The retinotectal projections after uncrossing the optic chiasma in *Xenopus* with one compound eye

By K. STRAZNICKY, R. M. GAZE and M. J. KEATING

From the Neurobiology Research Unit, Physiology Department, University of Edinburgh

**SUMMARY**

The nature of the retinotectal projection from a compound (NN or TT) eye in *Xenopus* raises certain problems concerning the mode of formation of connexions between the eye and the tectum. Each half of the compound eye appears to spread its connexions across the entire extent of the (apparently normal) contralateral tectum. This could indicate a certain plasticity in the way in which optic fibres can connect with the tectum. Alternatively, it is conceivable that each (similar) half of the compound eye is only able to innervate its corresponding half-tectum; in which case the uninnervated half-tectum could remain undeveloped and the innervated half-tectum could overgrow to resemble a normal tectum. This mechanism would preserve the idea of a rigidly fixed cell-to-cell specificity between retina and tectum.

In an attempt to distinguish between these two mechanisms (spreading or overgrown half-tectum) we have given each of a series of *Xenopus* embryos at stage 32 one compound eye (NN or TT). Then, shortly after metamorphosis, we uncrossed the optic chiasma and 6 months later recorded the retinotectal projections from each eye to the tecta. Thus by connecting up the normal eye to the suspect tectum, and the compound eye to the normal tectum, we used the normal side in each case to provide an indication of the degree of abnormality with which the other side was connected.

The results showed that a compound eye (NN or TT), connected to a normal tectum, gave a typical reduplicated map across the entire tectum, whereas the normal eye, when connected to the tectum which was previously innervated by the compound eye, gave an approximately normal projection across the whole of that tectum.

These results lead us to conclude that, in the *Xenopus* visual system, no strict cell-to-cell type specificity exists; rather, what is preserved throughout these experimental manoeuvres is the polarity and extent of the projection.

**INTRODUCTION**

During vertebrate neurogenesis the connexions that form between the eye and the primary visual centres (optic tectum in amphibians, fishes and birds; superior colliculus and lateral geniculate in mammals) are retinotopically organized in a highly consistent fashion. In lower vertebrates the optic nerve,
even though it is embryologically part of the central nervous system, will regenerate so as to restore neural connexion between the eye and the visual centres; and the regenerated retinotectal projection may also show a normal retinotopic organization, despite the extensive disorder induced at the site of section by the scar which results from the operation. These observations raise the question of how optic nerve fibres get to their correct destinations, both in neurogenesis and regeneration.

Sperry (1943, 1944, 1945, 1951, 1965) has proposed that this ability of the nerve fibres reflects the existence of a comprehensive system of cytochemical specifications among neurons. According to this hypothesis, which was developed with reference to amphibians and fishes, the ganglion cells of the developing retina undergo a form of differentiation which renders each cell different from all others; a comparable and matching cytodifferentiation takes place in the optic tectum and when the ingrowing optic nerve fibres reach the tectum the existence of matching cytochemical specifications on the optic axons and the tectal neurons produces specific intercellular affinities which enable appropriate synaptic linkages to form. What is postulated, then, is the specification of two spatial arrays of cells, the retina and the optic tectum, together with an intercellular recognition system. It has recently been suggested (Attardi & Sperry, 1963) that the intercellular recognition mechanism acts all the way along the optic paths (involving the optic fibres and the glial elements) and not only at the level of the tectum. This hypothesis of neuronal specificity has, in general, received support not only from the behavioural and histological experiments of Sperry and his collaborators, but also from histological studies on the developing visual system in the chick (De Long & Coulombre, 1965, 1967) and from electrophysiological observations on regenerating visual systems (see Gaze, 1970).

Sperry originally suggested (1943) that it was easiest to conceive of this retinal cellular differentiation as being orderly and continuous, so that the differences between cells lying far apart on the retina would be greater than those between cells that were closer together; and, furthermore, he suggested that the development of such a condition of cellular specification could be interpreted embryologically in terms of a polarized, field differentiation of the optic cup. Again, it was pointed out by Sperry (1945) that, to obtain a complete differential specificity of all retinal loci, the retinal field must undergo differentiation along at least two separate axes. He thought it possible that, as in the classical work of Harrison (1921) on the developing limb-bud, the anteposterior and dorsoventral axes of the retina were determined separately in the order given. This prediction about the separate times of nasotemporal (anteroposterior) and dorsoventral axial determination has since been borne out by the experiments of Székely (1954) on newts and of Jacobson (1968) on *Xenopus*.

