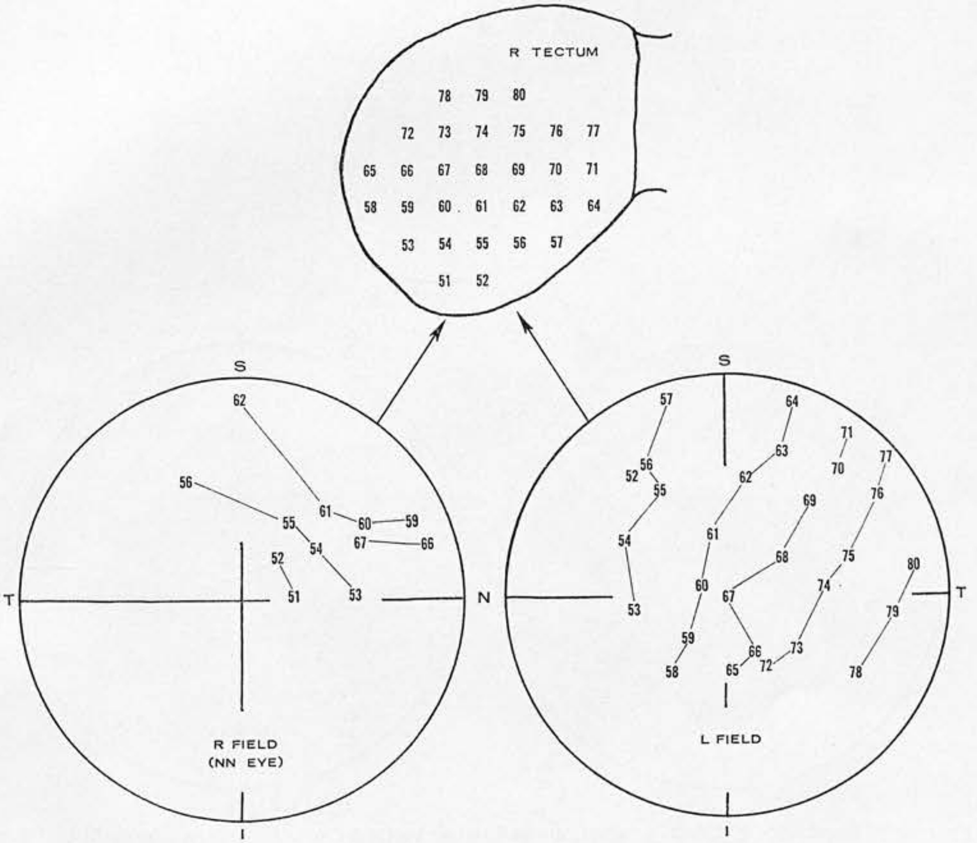

FIGURE 7. Expt. NN 3. Visual projections to the right tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

The ipsilateral projection from the compound eye was normal in XCN 1 (figure 11; compare with figure 1(*b*), bottom left). This ipsilateral projection in XCN 3 (figure 15) appears to be rotated 45° anticlockwise (compare with figure 1(*b*), top left). Because of deterioration of the animal during the recording experiment this projection is not available for XCN 2 or XCN 4.

The contralateral projection from the left, normal eye was normal in the three animals (XCN 1, 2, 3) in which it was obtained (figures 11, 13, 15). The projection in

XCN 3 (figure 15) appears to be rotated 45° anticlockwise (compare with figure 1(*b*), top right); the left eye in XCN 3 was normal however, and this point is considered further in the discussion.

The ipsilateral projections from the normal eyes (figures 10, 12, 14, 16) were in all cases abnormal; but as before, the map was internally organized in each animal. In XCN 1 (figure 10) and XCN 3 (figure 14) the abnormality involved a reversal of the map along the nasotemporal axis but the map was appropriately organized along the dorsoventral axis (compare figure 1(*a*), bottom right). In XCN 2 (figure 12) the ipsilateral map from the left eye arises mainly from a narrow strip of retina; it is reversed along the nasotemporal axis, normal along the dorsoventral axis, and rotated 45° in a clockwise direction. In XCN 4 (figure 16) the map is reversed along the nasotemporal axis, normal along the dorsoventral axis and rotated about 45° in a clockwise direction. A summary of the results of the eight animals with a right double-nasal eye is given in table 1.

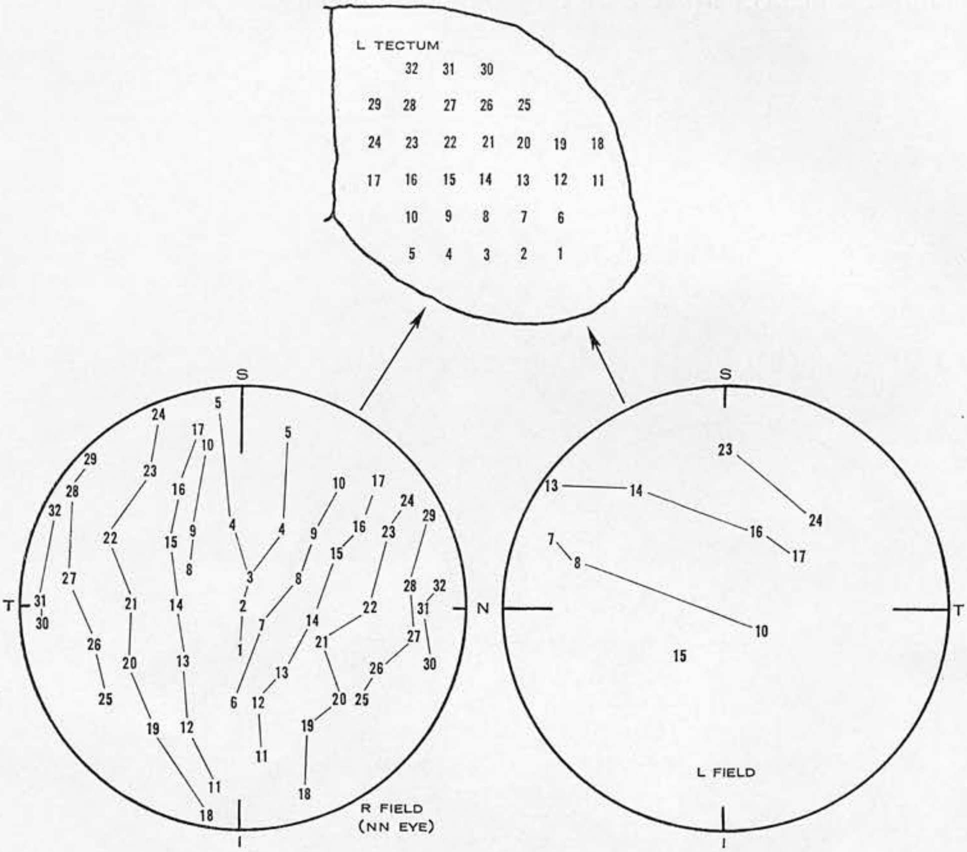

FIGURE 8. Expt. NN 4. Visual projections to the left tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

TABLE 1. VISUAL PROJECTIONS FROM THE NASOSUPERIOR QUADRANT IN ANIMALS WITH A RIGHT DOUBLE-NASAL COMPOUND EYE

		right (compound) eye		left (normal) eye	
animal	figures	contralateral	ipsilateral	contralateral	ipsilateral
NN 1	2, 3	rotated 90° clockwise	—	normal	rotated 90° clockwise
NN 2	4, 5	reversed N-T normal D-V 45° clockwise rotation	normal	normal	reversed N-T normal D-V 45° clockwise rotation
NN 3	6, 7	reversed N-T normal D-V 45° clockwise rotation	normal	normal	reversed N-T normal D-V 45° clockwise rotation
NN 4	8, 9	reversed N-T normal D-V no rotation	normal	normal	rotated 90° clockwise
XCN 1	10, 11	reversed N-T normal D-V no rotation	normal	normal	reversed N-T normal D-V no rotation
XCN 2	12, 13	reversed N-T normal D-V narrow strip of retina, rather mixed up rotated 45° clockwise	—	normal	reversed N-T normal D-V narrow strip of retina rather mixed up rotated 45° clockwise
XCN 3	14, 15	reversed N-T normal D-V no rotation	rotated 45° anti-clockwise	rotated 45° anti-clockwise	reversed N-T normal D-V no rotation
XCN 4	16	reversed N-T normal D-V rotated 45° clockwise	—	normal	reversed N-T normal D-V rotated 45° clockwise

(2) *Animals with a double-temporal compound eye*

Gaze *et al.* (1965) also investigated the ipsilateral projection in a series of animals which the right eye was a double-temporal compound eye. These results are examined further here.

In eight animals observations on the ipsilateral projection were made. In all eight animals the contralateral projection from the compound eye showed evidence of the reduplication that is characteristic of these compound eyes. In this case, however, in contrast to the double-nasal eyes, the projection from the nasal half-field (the original temporal retina) is normally oriented, whereas the projection from the temporal half-field (the transplanted temporal retina) is reversed along the nasotemporal axis.

The ipsilateral projection from the compound eye was investigated in four animals. It was approximately normal in all cases.

The contralateral projection from the normal eye was mapped in four cases. As expected, it was normal.

The ipsilateral projection from the normal eye was examined in seven animals.

This projection was organized normally in all seven cases. Figures 17 and 18 show the visual projections in one of these animals (TT 12).

(3) *Animals with one rotated eye*

The visual projections were mapped in two animals in which the right eye-cup had been rotated at stage 32.

In one animal (XRE 1) the eye had been rotated 90° clockwise whereas in the other (XRE 2) the rotation had been through 180°. The positions of the ventral iridial notch at the time of recording confirmed these rotations.

