<u>PATTERN FORMATION IN THE AMPHIBIAN RETINOTECTAL</u>
<u>SYSTEM</u>

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Someone saw Nasrudin searching for something
on the ground.
"What have you lost, Mulla?" he asked. "My
key," said the Mulla. So they both went down
on their knees and looked for it.
After a time the other man asked: "Where
exactly did you drop it?"
"In my own house".
"Then why are you looking here?"
"There is more light here than inside my own
house."

From Idries Shah
"The Exploits of the Incomparable
Mulla Nasrudin."

The work reported in this thesis was executed entirely by myself with the exception of of the experiments described in Chapter 6 which were performed jointly with Dr. R.M. Gaze and the experiments described in Chapter 7, section C in which the eye rudiment explants were prepared by Dr. J. Cooke.

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SUMMARY

The optic nerve of the lower vertebrates maps the neural retina onto the contralateral midbrain optic tectum in continuous and retinotopic order. Evidence is reviewed demonstrating that this mapping via nerve connections is ordered in the programme governing embryonic development, prior to the onset of visual function. The suggestion is discussed that the development of the map requires the acquisition by retinal ganglion cells of "neuronal specificities" which determine the positions in the retinotectal map to which their axons will project. The organisation of the map in the South African clawed toad Xenopus laevis is treated as a problem in embryonic pattern formation i.e. as a problem of the reliable formation of spatially ordered sequences of cell differentiation. The literature concerning the assembly of the map and in particular the developmental programme of the early eye rudiment is reviewed.

The behaviour of the retinotectal map following a variety of surgical interventions has been examined in the current study by electrophysiological recording. The results presented here fall into two classes: those dealing with the mechanisms of map assembly and those dealing with retinal pattern formation.

Map assembly has been investigated by examination of the visuotectal maps regenerated after removal of half a tectum in late tadpole stages and uncrossing the optic chiasma after metamorphosis. Contrary to previous findings with half tectal ablation in anurans, it was found that the axons deprived of tectal targets were able to compress onto the residual half tectum, synapsing with "foreign"

tectal sites.   This result brings the anuran data into line with the situation in teleosts, where such compression has been known for some time.   It is inferred that the failure to demonstrate compression in previous anuran experiments was due to insufficient elapsed time from operation to electrophysiological recording.

Pattern formation in the retina has been studied here following partial extirpation of the embryonic eye and following transection of the embryonic eye along the midline.   Mirror-reduplication of map order has been found after both of these operations.   These conditions for formation of these abnormal maps have been studied.   It was found that after partial extirpation, eye fragments which contained the central regions of the retina produced maps with normal order, while fragments which lacked these regions produced mirror-reduplicated maps.   This was however, only true of fragments in which the plane of ablation was parallel to the anteroposterior or dorsoventral axes of the eye.Fragments with planes of ablation oblique to these axes exhibited a wider variation in map order. These results are discussed with reference to similar findings on pattern formation in insect imaginal disc fragments and amphibian limb regeneration.   The occurrence of mirror-reduplication after midline transection was found to be strongly dependent on ionic conditions. It occurred after operation and healing in 25% solution but not after operation and healing in 100% solution.   This finding is discussed with reference to the role of healing rate and cell communication processes in retinal pattern formation.

The results presented here and those discussed in the literature review are interpreted in terms of a new model for retinal

pattern formation. This model suggests that positional information in the retina is specified on a radial (r, θ) rather than a Cartesian (x, y) coordinate system. Each cell or group of cells would have its position specified in terms of distance from the centre (r) and displacement around the circumference (θ). The model is compared with models for pattern formation in other systems. The limitations and predictions of the model are described.

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A.   PATTERN FORMATION, POLARITY AND POSITIONAL INFORMATION

Since the advent of the molecular biology "revolution", students
of developmental biology have concentrated largely on the problem of
cell differentiation; that is to say, the creation of the approximately
10^2 differentiated cell types out of a single cell (the zygote). An
extremely plausible schema has been developed within which the problem
of cell differentiation is reduced to the problem of differential
gene control (Davidson, 1969). The "operon" model of gene control in
prokaryotes (Jacob & Monod, 1961) has suggested broadly similar models
for coordinate control of gene batteries in eukaryotic development
(Britten & Davidson, 1969; Davidson & Britten, 1973). Considerable
knowledge has accumulated concerning the patterns of transcription
during embryogenesis (reviewed: Davidson, 1969) and study of these
patterns and their mode of control has come to occupy a central place
in modern developmental biology.