Observations relevant to our understanding of the phenomena of place-specification and intercellular recognition in retinal and tectal elements have
Retinotectal projections in Xenopus 525

come from the study of the projections formed by 'compound eyes' in Xenopus. The surgical formation of double-nasal (NN) and double-temporal (TT) compound eyes in Xenopus embryos, and the nature of the retinotectal projections in such animals, has been described in previous papers (Gaze, Jacobson & Székely, 1963, 1965; Gaze, Keating, Székely & Beazley, 1970). It has been found that, when a compound eye is formed by uniting two nasal or two temporal half-eyes in a Xenopus embryo at developmental stage 32 (Nieuwkoop & Faber, 1956), the fibre projection from the operated eye to its contralateral tectum, when mapped electrophysiologically after metamorphosis, is such as to suggest that the fibres from each hemiretina spread out to cover the entire extent of tectum which normally receives input from the whole eye.

In normal Xenopus, fibres from the temporal half of the retina go to the rostral part of the contralateral tectum and fibres from nasal retina go to the caudal part. Yet in an animal with a NN retina, fibres from each nasal hemiretina go to virtually the whole of the contralateral tectum, and comparably for fibres from the TT retina. Thus one and the same part of the tectum seems capable of forming appropriately organized connexions with either temporal or nasal retina. In each case the order or pattern of the projection is correct with reference to the original half-eye but the projection appears to have spread out rostro-caudally on the whole tectum. These observations suggested the existence of certain previously unsuspected plastic qualities in the neural relationship between the retina and the tectum (Gaze et al. 1963).

However, as was pointed out by Sperry (1965), there is an alternative explanation for the apparent nature of the retinotectal projection from NN and TT compound eyes, one which does not require such plastic qualities; and this explanation involves agenesis of half the tectum and hypertrophy of the other half.

It is well established that normally the differentiation and continued growth of the optic tectum is dependent upon the arrival of the incoming optic nerve fibres (Kollros, 1953; Larsell, 1931; McMurray, 1954). If one eye is removed before it establishes neural connexion with the tectum, then that tectum never thereafter receives its proper input of optic fibres (since retinotectal fibres are totally crossed in the amphibian optic chiasma); and, as a result, the tectum fails to develop properly and never acquires a normal complement of cells. Biochemical maturation of the tectum has also been shown to be partly dependent on the arrival of the incoming optic fibres (Boell, Greenfield & Shen, 1955).

In view of the known dependence of the optic tectum on the proper ingrowth of optic nerve fibres for its continued development, it could be argued that, since effectively the tectum contralateral to a compound eye receives fibres only from half the retina (each half being the same in terms of quality, or cell specificity), only the corresponding half of the tectum may go on developing. And since the developing half of the tectum would receive a double complement of fibres (being supplied by two similar hemiretinae), the half-tectum could respond by
growing larger than normal; it might in fact hypertrophy until it came to resemble a normal tectum in size and shape and we would not then be able to distinguish such an overgrown half-tectum from a normal one by visual inspection or by electrophysiological mapping. We may note that this hypothesis, that there is overgrowth of the half-tectum corresponding in 'specificity' to the type of hemiretina forming the compound eye, presupposes that, at this early stage of development, there is already a rigidly specified spatial relationship between the eye and the tectum. It supposes that, if we have a half-eye, it will only innervate the appropriate half-tectum.

We have here, therefore, two quite different interpretations of the nature of the projections from NN and TT compound eyes:

1. Spreading hypothesis, wherein the fibres from each half of the retina spread out, in order appropriate to the nature of the half-retina, across the entire extent of the tectum, which is presumed to be otherwise normal. This position, involving a certain plasticity in retinotectal relations, was that adopted by Gaze et al. (1963) in the original description of compound eye projections.

2. Overgrown half-tectum hypothesis, wherein the tectum, which appears normal, is held to be a hypertrophied half-tectum corresponding in specificity to the components of the compound eye (Sperry, 1965). In this view, retinotectal relations are rigidly determined and fibres from a half-retina do not spread over a whole normal tectum.

It is important, for our understanding of the mode of control of developing innervation-patterns, that we distinguish between these two postulated modes of innervation; and it is with this problem that the present paper is concerned.