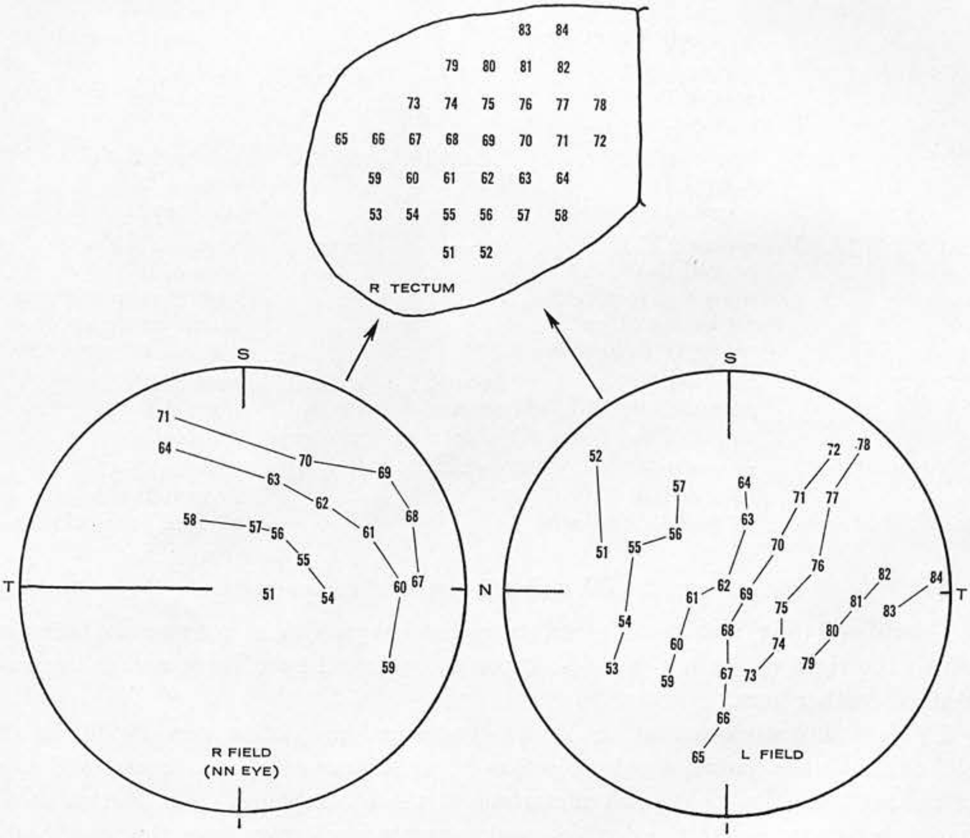

FIGURE 9. Expt. NN 4. Visual projections to the right tectum from both eyes. The right eye was compound (NN) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

The contralateral projections from the rotated eyes were, as expected, rotated; by 90° clockwise in the case of XRE 1 (figure 19) and by the full 180° in XRE 2 (figure 21). In the case of XRE 2 the condition of the animal was not very good and only a few points on each projection were determined. The number of points was, however, sufficient to provide information as to the general orientation of the maps.

The contralateral projection from the normal eye was normal in both animals (figures 20, 22; compare figure 1 (*b*), top right).

The ipsilateral projections from the rotated eyes (figures 20, 22) were normal whereas the ipsilateral projections from the normal eyes were in both cases abnormal. In the case of XRE 1 (figure 19) the ipsilateral projection from the left (normal) eye was rotated about 90° clockwise (compare with figure 1 (*a*), bottom right), whereas in XRE 2 (figure 21) it was rotated by 180°. These results are summarized in table 2.

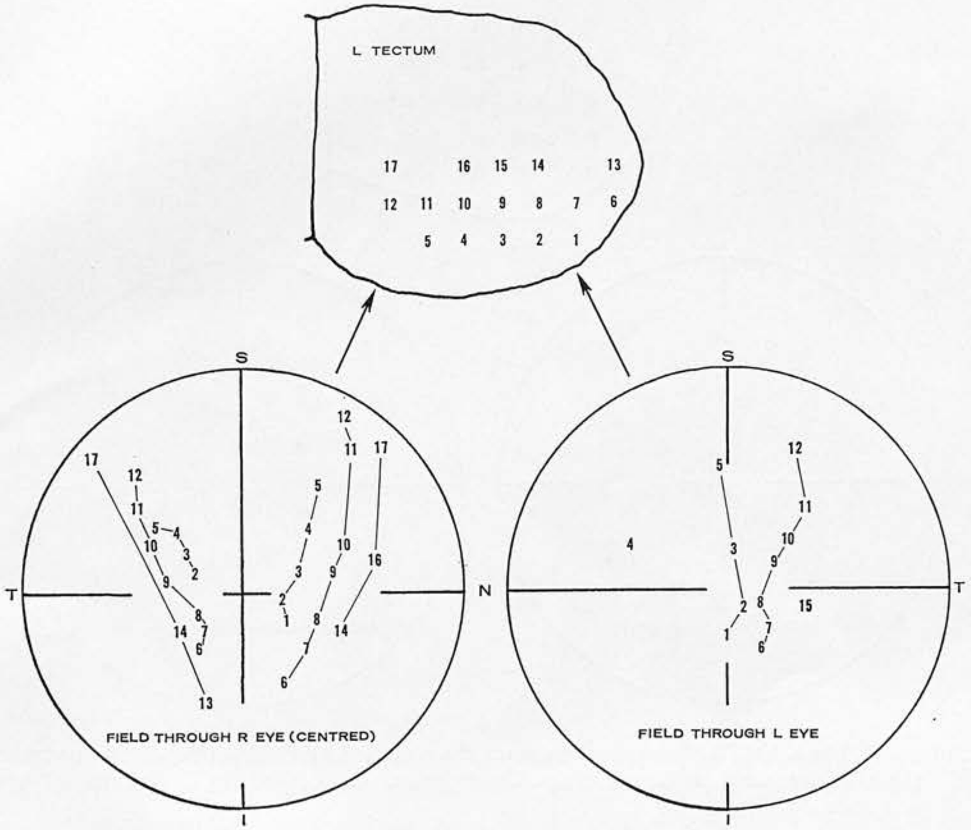

Figure 10. Expt. XCN 1. Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

(4) *Animals with one eye enucleated*

Observations were made on three animals in which one eye anlage had been removed before stage 29 of embryonic life. In addition one *Xenopus* (TT 5) from a previous series (Gaze *et al.* 1965) has been taken into consideration; in this animal the right eye, which was a double-temporal compound eye, failed to develop an optic nerve. Each of the four animals described in this section thus reached maturity with only one eye connecting to the brain.

In each case the contralateral projection from the remaining eye was normal. In all four animals, however, while ipsilateral responses were obtainable, the ipsilateral projection was abnormal. The abnormality in the ipsilateral projection was that the multi-unit receptive fields recordable at any given tectal position tended to be much wider than in a normal animal. Multi-unit fields of 45° to 50° diameter were commonly found, with no obviously better response in the centre than at the periphery. This meant that it was not possible in most cases to construct a meaningful map. Occasionally the microelectrode picked up single-unit responses and these had receptive fields of 5° to 8° diameter. However, when single units were recorded at different depths in the same electrode track they were found to come from widely different parts of the visual field.

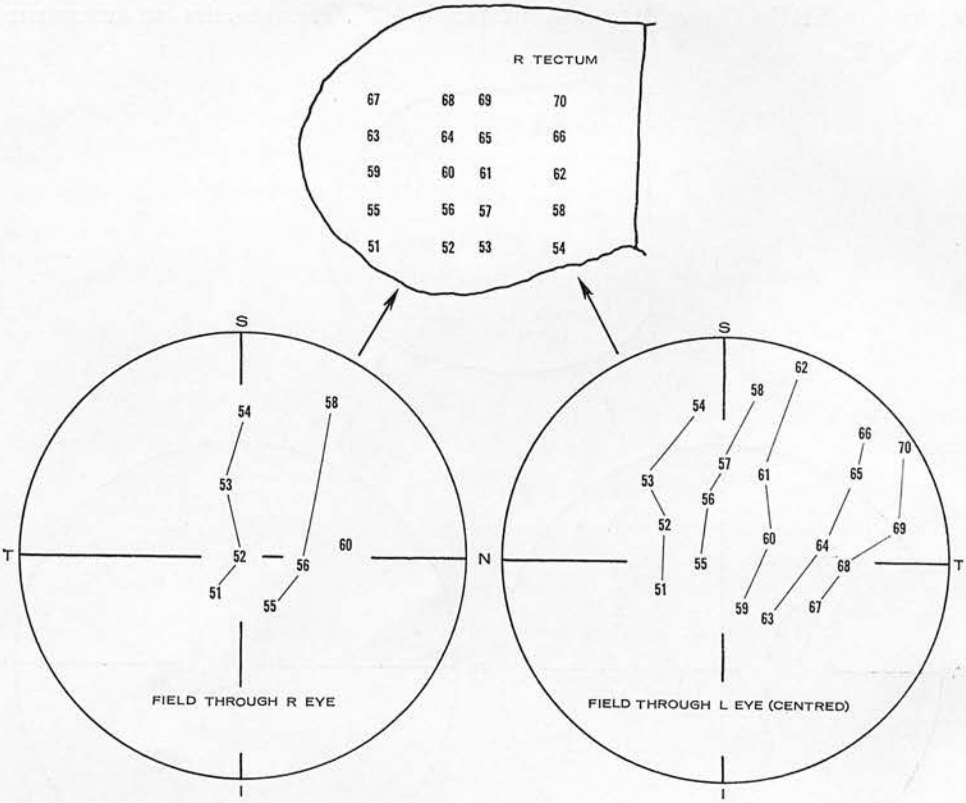

FIGURE 11. Expt. XCN 1. Visual projections to the right tectum from both eyes, mapped with the left eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

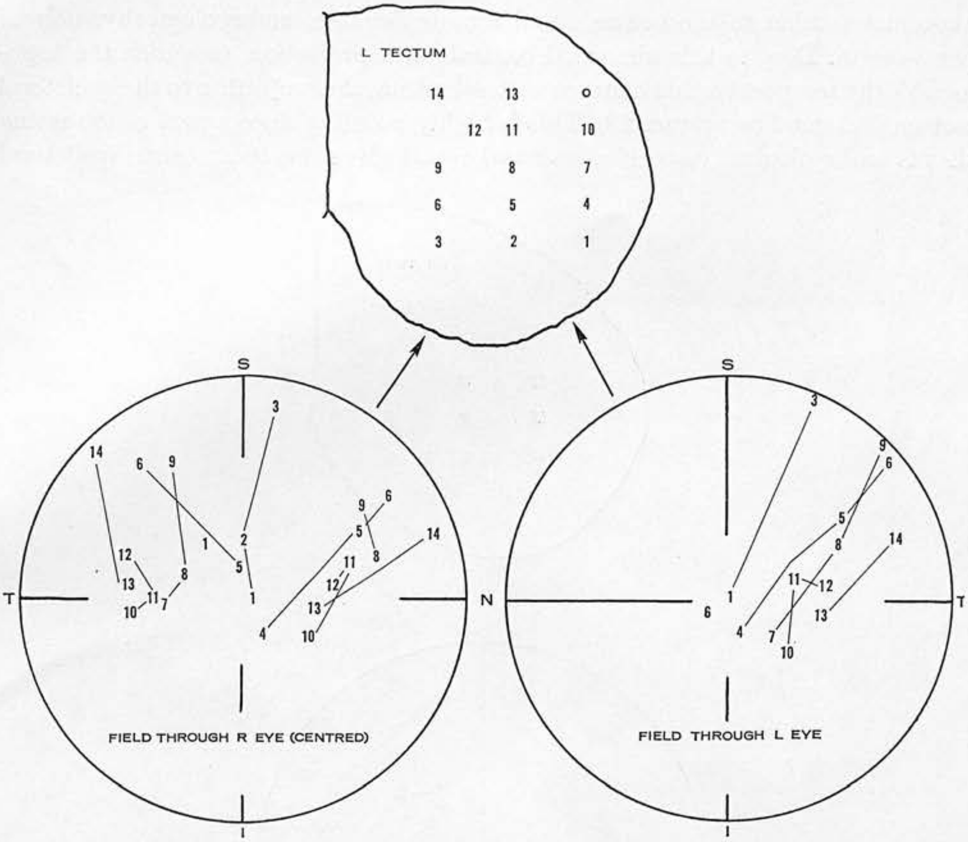

FIGURE 12. Expt. XCN 2. Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

DISCUSSION

In normal *Xenopus* the ipsilateral visual projection arises from the nasal visual field (temporal retina) and projects first to the contralateral tectum and then back, through an intertectal linkage, to the ipsilateral tectum. This is shown diagrammatically in figure 23. It may be seen that the tectal map of the retina or field in the normal ipsilateral projection is reversed along the nasotemporal axis in comparison with the contralateral projection; positions 1, 2, 3 run rostrocaudally on the contralateral tectum and caudorostrally on the ipsilateral tectum.