Valuable though this orientation has been, it has lead to a
marked neglect of the insights accruing from classical experimental
embryology. In particular it has largely stopped short of the analysis
of the initial acquisition by cells of developmental commitments, the
problem of determination. For the embryo does not simply form a
collection of 10^2 cell types, but 10^2 cell types in functional and
ordered spatial relation to each other. This aspect of embryogenesis
may be conceived of as a problem of establishing a spatial pattern of
differentiation commitments across a developing array of cells. The
greater part of the early development of some eggs (those classically
termed mosaic) is assimilable to the gene control schema outlined above.
Here asymmetric early mitoses can be conceived of as partitioning gene

control elements, pre-localized in the egg cytoplasm, differentially
between clonal compartments. In the terminology of classical
embryology, mosaic eggs have identical prospective potency and prospect-
ive fate. This simply indicates that the developmental options open
to early cells are identical with those they express in normal development.
However, another class of eggs (those classically termed regulative)
are characterised by a prospective potency wider than prospective fate.
This indicates that the developmental options open to the early cells
of these embryos are wider than those expressed in normal development.
A regulative system possesses the capacity, within certain limits, to
develop an orderly and normal pattern in a size-invariant fashion.
Control of pattern formation here regulates to accommodate to experiment-
-ally induced deletion or addition of cells. Such systems would seem
to require the existence of intercellular communication to establish
this coordinate spatial control of pattern. Here the problem of
pattern formation is not directly assimilable to the gene control
schema above. Indeed it seems likely that so-called "mosaic" eggs also
display a regulative phase earlier in development (Weiss, 1939). Rather
it stands as a problem in its own right. Recently several authors
have attempted to restore interest in this aspect of embryogenesis
(Waddington, 1966; Wolpert, 1969,1971; Goodwin & Cohen, 1969; Lawrence,
1970; Gaze 1970).

The bulk of the review that follows in subsequent chapters
concerns pattern formation in developing neural systems and will
concentrate on the evidence that this patterning is under epigenetic
control prior to the onset of function in a manner analgous to that in
non-neural embryogenesis. While it is not my intention to review in

an exhaustive fashion the literature on pattern formation, a sketch
of the relevant conclusions and ideas will facilitate comparison with
the data on neuroembryology.

A.1.   The Field Concept.

A region within which regulative communication exists may be
termed a field (for recent reviews of the field concept see: Waddington,
1966; Wolpert, 1969,1971; Cohen & Robertson 1972; Cooke 1975). Field
properties are displayed in early embryogenesis by the entire embryo.
Thus removal of 50% of the cells of an amphibian cleavage stage embryo
results in the remainder forming a more or less perfect but miniature
embryo. Subsequently a primary spatial pattern becomes determined,
mapping out the rough organ plan of the embryo. Deletion, amplification
or translocation of material between organ regions at this stage does
not result in the harmonious re-establishment of the primary pattern.
The regions of the pattern (primary organ rudiments) are "emancipated"
from global regulative control. Within the "emancipated" regions,
however, field properties may persist (or reappear) such that the
more refined spatial pattern of each organ is established through a
regulative spatial control system (see Harrison 1918,1921; Swett 1926).

A striking generalisation emerges from examination of the
many known field systems. Most fields are 50 - 100 cells in linear
extent and no normal field is known which exceeds 100 cells in linear
extent. Abnormally large fields created either by mutations (Waddington
1956; Bryant & Schubiger 1971) or by continued cell proliferation
(Gehring 1966; Nothiger & Schubiger 1966) do not form giant patterns
but duplications, the basic element of which retains normal size.

This striking size constancy of fields across a broad evolutionary spectrum (cellular slime moulds to amphibians and avians) is suggestive of common physical constraints on the patterning mechanism. Interestingly, calculations assuming patterning via passive diffusion of a "morphogen" (see discussion below) indicate size limits of this order of magnitude (Crick 1970)

A.2. Position Effects and Pattern Formation

With the discovery of the regulative phenomenon, it was recognized that cells must acquire differentiation commitments as a result of their position in an array (Driesch 1908). Wolpert (1969, 1971) has recently given a formal treatment to this observation. It is suggested that cell differentiation is initiated as a function of (1) position within the field (2) the developmental history of the cell and (3) the genetic characteristics of the cell. Cells in the field are thought of as having access to information about their position in the field, specified by some quantity which varies monotonically between the boundaries of the field. A variety of formal models have been constructed to simulate the transmission of positional information in different systems (Wolpert 1969; Wolpert et al 1971, 1974; Lawrence 1966, 1970; Goodwin & Cohen 1969; Wilby & Webster 1970; Summerbell et al 1973). The simplest of these is that based on passive diffusion of a substance produced at one boundary (source) and destroyed at the other (sink), resulting in the formation of a concentration gradient across the field (Lawrence 1966; Wolpert 1969). The value of substance concentration at either boundary is fixed and consequently the slope of the gradient will increase or decrease with loss or addition of cells. The differentiation of cells in the field

would be a response to the local concentration of the substance.
It will readily be seen that with boundary values fixed pattern form-
ation will occur in a size-invariant fashion. Other mechanisms would
involve the positional signal being propagated rather than diffusing
passively (Goodwin & Cohen 1969; Wilby & Webster 1970) or depending
on a developmental "clock" rather than a spatial map (Summerbell et
al 1973).