Fig. 1. Diagrams summarizing the experimental procedure. On the left before, and on the right after, uncrossing the optic chiasma. Each diagram shows the two optic tecta above and the two eyes below, seen from the dorsal aspect. Arrows from each eye indicate the optic nerves. The right eye is compound (NN) and the left eye is normal (✓). The right tectum is normal (✓) and the left tectum is suspect (?). In the right-hand diagram the hatched area represents the Millipore implant at the chiasma.
It seemed that the questions of whether the fibres from a half-retina can spread out over the whole rostrocaudal extent of a known normal tectum, and whether the tectum innervated by a compound eye is an overgrown half-tectum, should be answerable by uncrossing the optic chiasma in animals with one compound eye. In this way we should be able to cause the compound eye to innervate directly the normal tectum and the normal eye to innervate the suspect half-tectum (Fig. 1). After such an operation we would expect to obtain different results, according to whether retinotectal relationships display a certain plasticity or are rigidly determined. On the spreading hypothesis, we may expect that each half of the compound eye would project across the entire extent of the dorsal surface of the normal tectum, giving a projection identical to that of a compound eye connected to its contralateral tectum; and we should also expect that the normal eye would project in a normal fashion to the tectum which had previously been innervated by the compound eye.

On the other hand, according to the overgrown half-tectum hypothesis, we should expect that each half of the compound eye would give a projection which was confined to the appropriate half of the dorsal surface of the normal tectum; whereas the whole of the dorsal surface of the tectum previously innervated by the compound eye should show a projection only from that half of the normal eye which corresponds in type to the components of the compound eye. This is because the tectum originally innervated by the compound eye is, by hypothesis, a half-tectum in terms of its affinities for retinal connections.

The present experiments give a clear answer to one of the questions as posed. We wanted to know whether each half of a compound eye (NN or TT) could spread its connections in an orderly fashion over the entire dorsal surface of a normal tectum, and the answer is that it can. The second question, whether the tectum originally innervated by a compound eye is to be considered as an overgrown half-tectum in terms of cell specificities, is more difficult to answer unequivocally; but by demonstrating that compound eye connexions do spread over a normal tectum, we have removed the main reason for postulating that the tectum originally innervated by a compound eye should be an overgrown half-tectum.

Possibly the most useful outcome of these experiments is that consideration of the results requires us to attempt more precisely to define what we mean by the terms retina, tectum, matching, and indeed, the whole concept of neural specificity (to say nothing of the terms embryo, field and regulation). These matters we argue extensively in the discussion.

**METHODS**

Embryos of *Xenopus laevis* were operated on at stage 32 (Nieuwkoop & Faber, 1956) so as to produce an NN or a TT eye on one side. The operation has been described in previous papers (Gaze et al. 1963, 1965). The animals
were reared beyond metamorphosis, one month after which the optic chiasma was uncrossed. We had initially hoped that this could be done, in effect, by merely cutting the optic nerve on one side, near the chiasma. In *Rana* this operation gives rise, some months later, to the appearance of 'pattern 4' tectal reinnervation (Gaze & Jacobson, 1963; Gaze & Keating, 1970) in which the operated optic nerve comes to innervate both tecta with retinotectal fibres, rather than just the contralateral tectum as in a normal animal. However in *Xenopus* we were unable to achieve this result. Eventually we were successful following the implantation of a small piece of Millipore filter into the optic chiasma, at open operation through the roof of the mouth. This operation cuts the chiasmatic fibres and the implanted Millipore filter acts as a barrier to the regenerating fibres from both eyes and induces each eye to innervate its ipsilateral tectum (Fig. 2).

The operation of uncrossing the chiasma in this fashion was performed in 23 *Xenopus* shortly after metamorphosis. The animals were then kept for a further 6 months, to allow the optic nerves to regenerate, and the animals were then used for electrophysiological mapping of the retinotectal projections. The technique of doing this has been described in previous papers (Gaze *et al.* 1963, 1965; Gaze & Jacobson, 1963; Gaze *et al.* 1970).
RESULTS

Twenty-three animals, each with one compound eye, either NN or TT, were set up for electrophysiological mapping of the retinotectal projections. In seven animals we failed to obtain electrophysiological confirmation of the success of the original embryonic operation and we do not consider further these results. In the remaining 16 animals (9 NN; 7 TT) the projections were mapped in sufficient detail and the results are here described.