The results presented in this paper confirm the original observations of Gaze *et al.* (1965) that the ipsilateral projection from a double-nasal compound eye is normal. If one makes the reasonable assumption that the pathway from the compound eye to its ipsilateral tectum involves the same two stages as in the normal animal, namely initial passage to the contralateral tectum and then back to the ipsilateral tectum, then the ipsilateral projection from such an eye may be demonstrated diagrammatically as in figure 24. The topographically temporal retina projects in an abnormal fashion to the contralateral tectum because, embryologically, it is of nasal origin. Despite this abnormal contralateral projection, in which the topographically temporal retina behaves as nasal retina, the projection to the ipsilateral tectum is normal (see figure 23). This is highly puzzling since a part of the retina that is embryologically specified as nasal retina gives rise to a normal ipsilateral projection; yet in the normal animal the ipsilateral projection arises not from nasal but from temporal retina. In terms of retinal specificity one would not expect a double-nasal eye to give any ipsilateral projection at all, let alone a normal one.

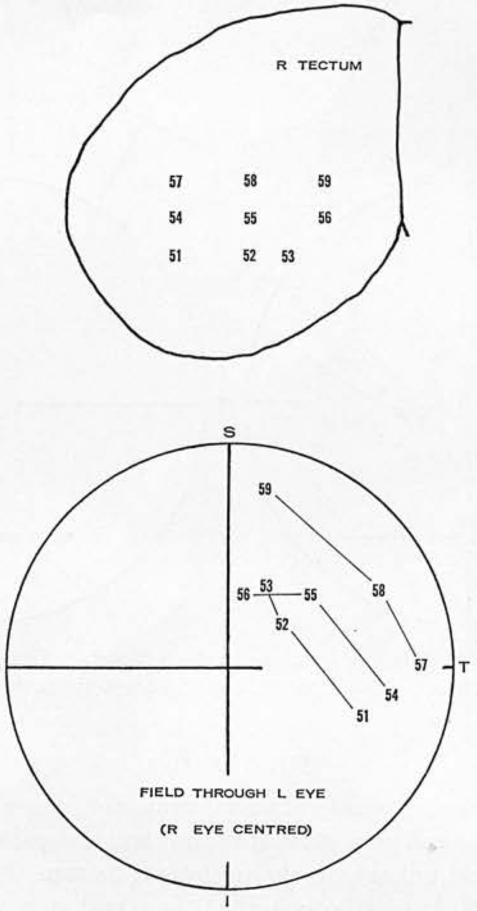

Figure 13. Expt. XCN 2. Projection from the left (normal) eye to the right tectum, mapped with the right eye centred on the perimeter. In this animal the right eye was compound (NN).

It seems therefore that a normal ipsilateral projection does not require an embryologically specified temporal retina. If this is so then perhaps the innate specificity mechanisms operate in two stages to produce the ipsilateral pathway. Thus, just as innate forces specify retinal and tectal neurons so that specific contralateral retinotectal linkages form, in the same way innate forces may perhaps specify appropriate intertectal neurons so that the most rostral neurons in the left tectum selectively connect with those more caudal in the right tectum and vice versa (figure 23).

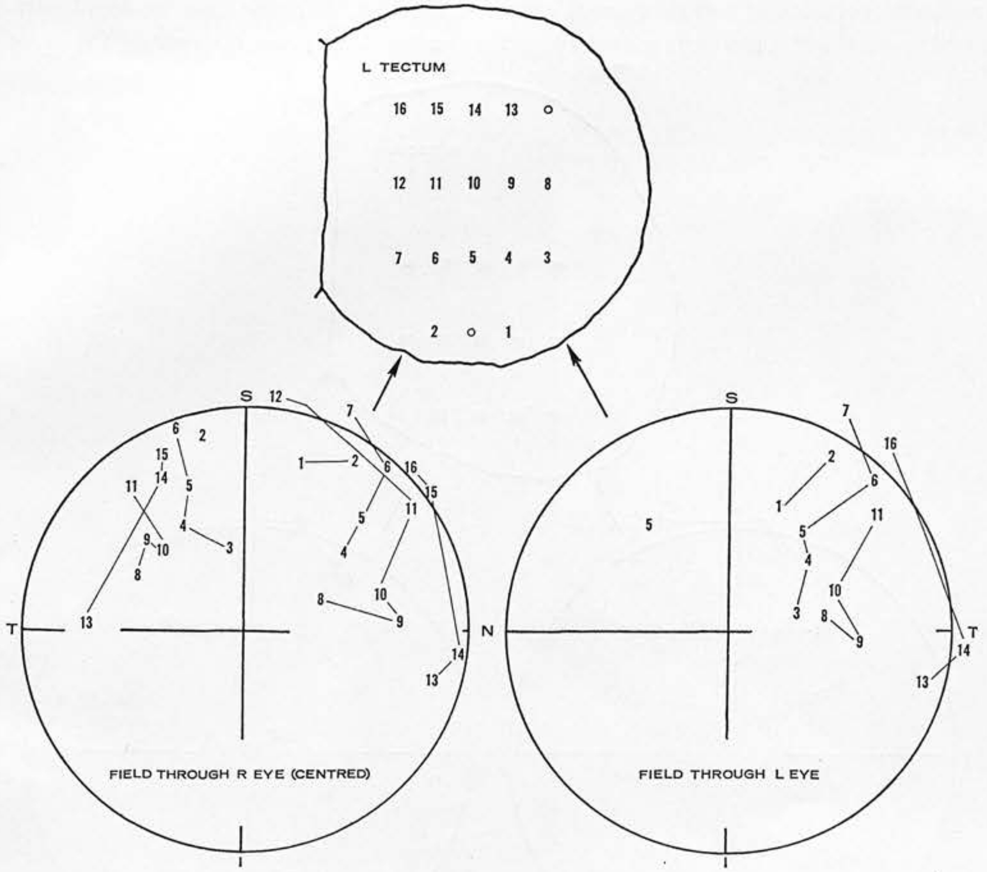

Figure 14. Expt. XCN 3. Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

If this were the mechanism operating then one would predict that the double-nasal compound eye would give an ipsilateral projection since the compound retina projects to the contralateral tectum and this input to the contralateral tectum would be transferred to the ipsilateral tectum through the specified intertectal linkages. If, however, these intertectal pathways were normally specified and of the pattern shown in figure 23, then any abnormality in the first-stage input to the contralateral tectum would, of necessity, be transferred to the ipsilateral tectum. A two-stage pathway involving first, a specified retinotectal stage and then a specified intertectal stage would produce an abnormal ipsilateral projection from a double-nasal eye. This is not found.

The ipsilateral projection actually recorded from a NN eye is illustrated diagrammatically in figure 24 and comparison of this diagram with figure 23 (the normal ipsilateral projection) shows that in the two situations the patterns of the intertectal linkages are quite different. In the case of the compound eye, the most rostral contralateral tectal neurons establish contact with the most rostral ipsilateral neurons. If a developmental specificity mechanism were operating, the most rostral contralateral neurons should connect with more caudal ipsilateral neurons (figure 23). This does not happen in animals with a double-nasal eye and this finding indicates that developmental specificity mechanisms of the type operating in the contralateral retinotectal projection are not at work in the formation of the specific neuronal connexions that constitute the intertectal pathway.

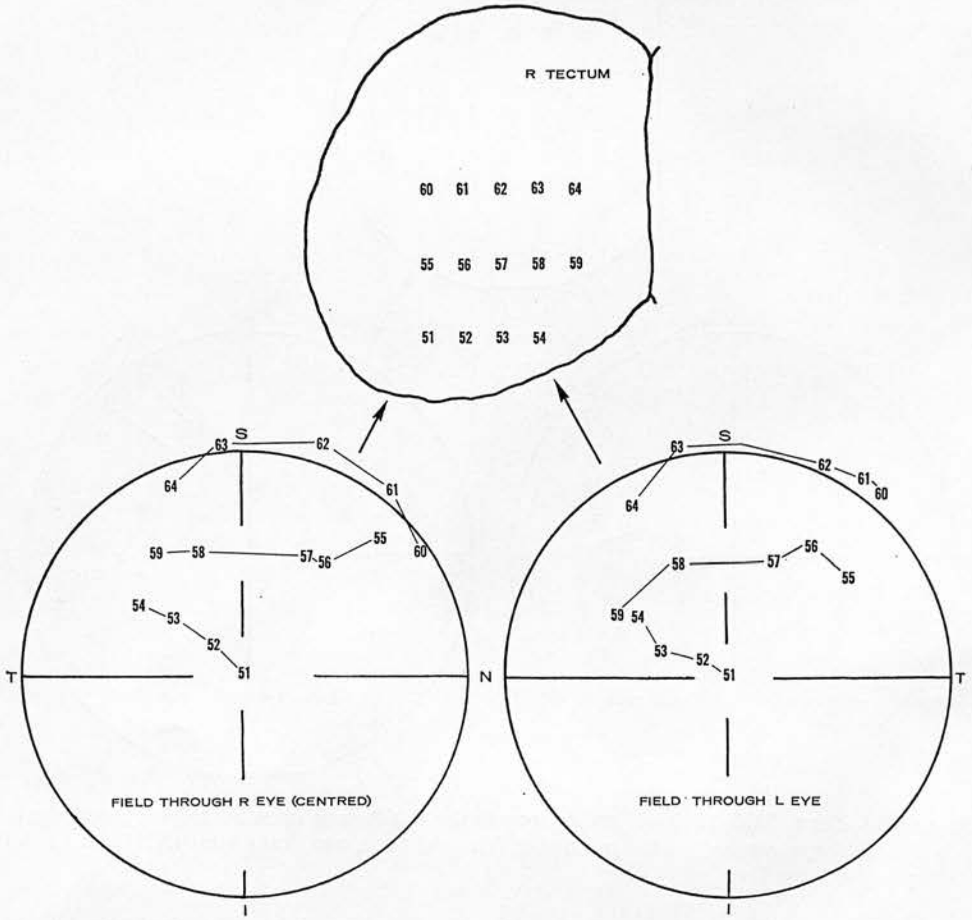

Figure 15. Expt. XCN 3. Visual projections to the right tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound (NN) and the left eye was normal.