Whatever the mechanism, the concept of positional information
introduces naturally two new definitions. Firstly, it allows a field
to be redefined as that population of cells which are having their
positional information specified with respect to the same boundaries.
Secondly, it introduces naturally a dissociation between those cell
state variables related to position in the field and those related
to the "interpretation" of that positional information as a commitment
to follow a particular developmental pathway. "Interpretation"
would be a function of developmental history and genetic character,
allowing an identical mechanism for specifying positional information
in different fields to result in qualitatively distinct patterns
(limb and retina for example).

Evidence for such a dissociation of cell-state variables comes
primarily from study of insect systems where tissues of differing
genetic and epigenetic character may be combined in the same pattern
field by means of genetic mosaics (Stern 1968), homeotic mutants
(review: Postlethwait & Schneiderman 1974; Gehring & Nothiger 1973)
or transplantation (Locke 1960; Stumpf 1968). In these experimental
situations the common observation is that the test tissues differentiate
in harmony with their positions in the field but in accordance with

their own developmental or genetic programme. Thus different pattern regions appear to have identical mechanisms for the specification of positional information but differing interpretation programmes. Similar results have been obtained with chick embryos from cross-transplantation of hind limb bud cells to differing proximo-distal levels of the wing bud (reviewed: Saunders 1972), and from cross-species transplantation of presumptive anterior ectoderm in amphibians (reviewed: Spemann 1938)

It should be noted that the observation that differentiation commitments are acquired in relation to cell position does not logically entail a theory of positional information. Two other candidates for the explanation of pattern formation should be briefly considered. These theories posit qualitative rather than quantitative differentials as underlying pattern formation (a controversy of some vintage: see Spemann 1938 and Child 1941). Prepattern theory arose from the work of Stern and his students on pattern formation in genetic mosaics (review: Stern 1968). The theory proposes the existence of "regional differences existing independently of and preceding the establishment of a subsequent pattern" (Stern 1968) and posits an isomorphism between pattern elements and pre-existing singularities in the prepattern landscape. Empirical test of the theory led to the observations on genetic mosaics quoted above. Contrary to expectation a distinct pre-pattern was not found to underly each distinct pattern. Stern was thus forced to postulate an invariant prepattern with the cells of each pattern field being competent to respond only to a subset of the singularities present, the remainder being cryptic in the given field. Such a reformulation of the theory leaves it little explanatory value and renders it empirically indistinguishable from positional information

theory. At the formal level only one class of models has been proposed that might account for the creation of prepatterns: those descending from the theoretical analysis of Turing (1952) which demonstrated the possibility of establishing standing waves of morphogen concentrations via instabilities inherent in auto- and cross- catalytic reactions. Doubts about the precision of patterns formed by such a mechanism have been raised by Maynard-Smith (1960) and by Bard & Lauder (1974). Furthermore, since the pattern "wavelength" is established by physical factors such as diffusion constants, themselves invariant, the model will not display regulative behaviour without the inclusion of additional assumptions (Geirer & Meinhardt 1972), involving a regulative gradient of the positional information type.

A more viable alternative to positional information theory is to be found in Rose's theory of polarized inhibition (Rose 1952,1970). On this theory, a field is composed of cells competent to activate any of a number of genetic subroutines in a polygenic programme. Each subroutine would underly one element of the pattern, and the total programme would constitute a genetic hierarchy with the top of the hierarchy being that set of genes determining the most distal pattern element. All cells will tend towards activation of this set of genes but once activated in a set of cells, an inhibitory signal is released preventing these genes being activated in the remaining cells. Similar inhibitory mechanisms will operate to restrict activation of the next subroutine and so on. Those cells with the highest metabolic rate would evolve most rapidly towards the activation of distal genes. A gradient of metabolic rate across the field would ensure spatial coordination of the pattern. Cells as they reached the stage in

development where they became competent to activate part of the
determination programme would form the most distal element in the
hierarchy for which they were not already receiving inhibition. In
many empirical situations Rose's model is indistinguishable from a
formulation of positional information utilizing substance gradients
(Webster 1971). In many sense it is a theory of positional information.
However it does make a number of distinct and testable predictions.
It predicts the finding of regionally specific inhibitors, a prediction
which seems to be borne out in some systems (reviewed: Rose 1970).
It further predicts an axial asymmetry in regulative behaviour, in that
cells may move up but not down the hierarchy. It therefore predicts
that distal fragments of a pattern will not form proximal elements may
regulate to produce distal elements. Such indeed is the finding from
Rose's experiments on _Tubularia_ fragments (Rose 1967). It is not
however true that most regulative systems display such asymmetry. This
then raises the question as to the generality of the mechanisms
proposed by Rose. For this reason the discussion in subsequent chapters
will be phrased in terms of positional information theory.