These animals showed, in greater or lesser degree of completeness, the following phenomena: the compound eye, now innervating the tectum which had previously been innervated by the normal eye, gave a typical compound projection across the tectum, with reduplicated field positions for each tectal position; and the normal eye, now innervating the suspect tectum which had previously been innervated by the compound eye, gave an approximately normal field projection across that tectum (Fig. 3).

The compound eye projection to the normal right tectum extended over the
complete rostrocaudal extent of the tectum in 14 animals (9 NN; 5 TT). The remaining two animals, both with one TT eye, did not show an ordered projection from the compound eye restricted to one or the other half of the normal tectum; in both cases the regeneration of the nerve from the compound eye

![Diagram of retinotectal projections](image)

**Fig. 4.** Retinotectal projections in an animal with a right TT compound eye and an uncrossed optic chiasma. Animal ChTT20. In this animal the left (normal) eye succeeded in regenerating fibres back to both optic tecta and a separate perimetric chart is given for the field projection from this eye to each tectum.
Retinotectal projections in Xenopus appeared to be incomplete, in that only a few points in the visual field gave a tectal projection (Fig. 4).

The projection from the normal eye to the suspect left tectum in 11 cases included virtually the entire extent of the dorsal visual field – which is what a normal projection to a normal tectum should do. In our experimental animals the suspect left tectum was shown to be able to receive a full-field coverage from the normal eye, regardless of whether the tectum had previously been innervated by an NN or a TT eye. Figs. 3, 5 and 6 show such results where the eye previously innervating the suspect tectum was NN. Comparable results from animals with a TT eye are shown in Figs. 4, 7 and 8.

In the remaining five cases the entire surface of the suspect left tectum was covered with an ordered projection coming largely from the temporal visual field (Figs. 9, 10). These five animals included four with an NN eye and one with a TT eye. The restriction of the projection in these cases to the temporal field was thus not linked exclusively to the presence of one type of compound eye.

In many animals of the present experimental series we have noticed that the projection from the normal eye to its ipsilateral tectum is somewhat distorted in
that the lines of field positions frequently slant in a temporo-superior direction in the field (instead of running vertically as in normal animals). This phenomenon was most marked in those five animals in which the projection was largely restricted to the temporal field (Figs. 9, 10), but could also be seen in cases where the field coverage from the normal eye was more complete (Figs. 3, 5, 6 and 11). Both the temporal-field predominance and the tendency for rows of stimulus positions to deviate from the vertical may perhaps reflect a systematic error in the way we set the animals up on the perimeter.

It may be seen from Fig. 9 that in this animal the projection from the NN eye is 'abnormal' in that the field positions comprise two sets which, while they are correctly ordered in the radial direction in the field, are rather badly mixed-up circumferentially. This phenomenon, which has also been seen with TT projections (Fig. 11), is reminiscent of the 'pattern 2' projections described by Gaze & Jacobson (1963) in regenerating frog visual projections. In the present cases, each half of the compound retina gives a projection pattern where the projection order is correct along the lateromedial axis of the tectum but mixed-up along the rostrocaudal axis.
Retinotectal projections in Xenopus

It may be seen that many of these visual maps show abnormalities in addition to those described above. In this connexion it is worth emphasizing three points: first, compound eyes are themselves essentially abnormal; secondly, we have

Fig. 7. Retinotectal projections in an animal with a right TT compound eye and an uncrossed optic chiasma. Animal ChTT23. In this animal the right (compound) eye succeeded in regenerating fibres back to both tecta and a separate perimetric chart is given for the field projection from this eye to each tectum.
found that TT eyes tend to be more abnormal than NN eyes; and thirdly, the projections we describe in this paper have additional abnormalities resulting from the section and regeneration of the optic nerves (Gaze & Jacobson, 1963).

In five animals the implanted Millipore filter turned out not to be an effective barrier against regrowth of optic fibres to their contralateral tectum. In such cases, of which Figs. 4, 7 and 10 are examples, regenerating fibres from one eye (in two cases the compound eye; in four cases the normal eye; in one case both eyes) formed two maps, one on each tectum. In the experiment illustrated in

Fig. 8. Retinotectal projections in an animal with a right TT compound eye and an uncrossed optic chiasma. Animal ChTT16.