The finding that in these animals with one double-nasal compound eye, the ipsilateral projection from the normal eye is always abnormal confirms the above conclusion. The basic abnormality in the majority of cases involves a reversal of the field along the nasotemporal axis (i.e. the field axis projecting along the rostrocaudal tectal axis) compared with the normal ipsilateral projection, and this is shown in figure 25. In this case the initial part of the projection from the normal (left) eye to the contralateral tectum is normal but the subsequent intertectal part of the pathway is abnormal. If developmental intertectal specificity mechanisms were operating, the ipsilateral projection from the normal eye should be normal; it can be seen from figure 25 that the pattern of intertectal linkages is again quite abnormal.

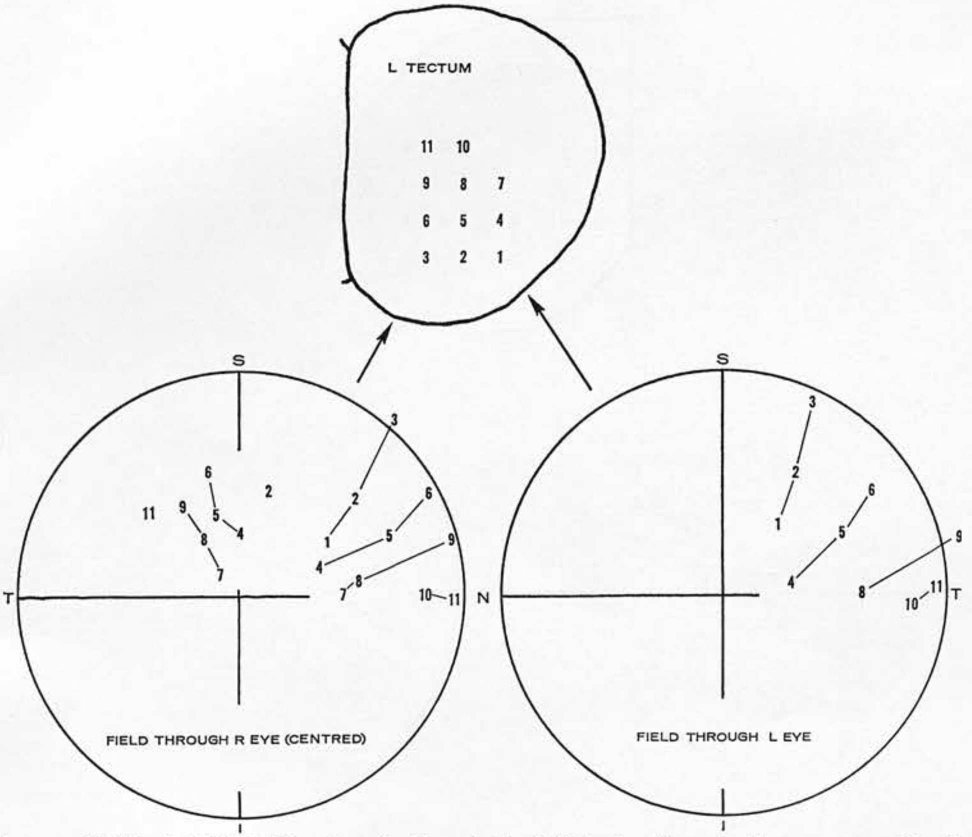

FIGURE 16. Expt. XCN 4. Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was compound and the left eye was normal.

In the animals with double-nasal compound eyes, therefore, there are two ipsilateral projections; one, from the double-nasal eye, has an abnormal initial pathway yet a normal ipsilateral projection; whereas the normal eye yields a normal initial contralateral pathway yet the ipsilateral projection is abnormal. These findings cannot be explained on the basis of a developmental specificity mechanism. It appears that mechanisms other than the well-established ones of neuronal specificity are at work in the formation of specific connexions in the ipsilateral system.

The key question seems to be why the ipsilateral projection from the normal eye is abnormal. The projections for the animals with a double-nasal compound eye are

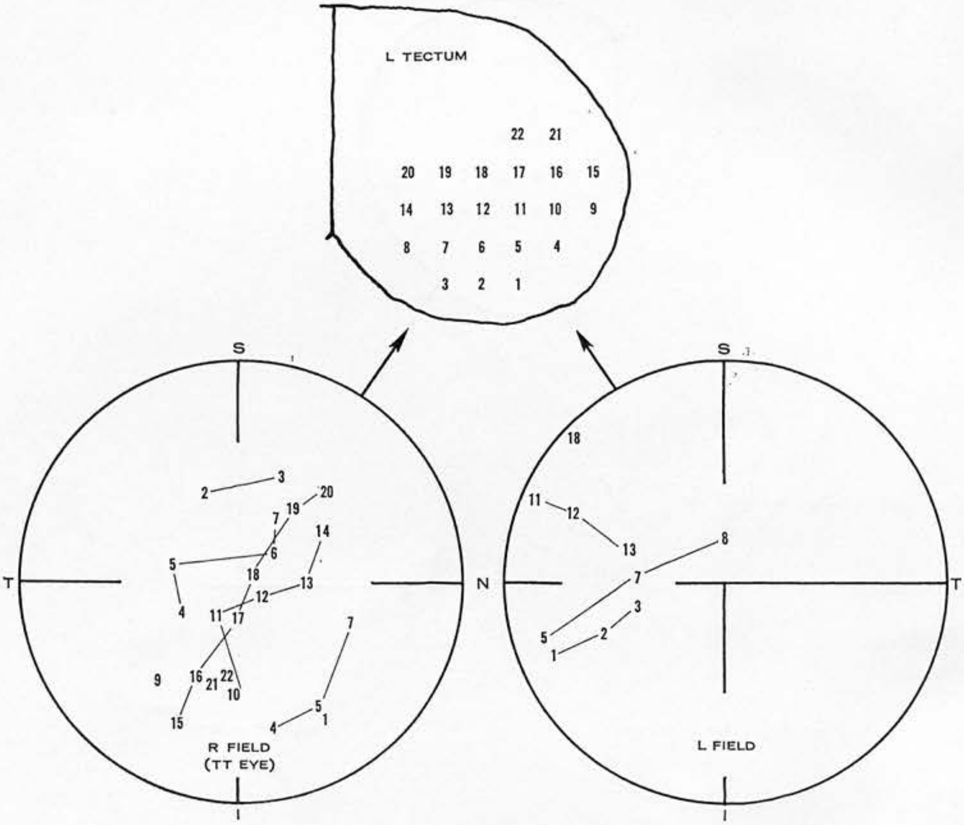

FIGURE 17. Expt. TT 12. Visual projections to the left tectum from both eyes. The right eye was compound (TT) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

summarized in table 1. It may be noted that the abnormality in the ipsilateral projection from the normal eye in animals NN 2, 3 is described in table 1 as reversal along the nasotemporal axis, whereas in the results section (p. 116) the reversal was described as occurring along an axis perpendicular to the rows of field positions. The reason for this anomaly is that we are gathering together the results from two series of experiments in one of which the ipsilateral projection from the normal eye was plotted with reference to the coordinates of the normal eye (NN 1, 2, 3, 4) whereas in the other series (XCN 1, 2, 3, 4) the ipsilateral projection from the normal eye was plotted with reference to the coordinates of the abnormal eye. In *Xenopus* the optic axis of the eye is directed upwards and outwards and hence the 'nasotemporal' planes for the two eyes are different. Because of this arrangement of the eyes, an axis perpendicular to the rows of positions in the ipsilateral map from a normal eye, when that eye is centred, would become the 'nasotemporal' field axis if the same map were plotted on the coordinates of the abnormal eye. This may be seen in figure 1. In figure 1 (*b*), top right, the ipsilateral projection from an eye with that eye centred is shown and it can be seen that the axis perpendicular to the rows of field positions is about 45° to the nasotemporal axis. In figure 1 (*a*), bottom right, the ipsilateral projection from an eye is plotted on the coordinates of the other eye and it can be seen that the axis perpendicular to the rows of field positions is now the nasotemporal axis.

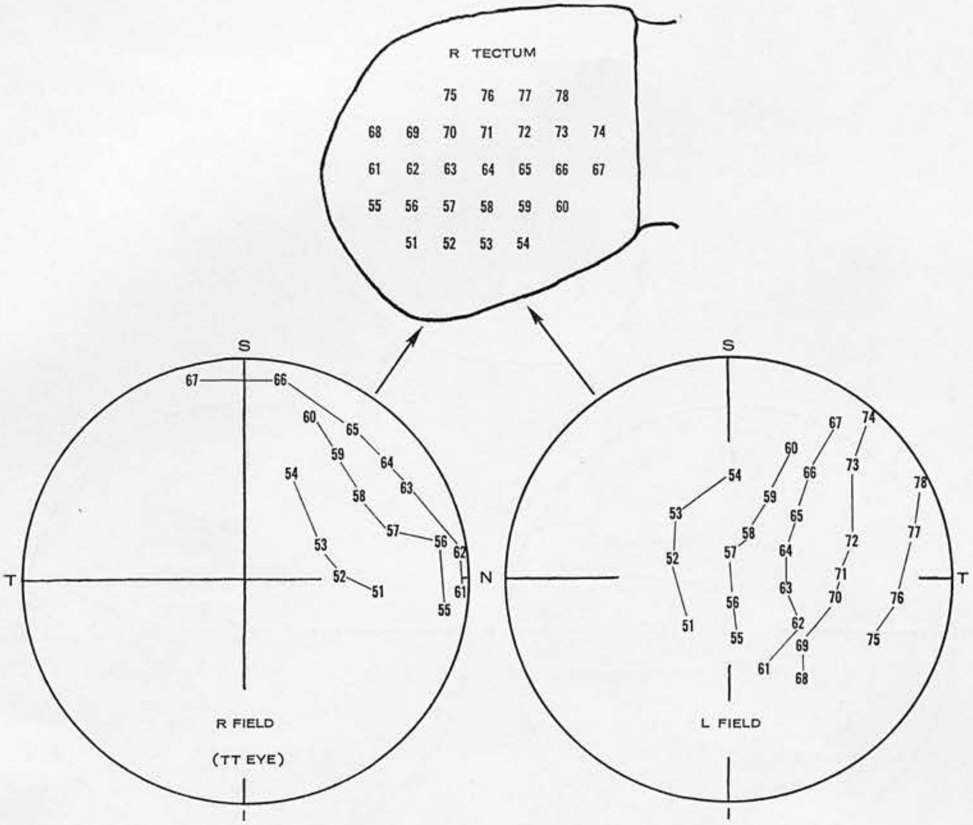

FIGURE 18. Expt. TT 12. Visual projections to the right tectum from both eyes. The right eye was compound (TT) and the left eye was normal. The right visual field was mapped with the right eye centred and the left visual field with the left eye centred.