A.3. Polarity

A further component of the pattern forming process which may
be seen distinctly from the specification of positional information
is the establishment of polarity in the field. The observation that
developing tissue shows a polarity in pattern formation was made at
least as early as the seventeenth century by Gilbert (quoted in
Oppenheimer 1967). Since that time many fields have been found to
acquire axes of asymmetry which correspond with the major axes of the
embryo. These polarized developmental axes determine the orientation

of the pattern and may serve to determine the location of boundary regions and the direction in which positional information is subsequently specified. Independent and temporally distinct polarizations in the order: anteroposterior, dorsoventral and mediolateral have been demonstrated in the amphibian and chick limb (Harrison 1921; Detwiler 1933; Swett 1937; Takaya 1941; Chaube 1959; Saunders 1972) the amphibian ear (Ogawa 1921; Choi 1933; Harrison 1936) and the amphibian retina (Szekely 1954; Jacobson 1968a; Eakin 1942). In addition polarization in the anteroposterior axis has been demonstrated in a number of other amphibian systems (heart, gills, ectodermal cilia and gut; reviewed: Huxley & De Beer 1934). An anteroposterior before dorsoventral rule in the acquisition of developmental asymmetries has also been observed in the epigenetic creation of clonal boundaries in the Drosophila wing disc (Garcia-Bellido et al 1973). Axes of asymmetry are also apparent in the inhomogeneities of many eggs.

While polarization of developmental axes may be a prerequisite for positional signalling, the two are not necessarily identical. Amphibian limb bud fragments with irreversibly polarized anteroposterior axes can nevertheless show size-invariant regulation of pattern (Harrison 1918, 1921; Swett 1926) Here clearly the axis is determined but not the positional values. Furthermore the acquisition by a region of organ specific differentiation commitments may precede the determination of axes (Chaube 1959).

A.4.   Developmental Decision Taking

A final generalisation concerning pattern formation may be offered about the number of developmental decisions being made at any

given time within a pattern field. The number of choices or decisions is generally small, often involving a bifurcation into only two cell types. For example, the axial mesoderm forms either notochord or somites; clonal analysis of chick limb bud mesenchyme (Abbot et al 1974) suggests the formation of two clonal compartments, one giving rise to either myoblasts or fibroblasts and the other either chondro-blasts or fibroblasts; the wing disc of _Drosophila_ has been shown to form increasingly restricted clonal compartments by a series of binary restrictions (Garcia-Bellido et al 1973). Interestingly Kauffman (1973) has recently proposed a model of the determination process in _Drosophila_ imaginal discs, based largely on transdetermination frequencies, which suggests the operation of bistable genetic circuits (see also Clayton 1953). It is therefore a tempting, if somewhat premature, suggestion that developmental decision taking may involve sequences of binary choices of differentiation commitments.

B.   NEUROBIOLOGY AND EMBRYOLOGY

Analytical study of the development of the nervous system poses two general questions: firstly, by what mechanisms are gene activities mapped onto the stereotyped patterns of neural connectivity which presumably underly species-typical behaviour modes; and, secondly, to what extent are these mechanisms similar to those operating in non-neural embryogenesis. It should, of course, be stressed at the outset that, in comparison between pattern forming systems (neural or otherwise), similarity at the level of phenomenological analysis need not imply identity of the fundamental mechanisms. It is possible that different developing systems utilize different components of

cellular behaviour (ionically controlled membrane excitatory states, metabolic oscillations etc.) to effect positional signalling. It is thus the virtue of a formal analysis that it allows us to see similarities which may exist beyond a possible mechanistic diversity.

B.1. Neurospecificity

The most extensively researched model system in neuroembryology is the retinotectal map of lower vertebrates, the projection via the optic nerve of the retinal ganglion cells onto the midbrain optic tectum. The map is retinotopically ordered (Sperry 1944; Gaze 1958) and inverted through 180° such that nasal (anterior) retina maps to caudal (posterior) tectum, temporal (posterior) retina to rostral (anterior) tectum, dorsal retina to ventrolateral tectum and ventral retina to medial (dorsal) tectum (see Fig. 1) . Like many other components of the basic integrative architecture of the nervous system, (reviews: Weiss 1941; Sperry 1951, 1963,1965; Gaze 1970; Jacobson 1970), the retinotectal projection is determined in ontogenesis, prior to the onset of visual function. The orderly nature of the retinotectal map was first observed by Stroer (1940) in a study of fasciculation in the Triturus optic nerve. It had also been known since the work of Matthey (1925) that the amphibian optic nerve possessed the capacity to regenerate with restoration of normal visual function. It was not until the work of Sperry, however, that the significance of these findings was realized. Sperry reasoned that regenerating (or growing) optic axons must display distinct "target" specificities for appropriate sites at the tectum such that the retinotopically ordered map could be reassembled in regeneration (or assembled in ontogenesis). The mechanics of map assembly, on this view, would involve a process of

FIGURE 1. Diagramatic representation of the <u>retino</u>tectal and
<u>visuo</u>tectal projection in the lower vertebrates. The projection
is shown from the left <u>retina</u> to the right tectum and from the
right <u>visual field</u> to the left tectum. The solid arrows indicate
the nasotemporal (NT) axis of the retina or visual field and its
representation on the tectum. The dotted lines represent the
dorsoventral (DV) axis of the retina or visual field and its
representation on the tectum. The rostral and caudal poles
of the tectum are indicated as R and C respectively. Note that
the visual field map is equivalent to a 180° rotation of the
retinal map. This is due to camera inversion of the visual image
on the retina.