Fig. 7 the TT eye directly innervated both tecta and the normal eye innervated its ipsilateral tectum. Both compound eye projections may be seen to be very similar to one another and both the compound eye and the normal eye project together across the same extent of the same (left) tectum. In Fig. 4 it is the normal eye which projects in similar fashion to both tecta. The animal in which both eyes project to both tecta is illustrated in Fig. 10. Thus the normal and the suspect tectum are equivalent in that each can form a closely similar projection from one eye, be it compound or normal; and each half of a compound eye is equivalent to a normal eye in that both the compound and the normal eye can project together to the one tectal area.
DISCUSSION

These experiments were undertaken to enable us to distinguish between the 'spreading' hypothesis and the 'overgrown half-tectum' hypothesis. A previous attempt to distinguish experimentally between these hypotheses by measurements of the retinotectal 'magnification factors' had led to results which were inconclusive (Gaze et al. 1965). In the event, it turns out that the present results are also inconclusive in that they fail to distinguish between alternative versions of the 'spreading' hypothesis; but even so they require us to re-evaluate our earlier concepts of neuronal specificity.

Fig. 9. Retinotectal projections in an animal with a right NN compound eye and an uncrossed optic chiasma. Animal ChNN1. See text.

For the sake of our discussion, the general results of the present experiments can be summarized in the following way: the compound eye spreads the connexion from each (similar) hemiretina over the entire normal tectum, no matter whether the compound eye is NN or TT; and, furthermore, the normal eye, which in its projection to its own normal tectum would give a full normal projection, also does so when forced to innervate the compound eye tectum. These results clearly accord with those expected on the basis of the 'spreading'
hypothesis and not with those predicted by the ‘overgrown half-tectum’ hypothesis.

The present experiments do not prove that the tectum previously innervated by the compound eye is a normal tectum. If, however, this tectum were to be an

Fig. 10. Retinotectal projections in an animal with a right NN compound eye and an uncrossed optic chiasma. In this animal both the right (compound) eye and the left (normal) eye succeeded in regenerating fibres back to both optic tecta and a separate perimetric chart is given for the field projection from each eye to each tectum. Animal ChNN3.
overgrown half-tectum, the finding that it can accept a full-field projection from a normal eye would lead us to the same conclusions as those that we reach when we consider the implications of the compound eye projection spreading over a whole normal (right) tectum.

Fig. 11. Retinotectal projections in an animal with a right TT compound eye and an uncrossed optic chiasma. Animal ChTT18.

Our experimental results thus make it quite obvious that the fibres from each hemiretina of a compound eye can spread over a whole normal tectum; or at least over a tectum that can simultaneously be receiving a complete projection from a normal eye. The nature of this spreading is not at all clear, however, and an analysis of the present results has led us to the retrospective realization that the spreading phenomenon can itself be the result of two entirely different mechanisms.

The difference between the two versions of the spreading hypothesis depends on the view we take of the nature of each hemiretina in the compound eye. Thus, just as Sperry (1965), in an attempt to account for the apparent spreading, called into question the nature of the tectum to which the compound eye projected, so we must now call into question the nature of each hemiretina of the compound eye.
Spreading mechanism I: retinal regulation

The operation to produce a compound eye is performed at a very early developmental stage and it is conceivable that the cells in each half of the developing eye undergo a form of embryonic pattern regulation. During development the normal eye connects up in such a fashion that the nasal extremity of the retina sends fibres to the caudal extremity of the tectum while the temporal extremity of the retina connects with the rostral extremity of the tectum. If we now halve the embryonic eye down the vertical midline (as in a stage-32 *Xenopus* when making compound eyes) and remove the temporal half, we are left with a half-eye that extends from the nasal extremity to the (old) midline; but the midline is now the temporal extremity of this half-eye and, in the compound eye situation, this part of the eye in fact behaves as if it were the normal temporal extremity; that is, it connects with most rostral tectum, and correspondingly for the transplanted half of the compound eye.

We could thus assume that the specificity-characteristics (in Sperry's sense) of the ganglion cells near the divided medial edge of each half-eye had undergone a form of pattern-regulation (although not size- or shape-regulation) and taken on the specificity-characteristics of the normal missing end of the eye. Thus instead of ganglion cells which are specified for the rostral end of the tectum lying at the temporal extremity of the normal eye, they will now lie at the temporal extremity of the half-eye, i.e. at the midline; likewise for the transplanted half-eye. Such a compound eye would thus comprise, in terms of neuronal specificities, two 'normal' eyes stuck together in this somewhat unusual geometric arrangement.