Thus in table 1 and in the subsequent discussion the ipsilateral maps from the normal (left) eye in animals NN 2, 3 are considered to show a reversal along the nasotemporal axis, this meaning a reversal along the nasotemporal axis of the abnormal (right) eye. This brings their description into line with that used for the corresponding projections in the animals of the XCN series. Since animals NN 1, 4 did not show an axis reversal in their ipsilateral projection from the normal eye this problem does not arise in the description of these maps.

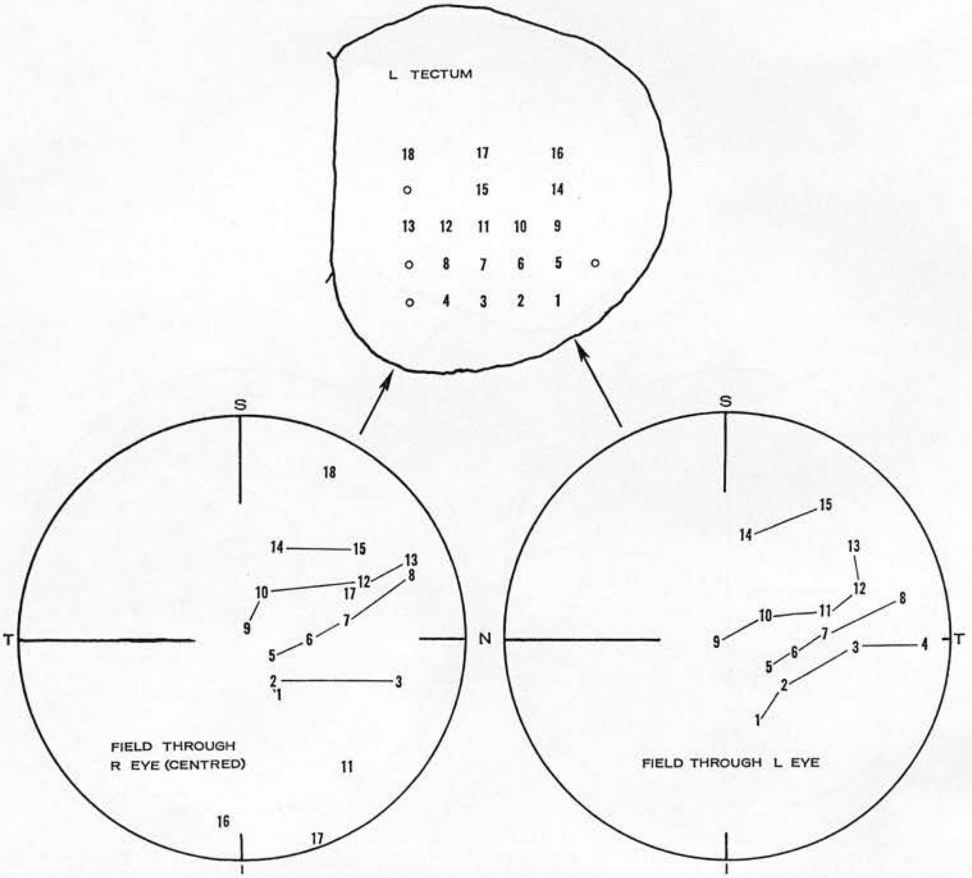

FIGURE 19. Expt. XRE 1. Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 90° clockwise, as seen from in front of the animal, and the left eye was normal.

The first point to emerge is that the ipsilateral projection from the normal eye is internally organized, not random. Some organizing mechanisms are thus at work but their nature is not immediately apparent. Since in all cases the ipsilateral projection is internally organized but abnormal, the next point to be considered is the nature of the abnormality. From table 1 the abnormalities of the ipsilateral projection from the normal eye may be summarized thus:

(*a*) reversal along the nasotemporal axis normal along the dorsoventral axis no rotation	2 animals XCN 1, XCN 3
(*b*) reversal along the nasotemporal axis normal along the dorsoventral axis rotated 45° clockwise	3 animals NN 2, NN 3, XCN 4
(*c*) reversal along the nasotemporal axis normal along the dorsoventral axis rotated 45° clockwise the projection arises only from a narrow retinal strip rather mixed up	1 animal XCN 2
(*d*) normal along the nasotemporal axis normal along the dorsoventral axis rotated 90° clockwise	2 animals NN 1, NN 4

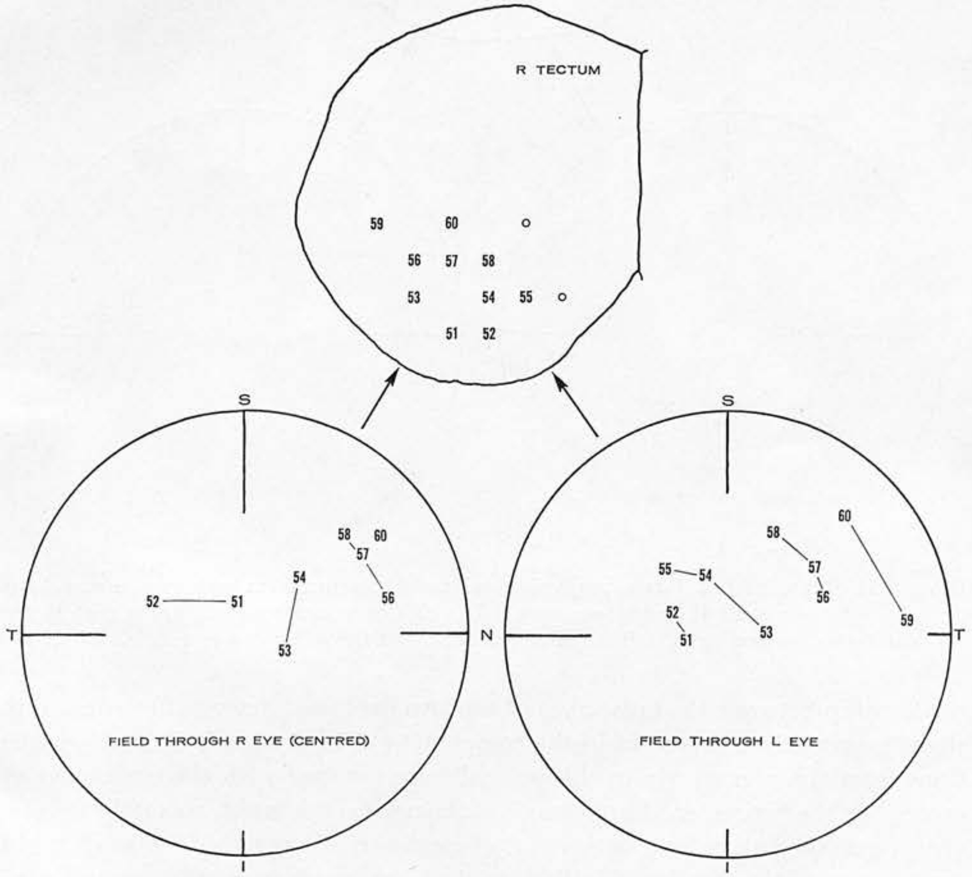

FIGURE 20. Expt. XRE 1. Visual projections to the right tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 90° clockwise, as seen from in front of the animal, and the left eye was normal.

Although some of the abnormalities are shared by several animals there is no over-all pattern to the abnormalities and it is certainly not immediately apparent that a single organizing influence has been applied to all these projections.

The contralateral projections from the normal eye were normal in all eight animals. The projection from the normal left eye in XCN 3 (figure 15) appears to be rotated 45° in an anticlockwise direction compared with normal but this is an artefact resulting from our method of presentation of the data. In this animal the relationship between the optic axes of the two eyes was abnormal because of the slightly unusual arrangement of the compound eye in the head. Since the projections from the normal eye in this animal were mapped with the compound eye centred on the perimeter, the optic axis of the normal eye is not in exactly the same position on the perimeter chart as that of most normal eyes and this is reflected in the rotation of the map by 45°. If the contralateral projection from the left eye in XCN 3 had been plotted with that normal eye centred on the perimeter it would have been found to be normal.

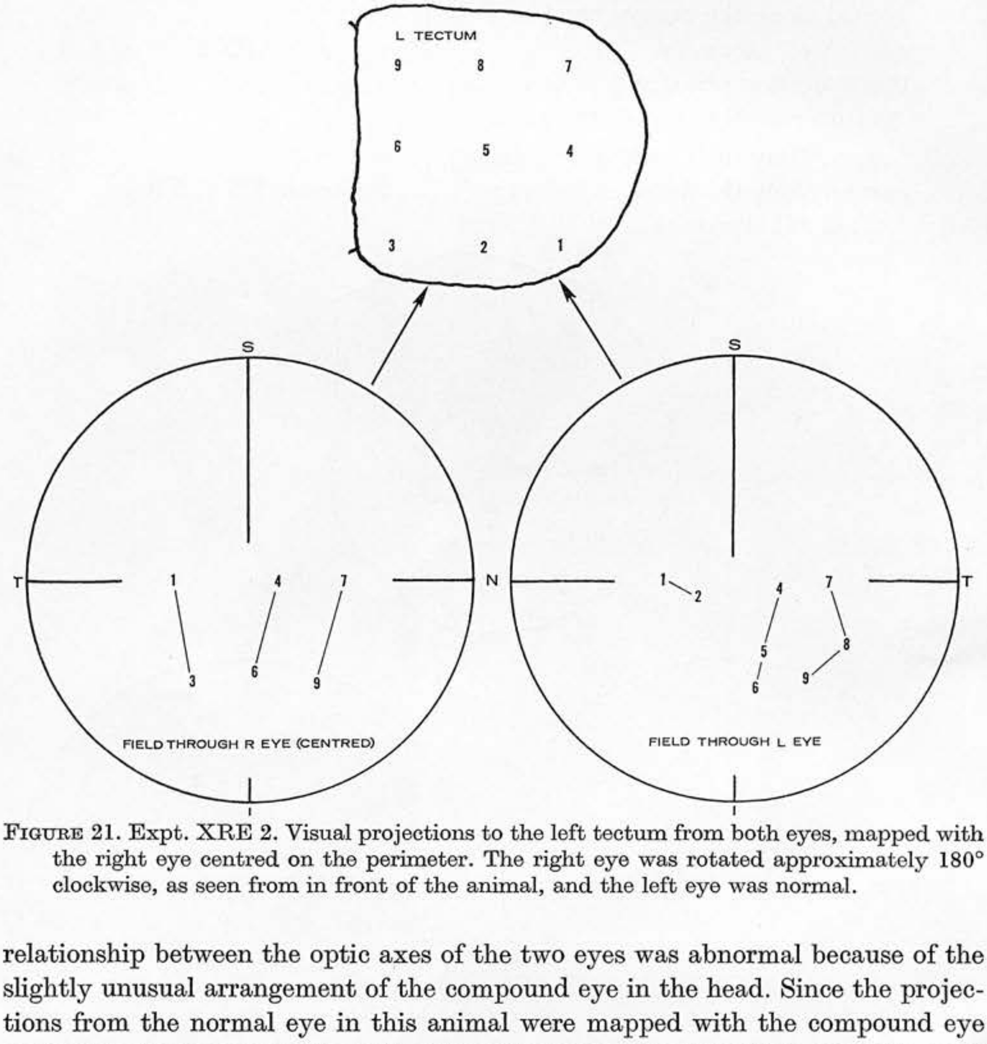

FIGURE 21. Expt. XRE 2. Visual projections to the left tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 180° clockwise, as seen from in front of the animal, and the left eye was normal.