L. RETINA
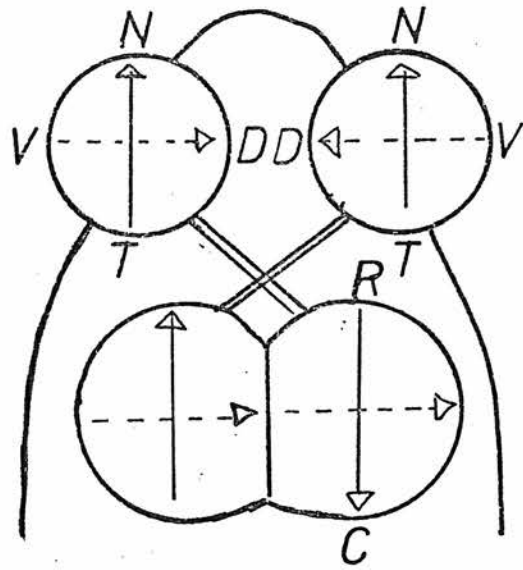
R. VISUAL FIELD

specific cell recognition, and the embryonic determination of the map would reduce to the process of acquisition by the map elements (retinal ganglion cells and tectal cells) of position-related target specificities, or <u>neurospecificities</u>. That is to say the assembly of the retinotectal map constitutes a problem in neural pattern formation. In a series of experiments, to be summarized below, Sperry confirmed the essential features of his interpretation and paved the way for a cell biological analysis of neural morphogenesis (a project still in its infancy).

Historical priority for the analysis of prefunctional epigenetic patterning of the basic integrative architecture of the nervous system belongs, however, to the work of Weiss (reviewed: Weiss 1936,1941) on the "homologous response" of transplanted amphibian limbs. He observed (Weiss 1922, 1926, 1937a,b,c,d) that the muscular response pattern of a limb transplant was synchronous and homologous with that of the adjacent host limb, even in cases where this pattern was positively dysfunctional for the animal (as in the case of reversed limbs). This provided the first firm evidence that connectivity patterns were established according to a developmental programme without reference to their functional outcome. Historical priority also belongs to Weiss for the first clear statement of the hypothesis that chemical "specificities" imparted to the nerve fibres determine their connections (Weiss 1936, 1941). His "modulation" hypothesis (Weiss 1937b, 1936,1941, 1942; Weiss & Hoag 1946) proposed that motoneurones terminated randomly in the limb and acquired specificity labels from the muscles on which they terminated (myotypic modulation). The real virtue of his work lay not so much in its truth or falsity but in its provision of a research tradition and stimulation of

experiments in a field which had hitherto been dominated by "heuristic" hypotheses. These suggested that functional feedback from initially random and diffuse connectivity patterns selected those connections which were of greatest benefit to the animal. Many tests of Weiss' hypothesis were subsequently undertaken, including the crucial nerve cross experiments (Sperry & Miner 1949; Sperry & Arora 1965; Mark 1965) and the essential prediction of random reinnervation has not been confirmed. Recent analyses of cutaneous local sign in skin rotation experiments (Jacobson & Baker 1968,1969; Sklar & Hunt 1973) following on from the work of Miner (1956) have also failed to substantiate the hypothesis. Nevertheless it is from this historical context that the work of Sperry emerges.

Sperry's innovation in neurospecificity theory was the suggestion that growing axons possess intrinsic destination specificities rather than having their specificity conferred on them by their termination site (Sperry 1951,1963,1965: reviews). Utilizing behavioural mapping of visual responses (optokinetic reflexes and lure location) in adult amphibians and fish, he provided systematic tests of such a neurospecificity hypothesis. He reconfirmed (Sperry 1943b, 1944) the restoration of visual function following nerve section and in subsequent experiments went on to examine the effects of various mismatch situations on map assembly. Rotation of an eye by 180° (Sperry 1943a) resulted in persistently inverted visual responses, which, as in Weiss' experiments, were not corrected by learning over time. Contralateral transplantation of an eye reversing only the nasotemporal axis resulted in inverted responses in that axis and correct responses in the dorsoventral axis (Sperry 1945). Cutting the optic chiasma and cross-uniting pre- and post- chiasmatic stumps to produce ipsilateral

(instead of the normal contralateral) connections, resulted in inversion of responses about the mid-saggital plane (Sperry 1945). As mentioned above, nerve cross experiments were also performed in other systems (Sperry & Miner 1949; Sperry & Arora 1965; Mark 1965) with the result that a target specificity of the nerve for its appropriate muscle was demonstrated thus extending the generality of the earlier findings. Extension of the work on the retinotectal system to teleost fish (Sperry 1948) yielded additional confirmation of the earlier amphibian work. Thus geometrical mismatch between nerve and target had no effect on the connectivity pattern finally achieved.