If retinal regulation of this sort were to occur following the operation to produce a compound eye, then the results obtained in the present experiments after uncrossing the optic chiasma are what we would expect on the basis of a Sperry-type neuronal specificity mechanism. After uncrossing the chiasma we should expect that each half of the compound eye, since each is a whole eye in terms of specificities, would connect across the entire extent of the ipsilateral (normal) tectum; thus there should occur a reduplicated field projection of the sort one normally finds when a compound eye innervates its proper tectum. And conversely, we should expect the normal eye to make a normal map across its own ipsilateral tectum, which was formerly connected to the compound eye. From this analysis it can be seen that the results of uncrossing the optic chiasma in animals with one compound eye could be explained on the assumption that retinal regulation has occurred and that the compound eye consists of two small eyes put together with opposite nasotemporal polarities.

Spreading mechanism II: plasticity of connexions

An alternative explanation of the results of the present experiments is available if we postulate that retinal regulation does not occur and that the ganglion cells
of each hemiretina in the compound eye retain their original ‘place-labelling’. In this case, the results of uncrossing the chiasma demonstrate that the optic axons from a half-eye can establish connexions across a whole normal tectum. This would imply that the intercellular recognition mechanism between optic nerve terminals and tectal elements is more modifiable than was previously thought. Thus the tectal connexions of an optic nerve fibre may not be determined solely by the location in the retinal field of its ganglion cell, but may be governed by more catholic rules dependent upon the nature of the total retinal and total tectal systems available to be matched.

It is not possible, on the basis of the results reported in the present paper, to distinguish conclusively between the two alternative mechanisms of spreading; retinal regulation or plasticity of connexions. We believe, however, that the trend of the evidence supports the mechanism of connexion-plasticity rather than that of retinal regulation. While the idea of retinal regulation in compound eyes is attractive, there are certain objections to accepting regulation as accounting for the results of uncrossing the chiasma. First, there are other experimental situations (projections from half-eyes, Straznicky, Gaze & Keating, in preparation; projections from double-ventral compound eyes, Straznicky, Keating & Gaze, in preparation) where we apparently do not find regulation following operation at comparable larval stages. Secondly, on the assumption of retinal regulation, the mode of growth of the eye requires that the regulative phenomenon continues to manifest itself until after metamorphosis.

Straznicky & Gaze (1971) have shown that in normal Xenopus retinal growth occurs throughout larval life and metamorphosis, continuing until some unknown time after metamorphosis. The retina grows at its edges, not in the centre. Cells are continuously being added at the retinal ciliary margin. The oldest retinal cells are therefore those in the middle of the retina, and these oldest cells normally possess the place-labelling appropriate to middle retinal cells. The retinal cells which in the adult occupy the nasal and temporal poles of the eye and possess the most marked degrees of nasal and temporal place-labelling, are the youngest retinal cells.

The concept that each hemiretina of the compound eye regulates its ‘specificity-structure’ requires that the cells in the middle of the eye acquire throughout larval life ever increasing degrees of nasal or temporal (depending on the nature of the compound eye) ‘specificity’. However Feldman & Gaze (in preparation) have shown, using autoradiographic techniques, that the compound eye, like the normal eye, grows by the addition of cells at the ciliary margin. The cells at the centre of the compound eye are, therefore, the oldest cells in the retina. If retinal regulation is occurring these cells must throughout larval life be continuously changing their ‘specificity name’ as the eye grows, from that of middle retinal to that of either most temporal retinal cells (in the case of an NN eye) or most nasal retinal cells (in the case of a TT eye). In the developing normal eye this does not have to happen, since as the retina adds the cells at its edges,
these new cells may be considered to acquire the new specificities appropriate to their situations. But in the compound (as in the normal) eye, the cells at the centre of the retina are probably the original ganglion cells dating from the beginning of eye development. Thus, since the cells do not disappear and become replaced by new ones bearing the needed new specificities, we have to consider that the same cell changes its specificities as the eye increases in size, and this process would have to continue until after metamorphosis. Regulative phenomena, of the type we are considering, customarily operate only in early embryonic life; and the requirement that in the case of the compound eye retina such regulation would have to continue into the adult life of the animal argues against acceptance of the idea that the retina is regulating its specificity-structure. It must also be pointed out that in the case of the compound eyes, the polarity of the specificity-structure does not regulate; thus in the transplanted hemiretina the nasotemporal polarity is reversed by the embryonic operation and it remains reversed into adult life.