The contralateral projections from the double-nasal eyes were, of course, abnormal (table 1), as would be expected on the basis of neuronal specificity. The nasosuperior part of the visual field is that which gives rise to the ipsilateral projection; if we examine the nasosuperior quadrant of the visual field projection through the compound eye to the contralateral tectum, and express it in terms of the normal projection from that quadrant, we obtain the following results:

(*a*) reversal along the nasotemporal axis normal along the dorsoventral axis no rotation	3 animals NN 4, XCN 1, XCN 3
(*b*) reversal along the nasotemporal axis normal along the dorsoventral axis rotated 45° clockwise	3 animals NN 2, NN 3, XCN 4
(*c*) reversal along the nasotemporal axis normal along the dorsoventral axis rotated 45° clockwise the projection arises only from a narrow retinal strip rather mixed up	1 animal XCN 2
(*d*) normal along the nasotemporal axis normal along the dorsoventral axis rotated 90° clockwise	1 animal NN 1

This analysis suggests that there is, after all, system in the abnormality of the ipsilateral projection from the normal eye. *In seven cases out of eight (the exception is NN* 4) *the abnormality in the projection ipsilaterally from the normal eye reflects the abnormality in the contralateral projection from the double-nasal eye to the same tectum. Similarly, the normality of the ipsilateral projection from the double-nasal eye to the right tectum reflects the normality in the contralateral projection from the normal eye to that tectum.*

In this context it is interesting to note that in animal XCN 3 the ipsilateral projection from the double-nasal eye (figure 15) is rotated 45° anticlockwise, reflecting the abnormality in the contralateral projection from the normal eye. Since the rotation of the field through the left eye is really an artefact, as already explained, there is no obvious reason for the ipsilateral projection from the double-nasal eye to be thus rotated since the right eye is centred on the perimenter. This finding that the ipsilateral projection from the double-nasal eye is so rotated indicates the dependence of this projection upon the contralateral projection from the other eye. Thus the ipsilateral projection from the double-nasal eye reflects the normality or otherwise of the contralateral projection from the normal eye.

It is thus possible to discern an over-all organizing influence operating in the formation of the ipsilateral visual projection. *It seems that the ipsilateral projection from one eye is dependent upon the input to the same tectum from the contralateral eye.*

The ipsilateral projection appears to require an interaction at the tectal level between the two eyes.

The next stage of the analysis involves a consideration of the type of interaction that is occurring at the tectal level. Gaze *et al.* (1965) suggested that perhaps the direct contralateral input from the double-nasal eye somehow altered the receptivity of its tectum for fibres from the other tectum. Is it possible that the ingrowing optic nerve fibres alter or 'modulate' (Weiss 1936; Sperry 1951*b*; Jacobson & Baker 1968) the tectal neurons on which they terminate, thus altering the receptivity of these neurons to the intertectal fibres? In other words, are the ingrowing optic fibres from the abnormal eye 'respecifying' the left tectum so that the ipsilateral input to the left tectum is altered appropriately?

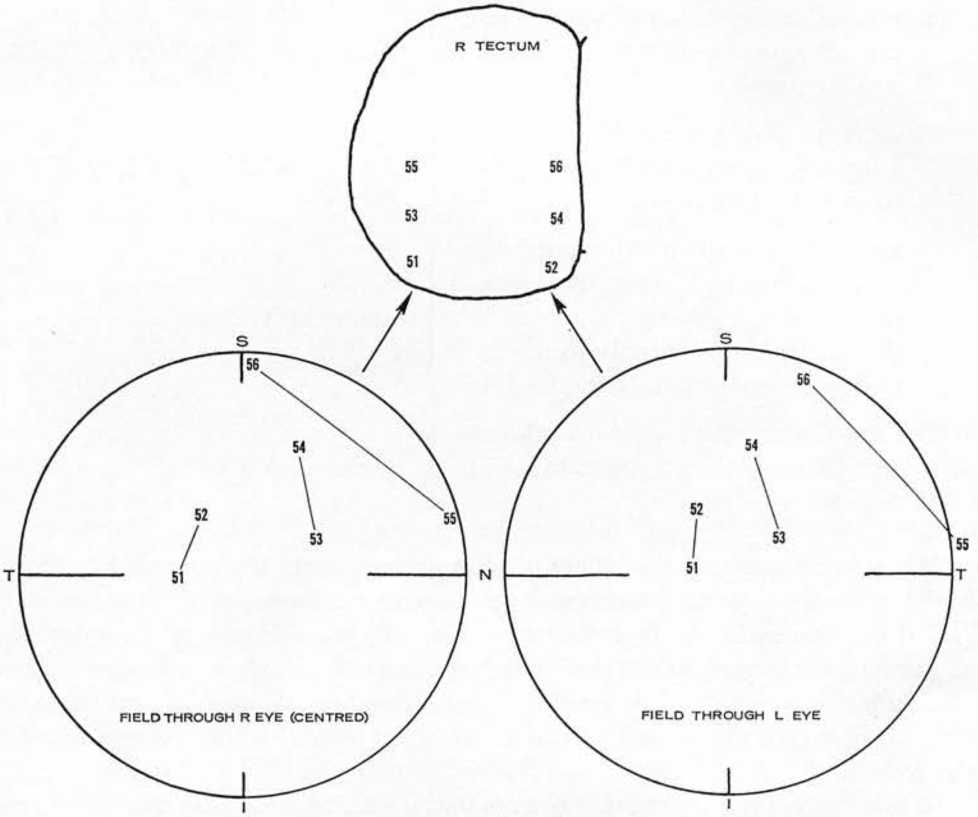

FIGURE 22. Expt. XRE 2. Visual projections to the right tectum from both eyes, mapped with the right eye centred on the perimeter. The right eye was rotated approximately 180° clockwise, as seen from in front of the animal, and the left eye was normal.

If this were the mechanism then one would predict that the ipsilateral input from the normal eye would depend upon the contralateral input and this does indeed occur. At the level of detail, however, the mechanism breaks down. If one postulates that the nasal optic nerve fibres from the double-nasal eye alter the tectal neurons on which they terminate and convert them to 'nasal' tectal neurons, then these neurons will only accept intertectal fibres that would normally end on 'nasal' tectal neurons. In the normal animal, however, the intertectal fibres go only to the rostral part of the tectum, where they connect with 'temporal' tectal neurons. The caudal 'nasal' tectal neurons do not receive an intertectal input. Thus a modulation or respecification hypothesis would predict that the normal eye should have no ipsilateral projection in these animals, rather than an abnormal one. A consideration of the results in animals with a rotated eye furnishes even more telling evidence against such a concept and this will be considered when discussing those results.

TABLE 2. VISUAL PROJECTIONS IN ANIMALS WITH ONE ROTATED EYE

		right (rotated) eye		left (normal) eye	
animal	figures	contralateral	ipsilateral	contralateral	ipsilateral
XRE 1	19, 20	rotated 90° clockwise	normal	normal	rotated 90° clockwise
XRE 2	21, 22	rotated 180°	normal	normal	rotated 180°

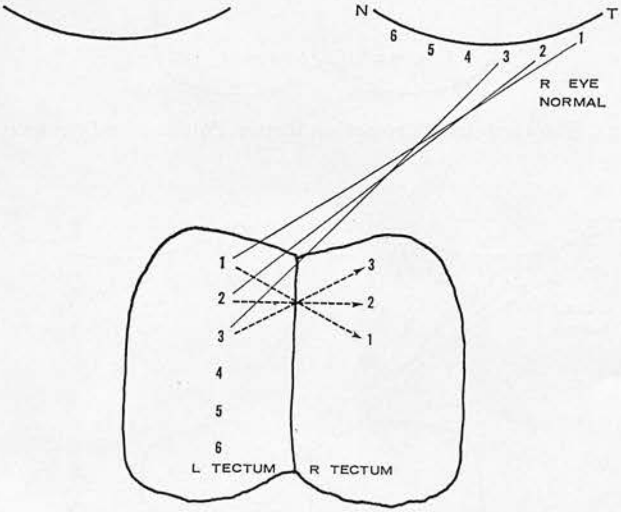

FIGURE 23. The normal visual projection to the ipsilateral tectum. The ipsilateral projection involves, first, passage of impulses from the retina to the contralateral tectum, then passage back to the ipsilateral tectum via an intertectal linkage. See text.

In figures 23 to 29 the diagrams of the retinotopic projections are idealized and simplified. In particular, in figures 23 to 28 the ipsilateral projection is shown as arising only from nasal field and extending only to rostral tectrum; whereas in fact the binocular field (and hence the ipsilateral projections) involves rather more than half the visual field of each eye. These simplifications do not invalidate the principles illustrated in the diagrams.

Since the interaction between the two eyes that appears to occur at the tectal level cannot be explained on the basis of the specificity type of the incoming optic nerve fibres, we are led to the conclusion that this interaction is independent of the prefunctional growth mechanisms of neuronal specificity. If this conclusion is valid, then an alternative form of interaction at the tectal level must be sought. Since the evidence appears to rule out prefunctional mechanisms, we must next consider the possible role of functional processes. There exists a functional visual input from

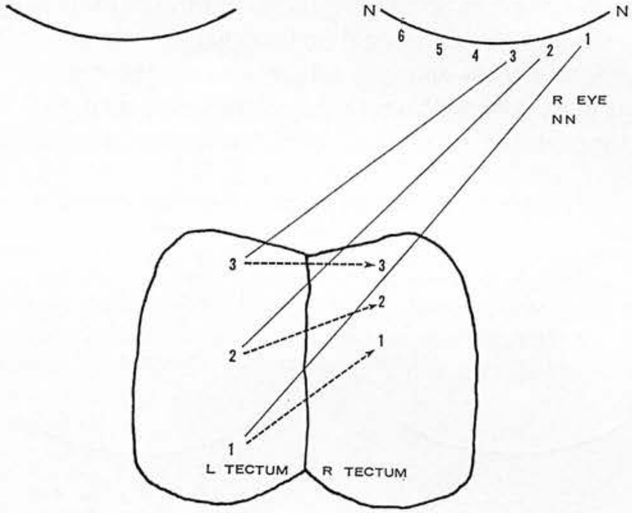

Figure 24. The ipsilateral projection from a double-nasal compound eye.