To account for these results Sperry (1945,1951,1963,1965) proposed a model of neural pattern formation and retinotectal map assembly, the "chemoaffinity" hypothesis, which invokes mechanisms similar to those thought to operate in better characterised non-neural cases of pattern formation (Harrison 1921, 1936,1945; Swett 1937) and morphogenesis (Townes & Holtfreter 1955).

B.2. Retinotectal Connectivity and the Postulation of Chemospecificity

Sperry's chemoaffinity hypothesis made a number of postulates: firstly, he postulated that both retina and tectum acquired a neurospecificity pattern in the course of a polarized field-like differentiation (Sperry 1945,1951). The retina being essentially a two dimensional cell sheet, two pattern axes would be sufficient to specify uniquely the position of each retinal locus, and he suggested by analogy with somatic pattern formation (Harrison 1921,1936; Swett 1937) that these axes would be the anteroposterior and the dorsoventral.

The tectum was also presumed to undergo field differentiation in the same two axes. Secondly, he postulated that matching between optic terminals and tectal elements was mediated via macromolecular labels, whose synthesis was the response to retinal and tectal field polarization. Similarly labelled retinal and tectal elements would form a stable synaptic connection. Again analogy to somatic morphogenesis mechanisms is apparent in the notion of cell affinities as mediating the pattern of cell contacts (Townes & Holtfreter 1955). The notion of chemodifferentiation resulting from position-related pattern formation is clearly analogous to positional information theory (discussed previously).

The chemoaffinity version of the neurospecificity hypothesis has occasioned some controversy in its exegesis in the hands of different authors (e.g. Sperry 1963; Gaze & Keating 1972; Yoon 1971; Hunt & Jacobson 1973c), the controversy arising essentially from the issue of the precise mode of acquisition of neurospecificity markers and the precise nature of their deployment. Since this has led to further confusion as to precisely which hypothesis was being tested by whom, it may be as well at this point to outline a brief history of the hypothesis, as I see it.

In the first place, I will define a neurospecificity hypothesis as one which argues that in the development of neural projection systems (e.g. retinotectal or neuromuscular systems) there is a tendency towards invariance of the connectivity pattern which pre-exists and is independent of function. As summarized above, neuro-specificity hypotheses of somewhat different natures had their origins in the thought of Weiss and Sperry. By 1945 Sperry's chemoaffinity

hypothesis had begun to take shape, though not yet in an unambiguous
form. The short outline of the hypothesis given above leaves open a
number of possibilities: were the identities of different loci stable
and independent of each other in all contexts (e.g. would the field
regulate for loss or addition of material and if so, over what period)?:
were these identities deployed independently of each other during map
assembly (e.g. would matching between a given retinal element "A" and
tectal element "A" occur irrespective of the presence or absence of
other elements)? In other words were the acquisition and deployment
of neuronal specificities context sensitive or context independent
(Hunt & Jacobson 1973c)? By 1963 (Sperry 1963; Attardi & Sperry 1963)
the hypothesis had reached a "stronger" formulation. The matching
of similarly labelled retinal and tectal elements was now seen as
involving a non-contextual stability and deployment of neuronal
specificities. That is, retinal element "A" would form stable synapses
only with tectal element "A" irrespective of the available extent of
other retinal and tectal elements. This variant of the hypothesis
provided the rationale for the "crucial" size disparity experiments
of Attardi & Sperry (1963). This experimental strategy, which was to
provide a paradigm for much of the work in the field to the present
day, involved the ablation of selected regions of the goldfish retina,
thus leaving vacated tectal sites, and examination of the pattern of
connectivity formed after regeneration of the optic nerve. Concordant
with the predictions of the "strong" formulation of the hypothesis,
Attardi & Sperry found that optic fibres had regenerated to their
appropriate tectal sites, crossing vacated sites without synapsing,
to reach their targets.

It must however be recognized that what was under test in the

Attardi & Sperry experiments and in subsequent work was not <u>the</u> neurospecificity hypothesis or even <u>the</u> chemoaffinity hypothesis, but rather a specific form of the general hypothesis. This has been variously stressed by several authors (Gaze & Keating 1972; Hunt & Jacobson 1973c). It is also important to realize that "Sperry's hypothesis" is a specific development of the more fluid and ambiguous concepts expressed in his earlier papers.