For these reasons we regard the retinal regulation version of the spreading hypothesis as unlikely. We therefore consider the alternative spreading mechanism, connection-plasticity, as the more likely explanation of our results. If the retina is not regulating its specificity-structure, then the middle retinal cells, at the junction of the two hemiretinae, possess the ‘place specificity’ normally acquired by cells in the middle of the retinal field. In the case of a double-nasal compound eye, these middle retinal cells connect selectively with the rostral pole of the tectum, whereas in the case of a TT compound eye the middle retinal cells project selectively to the caudal pole of the tectum. It would appear that the retinal ‘place specificity’ of the ganglion cell does not irrevocably determine the tectal site with which that ganglion cell will connect.

This conclusion, if correct, requires a modification of Sperry’s theory of specificity. In Sperry’s view the specification of the retina resulted, not only in the acquisition by a retinal ganglion cell of a particular retinal ‘place specificity’ but also, because of the rigid rules governing retinotectal connexions, the presence of a particular retinal ‘place specificity’ was exclusively tied to a particular ‘address’ in the optic tectum.

We must accept that, during development, the retina becomes axially polarized at a certain stage, and at different times across the three major axes of space. If we consider the nasotemporal axis of the retina, polarization implies that there is a difference between nasal and temporal ends of the tissue. Now obviously there will be an array of ganglion cells along this axis of the retina and for our argument we need a term to indicate the position of a cell along this array. We need a term to identify a cell in terms of its ‘positional information’ in Wolpert’s (1969) sense. Historically, the term ‘specificity’ has been used partly in this sense; but the word specificity has become unavoidably associated with the concept of fixed tectal addresses for specified retinal ganglion cells. We would suggest that, for this reason, the term specificity has now become
Retinotectal projections in Xenopus

misleading (in view of the present results and those of Gaze & Sharma, 1970) and should be abandoned in relation to the retinotectal system. We propose instead that there are three separable concepts to be dealt with here, rather than the interlinked retina/fibre/tectum system that was inherent in the original use of the word specificity.

We would propose that the three elements of this system have to be considered separately, viz (1) positional information of the retinal ganglion cells; (2) the nature of the rules for mapping retina on to tectum; (3) positional information of the tectal cells.

Now these three aspects of the system are conceptually separable; which is not to say that they have been (or could be) experimentally separated in the present type of experiment. But we consider this conceptual separation necessary for the further analysis of various other experiments in progress.

Originally, ganglion cell specificity (and matching tectal cell specificity) was postulated in order to account for the invariant connectivity-pattern observed between retina and tectum. The present results indicate that the connectivity-pattern (in Xenopus) is not invariant. It can alter; but it does so in an orderly fashion. Seemingly, therefore, what we need to find are the rules for connecting the retinal array to the tectal array, and not any series of individual cellular specificities. If by the term 'middle retinal specificity' we merely imply that a cell is mid-retinal in position, then our statement is tautologous; if, on the other hand, we imply that cells bearing this specificity always behave like middle retinal cells in a normal eye, this is in fact not the case. What is preserved in most (if not all) experimental situations is the topological and not the geometric mapping of the retina onto the tectum.

It would seem likely that whether certain retinal and tectal elements will link synaptically is influenced by the overall nature of the particular retinal and tectal systems being matched. The matching process may be viewed as relative rather than absolute, and such that the most nasal available retinal fibre projects to the most caudal available tectum, while the most temporal available retinal fibre projects to the most rostral available tectum. Fibres from intermediate points on the retina would arrange themselves in an ordered fashion at intermediate sites. Thus the recognition process between a retinal fibre and a tectal cell must also involve processes which take into account the presence or absence of other retinal fibres and tectal elements. Perhaps the process involves competition between retinal fibres for several favoured tectal sites, with the less successful retinal fibres connecting at positions progressively further removed from the more favoured sites.

It seems that what is important to the developing and regenerating retinotectal projection is polarity and extent; given these two constraints, the retinal and tectal systems tend to match up in a plastic (or elastic) fashion. It now becomes important for us to investigate further those cases where this does not occur, and to try to elucidate further the rules governing the retinotectal mapping.
REFERENCES


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