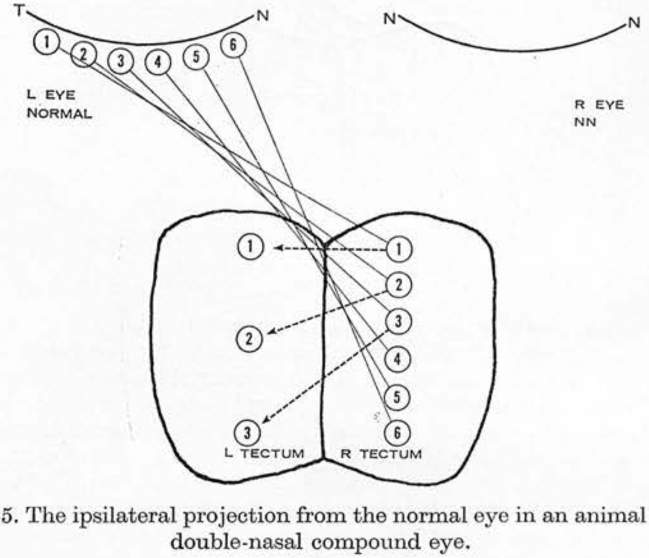

Figure 25. The ipsilateral projection from the normal eye in an animal with one double-nasal compound eye.

each eye to its contralateral tectum; perhaps it is an interaction between these two functional inputs that determines the precise topography of the intertectal stage of the ipsilateral projection.

At this point it is relevant to consider an observation made by Gaze & Jacobson (1962) in their investigation of the ipsilateral projection in the normal animal. The binocular part of the visual field (the nasal field) is seen by the temporal retinae of both eyes; and Gaze & Jacobson found that within the binocular part of the field any one point in visual space projects to each tectum through both eyes. They also observed that one point in visual space projected through both eyes to the same point on the tectum. This is illustrated experimentally in figure 1 and diagrammatically in figure 26. The point *X* stimulates the retina of the right eye at position 1 and the left eye at position ③. Position 1 of the right eye projects contralaterally to position 1 on the left tectum whereas position ③ of the left eye projects ipsilaterally to position ③ on the left tectum. Positions 1 and ③ on the left tectum are identical and thus the point *X* projects to the same point on the tectum through the two eyes. Similarly the contralateral projection from position ③ of the left eye to the right tectum and the ipsilateral projection from position 1 of the right eye to the right tectum are to the same right tectal point (it may be noted that the points on the two tecta are not necessarily symmetrical). Similar arguments apply to the tectal projections of positions *Y* and *Z* of visual space.

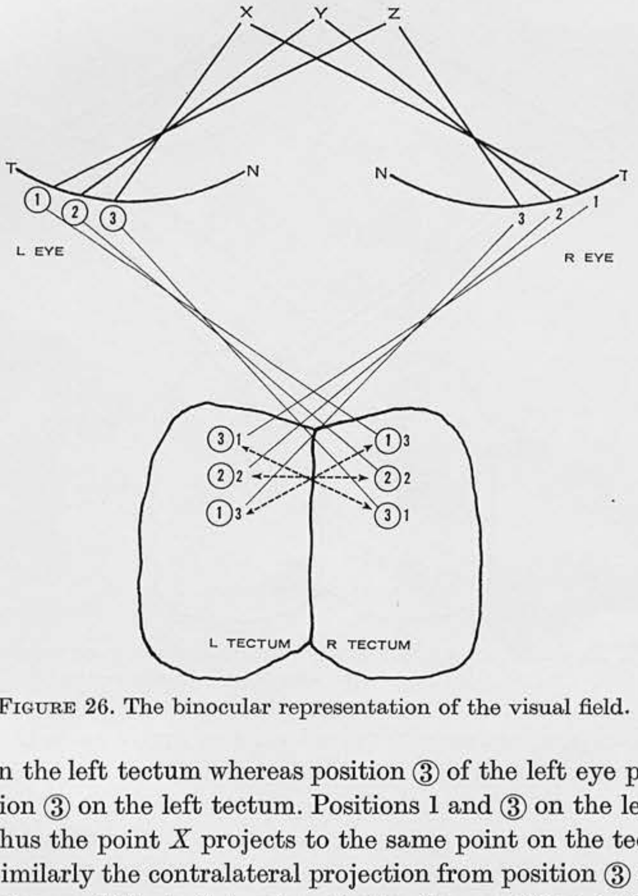

FIGURE 26. The binocular representation of the visual field.

It was formerly thought that this arrangement was the end result of innate growth processes which determined the pathways of the retinal projections involved. We have shown, however, that innate specificity mechanisms cannot explain the results obtained in animals with one double-nasal eye. It was therefore suggested (Keating 1968) that the feature outlined above, whereby the same point in visual space projects through both eyes to the same tectal point, rather than being the end-product of ontogenetic processes of neural pathway determination, may itself be the mechanism that produces the pattern of ipsilateral visual projections seen in the adult. The hypothesis advanced here is that at some stage of development the point X stimulates position 1 on the right retina and position ③ on the left retina; from these retinal positions impulses pass by the contralateral pathways to point 1 on the left tectum and point ③ on the right tectum. These two points, one on each tectum, thus receive similar spatiotemporal patterns of excitation. *The ipsilateral projection from point* 1 *of the right eye crosses to point* 1 *of the left tectum and then recrosses to that point on the right tectum (point* ③*) which is simultaneously receiving a similar spatiotemporal pattern of excitation from the same point in visual space. Points on the two tecta receiving similar excitation patterns become specifically linked.*

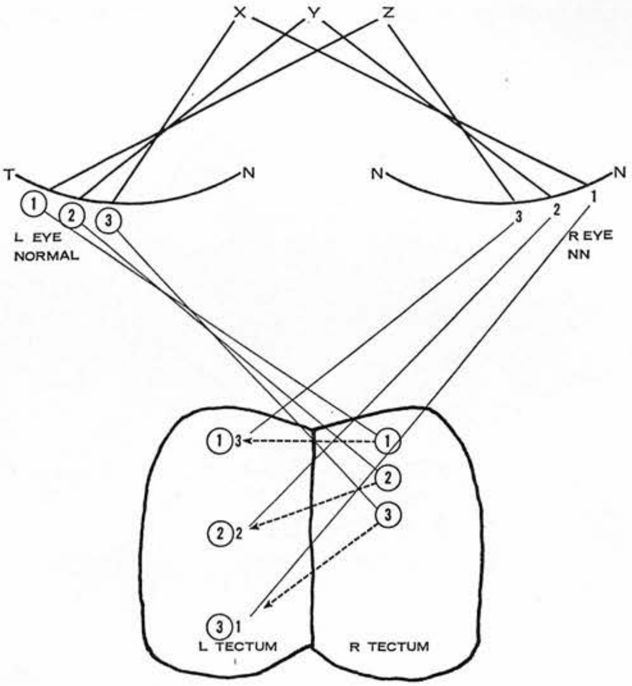

FIGURE 27. The ipsilateral projection from the normal eye in an animal with one double-nasal compound eye. The field projection.

This mechanism could explain the formation of specific connexions in the normal animal. Can it explain the projections seen in animals with a double-nasal eye? Such a mechanism would mean that, as in the normal animal, the ipsilateral pathway involves passage to the contralateral tectum and then back to that point on the ipsilateral tectum that has just been stimulated through the other eye. This system would obviously imply that if the contralateral projection from that other eye were for some reason abnormal, then the ipsilateral projection from the first eye would also be abnormal and the abnormality would be the same in both cases. This is what is seen in animals with a double-nasal eye.

Figure 27 illustrates this point. If the suggested mechanism is operating then position *X* will stimulate position ③ on the left (normal) retina which projects contralaterally to position ③ on the right tectum. At the same time position *X* will stimulate position 1 on the right retina which projects contralaterally to position 1 on the left tectum. According to hypothesis, the ipsilateral projection from position ③ of the left retina will be to position 1 of the left tectum. Similar considerations apply to stimulus positions *Y* and *Z*. Thus the hypothesis here suggested would predict that the ipsilateral projection from the normal left eye to the left tectum would reflect the abnormality in the contralateral projection from the right double-nasal eye to the left tectum. This abnormality is seen experimentally (table 1).

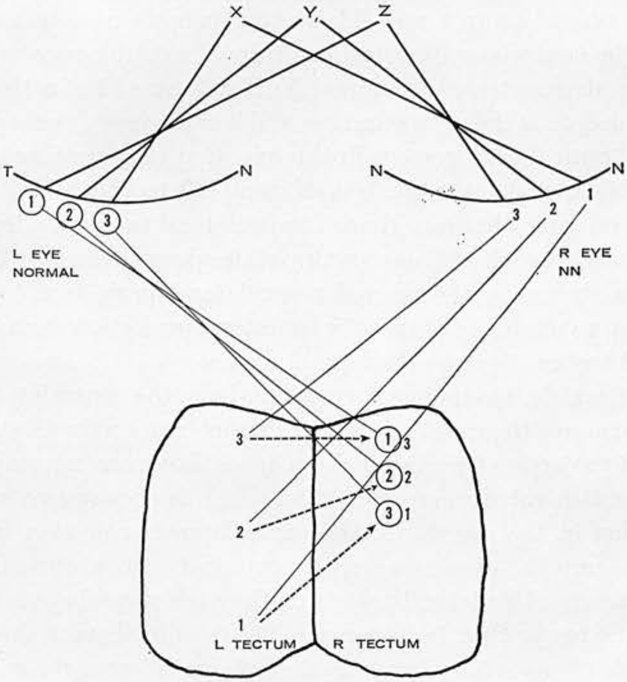

FIGURE 28. The ipsilateral projection from a double-nasal compound eye. The field projection.

Similar considerations applied to the ipsilateral projection from the double-nasal eye (figure 28) lead to the prediction that the ipsilateral projection from the double-nasal eye to the right tectum should reflect the normality of the contralateral projection from the normal eye to the right tectum. This is what occurs.

It seems, then, that the hypothesis of functional interaction between the two points on the tecta that are receiving similar spatiotemporal patterns of visual input can explain the observed relationships between the four visual projections both in normal animals and in animals with one double-nasal compound eye.

It is, of course, an essential requirement of the hypothesis that a point in visual space projects through both eyes to the same tectal points. In the new series of double-nasal *Xenopus* (XCN 1, 2, 3, 4) the mapping method was adapted to demonstrate this feature. It can be seen from the results in these animals that, to a close approximation, one point in visual space does project through both eyes to the same point on one tectum and this feature persists despite a variety of abnormalities in the contralateral projections from the double-nasal eye.

This is clearly demonstrated in animal XCN 2 (figure 12). In this *Xenopus* we observed a double lens in the compound eye and it can be seen, perhaps as a product of the abnormal optical arrangement in this eye, that only a rather narrow strip of the nasal visual field projects to the contralateral left tectum and the projection is rotated by 45° and somewhat mixed up. The ipsilateral projection from the normal eye (figure 12) reflects each of these abnormalities despite the fact that this eye is quite normal as shown by the normal contralateral projection (figure 13). Unfortunately we were unable to obtain the ipsilateral projection from the compound eye to the right tectum because the animal died.