CHAPTER 2: THE SELF-ASSEMBLY OF THE RETINOTECTAL PROJECTION:

A REVIEW OF THE LITERATURE

A   <u>SIZE DISPARITY EXPERIMENTS</u>

The work of Attardi & Sperry (1963) had suggested an
invariance of connectivity pattern between retinal and tectal elements.
Retinal element "A" would synapse always with tectal element "A",
irrespective of the presence or absence of other elements. Other
authors exploiting size disparity paradigms (Gaze et al. 1963,1965;
Gaze & Keating 1972) argued that the connectivity pattern was not
established by such a point-to-point specificity, but rather a system-
to-system specificity ("systems matching": Gaze & Keating 1972).
According to this view the relative ordering of fibres (the topology
of the map) is always conserved but not the point-to-point connectivity
(the geometry of the map). The map will expand or compress so as to
project the entire extent of retina onto the entire extent of tectum.
This view predicts the formation of "inappropriate" connections in
size disparity experiments.

Gaze et al. (1963,1965) introduced the "compound eye" (Szekely
1957) in <u>Xenopus</u> as a variant of the half eye paradigm used by Attardi
and Sperry in goldfish. This technique involves the recombination
in early larval life (Nieuwkoop & Faber stage 32) of identical half
retinae with opposite polarity. Thus a "double nasal" compound eye
results from substitution of the temporal half of one eye with the
nasal half of the other. This produces a composite eye with embryo-
logically nasal poles at either extremity of the anteroposterior axis
and a temporal (least nasal) region in the vertical midline of the eye.
Similarly a "double temporal" eye results from the substitution of
the nasal region of one eye by the temporal region of the opposite
eye. On the Attardi and Sperry (1963) hypothesis each half of a

"double nasal" eye would be expected to map in register, and with mirror-image polarity, to the caudal half of the tectum only. In fact, while each tectal locus did indeed receive input from two visual field loci disposed symmetrically about the vertical midline of the field, the entire tectum was covered. There was no deafferented region in the rostral half of the tectum. Similarly, "double temporal" eyes mapped to the entire tectum rather than just to the rostral half. Gaze et al. (1963,1965) interpreted these results as a "spreading" of fibres into vacant and "inappropriate" tectal regions. Sperry's (1965) suggestion that this reflected "appropriate" mapping to hypertrophied half tecta was refuted by uncrossing the optic chiasma (Straznicky et al. 1971) allowing each tectum to receive input from both eyes. The "experimental" tectum was found to be capable of receiving a complete input from the normal control eye, thus substantiating the suggestion that it was a normal tectum (at least in terms of Sperry's proposed rules of mapping).

This result however, as the authors commented, left open the possibility that the two halves of the compound eye had regulated their specificity structures to yield a normal structure in each half. Thus from this work one is left with two possible classes of explanation: contextual map assembly ("systems matching"; Gaze & Keating 1972) or regulation of retinal specificities (Yoon 1971; Meyer & Sperry 1973). The apparent conflict in the results of retinal size disparities in Xenopus and goldfish was reinvestigated, in a repeat of Attardi & Sperry's work by Horder (1971) and Yoon (1972b). Here again complete tectal coverage was obtained. The divergence between the results of Attardi & Sperry and those of Horder and of Yoon may be attributable to three possible causes:

1) Attardi & Sperry used histological techniques which
    would only have picked up myelinated fibres (Gaze 1970)
    and the possibility remains that unmyelinated fibres had
    found their way to the "uninnervated" tectum.

2) Attardi & Sperry allowed only 17-25 days to elapse after
    surgery before histological analysis. A longer time
    period may have shown complete tectal coverage (Gaze 1974).

3) Attardi & Sperry in most cases removed only retina, being
    careful to leave the pigment epithelium undamaged, whereas
    Horder and Yoon destroyed both tissues. If regulation plays
    a part in the spreading phenomenon it may be that it
    requires the triggering effect of pigment epithelium trauma
    (Cronly-Dillon (1968) has shown restoration of retinotopic
    map ordering after regeneration of the retina from pigment
    epithelium in newts).

The results of tectal size disparities have also been investigated
(Jacobson & Gaze 1965; Gaze & Sharma 1970; Sharma 1972; Yoon 1971, 1972a;
Straznicky 1973; Meyer & Sperry 1973). Early attempts with goldfish
(Jacobson & Gaze 1965) with half tectal removal in the rostrocaudal
axis resulted in only the appropriate half projection on the tectum.
However Gaze & Sharma (1970) found compression of the whole projection
in goldfish with half tectal removal in the mediolateral axis. These
authors concluded that compression was possible in the one axis but not
the other. Subsequently Yoon (1971, 1972a) obtained compression in
both axes and concluded that the failure to demonstrate this in the
earlier work had been due to damage to the optic tracts. Yoon further
showed that the compression was reversible by using tectal implantation
of tantalum foil or gelatin barriers which were removable. Thus again