In animals with a double-temporal compound eye the contralateral projections from the abnormal eye (figure 17) show the characteristic reduplication. The binocular nasal field projection through such an eye is, however, normally oriented on the tectum since it involves temporal retina which is occupying its normal topographical position in the eye. Since the contralateral projection from the nasal field in double-temporal eyes is normally oriented, the postulated mechanism would produce a normal ipsilateral projection from the normal eye in these animals, in marked distinction to that from normal eyes in animals with one double-nasal eye. *The ipsilateral projection from the normal eye in animals with one double-temporal eye is normal* (figure 17). In the particular example shown (TT 12) it can be seen that while the over-all contralateral projection from the nasal field is normal (figure 17), three field positions (11, 12, 13) are rotated about 90° clockwise. If one examines the ipsilateral projection from the normal eye in this animal one can see that its over-all pattern is normal but the corresponding field positions (11, 12, 13) are also rotated by 90° in a clockwise direction, as would be predicted on the hypothesis of functional interaction.

The fact that in animals with a double-temporal eye the ipsilateral projection from the compound eye is normal again accords with the hypothesis, since the contralateral projection from the normal eye is normal. Thus the hypothesis of functional interaction can explain the formation of specific connexions in the ipsilateral projection in normal animals and in animals with one double-nasal or double-temporal eye.

In animals with one rotated eye the hypothesis would predict that since the contralateral projection from the rotated eye is rotated then the ipsilateral projection from the normal eye should be rotated and to the same degree as the rotation of the abnormal eye. Similarly, since the contralateral projection from the normal eye is normal, the ipsilateral projection from the rotated eye should be normal. Examination of figures 19 to 22 and table 2 indicates that the experimental observations accord with the predictions from hypothesis.

The evidence appears to establish beyond doubt that a functional interaction between the visual inputs from the two eyes, occurring at the tectal level, operates in the establishment of specific neuronal connexions in the ipsilateral visual projection. The results from rotated eyes furnish clear proof that this interaction is not a product of developmental forces such as modulation or respecification.

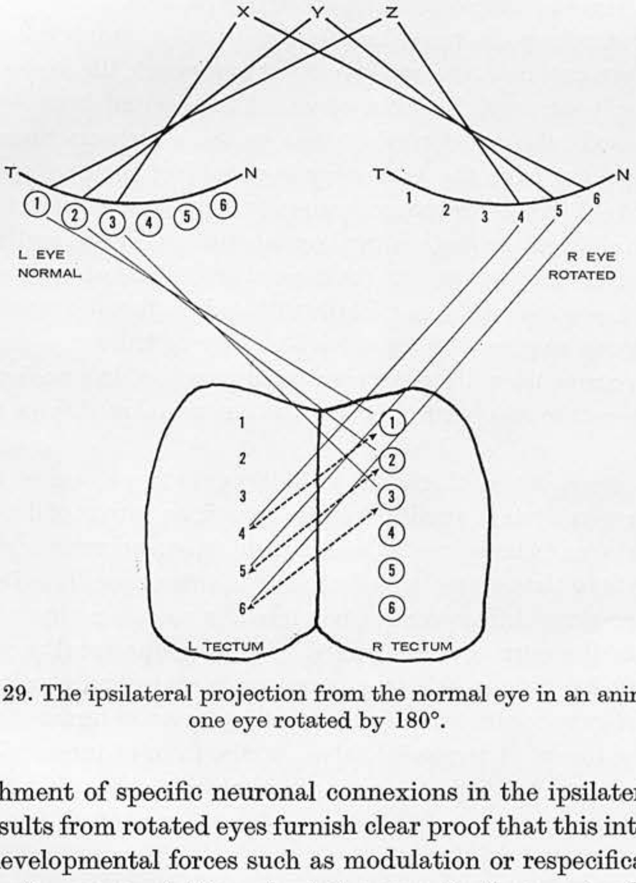

FIGURE 29. The ipsilateral projection from the normal eye in an animal with one eye rotated by 180°.

A diagrammatic representation of what occurs in these animals is shown in figure 29. A mechanism involving modulation or respecification would suggest that the intertectal fibres arrive at tectal neurons which have been appropriately specified by the incoming optic nerve fibres. In normal animals the intertectal fibres arrive at 'temporal' tectal neurons in the rostral tectum. In the animal with a 180° rotated right eye (figure 29) the rostral part of the left (contralateral) tectum still receives embryologically specified 'temporal' optic nerve fibres, even though these fibres are now stimulated by activity in the temporal visual field instead of nasal field as they would be in the normal animal. Since 'temporal' fibres arrive at the rostral left tectum, a respecification or modulation hypothesis would predict that the intertectal fibres should arrive at these neurons (positions 1, 2, 3) as they do in the normal animal. The diagram shows that this does not occur. The intertectal fibres from the normal right tectum arrive at caudal 'nasal' tectal neurons and not at rostral 'temporal' tectal neurons. Again it can be seen from figure 29 that the origin of the intertectal fibres from the left tectum to the right tectum is caudal 'nasal' tectum and this is quite different from that seen in the normal animal and inappropriate to a theory involving modulation or respecification at the time of ingrowth of the optic nerve fibres.

As a further check on the hypothesis of functional interaction a number of animals had one eye-cup removed at stage 28 of embryonic life and were allowed to develop with only one eye. No form of visual interaction between the two eyes thus occurred and one might predict that in these animals there would be no ipsilateral projection from the remaining eye. In fact such an eye does give ipsilateral responses but these responses generally come from a wide area of the visual field and the ipsilateral projection in these animals is diffuse and not the precise retinotopic projection that is seen in the normal animal. One is led to the conclusion that the growth process produces initially diffuse intertectal connexions and these diffuse connexions require the functional modifying influence envisaged in the hypothesis to narrow them down to specific connexions. In enucleate animals the initially diffuse connexions persist because the functional modifying influence never appears.

The picture emerging is, therefore, that the growth process in the developing amphibian nervous system produces initially diffuse intertectal connexions and then, at some stage of the animal's development, there occurs interaction between the visual inputs to the two tecta as described in this paper. This functional interaction transforms the diffuse connexions into the precise retinotopic connexions that are seen in the normal adult animal. The developmental period over which interaction may function in this way is presently under investigation.

Animal NN 4 gave results which we cannot interpret in terms of the present (or any other) hypothesis. It is possible that, at the time of interaction, the spatial relationships between the two eyes were different from those obtaining at the time of recording. Even on this assumption however we are not able to put forward a satisfactory explanation of this result.

Another point of discussion emerges if we consider further figure 28. From the figure we have demonstrated that the intertectal linkages from the left tectum arise from positions 3, 2, 1 on that tectum and cross to the right tectum in the manner shown, thus producing a normal ipsilateral projection from the double-nasal right eye. It should be remembered, however, that the original nasal retina also projects to the left tectum and that each of the tectal positions 3, 2, 1, in addition to receiving the input shown in the diagram, also receives an input from a mirror-image position on the original nasal retina. Thus each left tectal position receives a double input from its contralateral eye. Why are not both inputs conducted to the ipsilateral tectum so that the ipsilateral projection from the right compound eye consists of a normal projection from the nasal field and a mirror-image projection from the temporal field?

The answer to this question appears to be that, occasionally, this is what one does find (Gaze *et al.* 1963) but that in the majority of cases this is not so. In the present series of experiments, apart from an occasional reduplicated point (e.g. point 5 in the projection from the left eye to the left tectum in figure 14), the ipsilateral projection from the compound eye does not consist of two mirror-image field positions per tectal position.

It would seem that the two inputs from mirror-image retinal positions, although going to the same tectal locus, do not both excite the same neuron. Only that neuron receiving input from the nasal (binocular) field is receiving a pattern of excitation similar to that from the same stimulus via the left eye to the right tectum; thus only that neuron forms the intertectal link. Another neuron, at the same left tectal locus, receiving visual input from the mirror-image position on the retina, is excited by stimuli in the temporal (non-binocular) field. When this other neuron receives a specific pattern of excitation from a stimulus in the temporal field, it does not form an intertectal linkage because there is no similar pattern of excitation on the right tectum as the stimulus cannot be seen by the left eye.

Finally there would seem to be one other possibility to consider for the formation of the specific intertectal linkages and this involves a delayed respecification ('modulation') of the retinal ganglion cells. The original specification of the ganglion cells occurs between stages 28 and 32 and tectal specification appears to occur independently and in parallel fashion. Suppose that at some later stage, perhaps after metamorphosis, the structure surrounding the eye exert a further specifying effect on the ganglion cells, allowing the respecified 'temporal' cells to give rise to and receive intertectal connexions. In this case a rotation of the eye by 180° occurring before the final respecification could result in a normally oriented ipsilateral field projection via the rotated eye and an ipsilateral field projection rotated 180° via the normal eye, as in figure 29.

Such a process of delayed respecification of the ganglion cells could thus account for the over-all field orientations observed experimentally, There are two major objections to this respecification hypothesis, however, which seem to us to exclude it as the cause of the field-rotation effects. First, such a respecification, in order to produce the results observed, would have to operate up to and including the time when the eye reaches its final position in the body; that is, after metamorphosis. At this late stage of development, the eye is freely movable in the orbit and it seems highly unlikely that the eye muscles or other orbital structures could exert an adequate respecifying influence on the ganglion cells. Secondly, while respecification could account for the overall field orientations, it could not account for the small individual variations in the ipsilateral projections, which are found to follow closely the variations in the contralateral projection to the same tectum. Such a case as animal XCN 2 (figure 12), where the abnormality in the contralateral projection from the operated eye appears to be related to the optical abnormalities in this eye, and the ipsilateral projection via the normal eye to the same tectum reflects the same abnormalities, can not be explained in terms of late respecification of the ganglion cells of the operated eye.

While we believe that the arguments presented here indicate that delayed respecification does not account for our findings, conclusive evidence against such a mechanism does not yet exist. The crucial experiments involve rearing animals either in the dark or with controlled optical abnormalities. These experiments are in progress.

Preliminary histological observations (G.S.) indicate a binocular input to the lateral geniculate body and other diencephalic nuclei, some of which project to the tectum. It remains for future work to reveal the relationship between these diencephalic nuclei and the tectal visual inputs described in this paper.

The evidence we have put forward from experiments on double-nasal eyes, double-temporal eyes, rotated eyes and single eyes, suggests a major role for functional interaction in the formation of the retinotopic intertectal projection. The type of mechanism proposed immediately brings to mind suggestions that have been made from time to time concerning the mode of formation of conditioned reflexes (Grastyan 1967). The importance of functional interaction in the preservation of the normal binocularity of cells in the cat's visual cortex has been demonstrated by Hubel & Wiesel (Hubel & Wiesel 1963, 1965; Wiesel & Hubel 1963, 1965). These authors found that the absence of normal binocular interaction during a critical period of the postnatal life of the kitten led to a permanent loss of normal responsiveness in various cortical visual cells, The present results, however, appear to demonstrate for the first time the formation of a new, precise, spatial arrangement of fibres as a result of functional interaction between the two eyes.

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