the results from such experiments allow of a dual interpretation: either contextual map assembly or tectal regulation (Yoon 1971, 1972a). However, the results of similar experiments in anurans have given a somewhat different answer. Straznicky (1973) using Xenopus and Meyer & Sperry (1973) using Hyla regilla ablated half a tectum in post-metamorphic juveniles (Straznicky) and in adults (Meyer & Sperry). Here an input from only the appropriate half of the retina was obtained, consistent with a context invariant point-to-point specificity. Meyer & Sperry argue that the discrepancy between the retinal and tectal size disparity experiments and between the anuran and goldfish work allow of a simple interpretation. They argue that embryonic systems have the capacity to regulate in a field-like fashion and that this regulation will be displayed in the Xenopus compound eye paradigm (the operation being done at Nieuwkoop & Faber stage 32) and in goldfish size disparity experiments, since the goldfish continues actively to grow throughout much of its adult life. They argue however, that tectal operations in anurans, done at stages when growth has ceased, will eliminate such regulative ability and generate the results seen. Thus they propose a variant of the 1963 hypothesis: that the deployment of specificity labels is context independent and that the acquisition and maintenance of these labels is, in embryonic and growth phases, context dependent in the sense of being capable of undergoing field regulation.

At this point one of the methodological problems inherent in the system becomes apparent. If one wants to design an experiment to distinguish between the contextuality and regulation variants of the hypothesis, one is confronted with a three variable system (retinal specificity, tectal specificity and rules of map assembly) in which each of the variables has to be assayed by the others.

Thus the retinal properties are assayed in terms of connectivity with the tectum, tectal properties assayed in terms of input connections from the retina and the rules of map assembly (which may themselves not be invariant) inferred from the connectivity pattern. A priori arguments have been raised against the regulative hypothesis (Feldman & Gaze 1972; Gaze 1974) but concrete experimental evidence is hard to come by. Two pieces of evidence tell against the regulative hypothesis. Sharma (1972) created tectal size disparities in the goldfish by removing rostrocaudal median strips, leaving one third of the rostrocaudal tectal extent on each side of the strip. If the tectum indeed were to regulate (Yoon 1971, 1972a) the contralateral eye would be expected to either compress singly into one of the strips or to send a complete double projection to both strips. In the event, neither of these outcomes were observed. Rather a single projection distributed itself retinotopically over the remaining tectum (both strips) ignoring the gap in the middle. The second piece of evidence again concerns tectal "regulation" in goldfish. Cook & Horder (1974) followed the time course of map compression after hemitectal ablation, obtaining the same gradual compression as reported by Yoon. They then sectioned the optic nerve and followed the time course of reestablishment of the map. Here they found that again only the appropriate half retina at first projected to the half tectum, followed by the same gradual compression as after simple hemitectal ablation. This result is difficult to reconcile with a regulative hypothesis, which would predict immediate compression of the projection after regeneration. Equally, however, it is difficult to reconcile with the systems matching (Gaze & Keating 1972) variant of a contextual hypothesis of map assembly. It would seem most likely that both retina and tectum do possess differentiated specificity structures

which may normally direct map assembly on a cell-to-cell matching

basis. However, after the creation of size disparities this

mechanism may not determine the map configuration that is thermo-

dynamically most stable (Steinberg 1963) and thus the minimum energy

configuration of the system will result from an interaction of point-

to-point specificity matching and contextual (competitive ?) map

assembly. It should be noted, however, that there are no grounds

for asserting the generalizability of these data, which may therefore

not apply to the retinal specificity structure or to Xenopus. It is

still necessary to have a more direct assay of the specificity

structures of experimental tissues.

B.   MODE OF MAP ASSEMBLY: STUDIES ON DEVELOPMENT AND REGENERATION

In the preceding section, discussion turned on the question of

the specificity of map assembly: the invariance or otherwise of the

connections formed by a given optic terminal with tectal elements

under a variety of experimental situations. These experiments

comprise attempts to analyse the context sensitivity of the final

map configuration. It is also relevant to this question to understand

the mechanics of map assembly: the process whereby an incoming optic

fibre complement sorts out at the tectum. Attempts have been made

to follow this process in time course studies of regeneration in

Xenopus (Gaze & Jacobson 1963; Gaze & Keating 1970) and in Triturus

(Cronly-Dillon 1968) and in time course studies of Xenopus development

(Gaze, Chung & Keating 1972; Gaze, Keating & Chung 1974). These

studies, together with others, implicate a competitive process in

map assembly. That is, the fibres do not seem to home in directly

on their appropriate tectal site irrespective of the presence and

behaviour of the rest of the fibre population, but rather the fibres "unscramble" themselves to form the map through a mode of competition for synaptic sites.

B.1. Regeneration Studies

The classical work of Sperry had already indicated that the cut optic nerve of amphibians and teleosts would regenerate and re-establish appropriate functional connections with the tectum. However, Sperry undertook no time course studies to follow the reassembly of the map. The ideal design for tracing this time course would be serial recording from the same animal as the map reassembled. Due to the difficulty of keeping adult animals alive after electrophysiological recording, however, the experiments to date have involved recording from different animals at various stages after optic nerve section. Such a study is then burdened with the possibility that the map configurations observed at later and later time from the date of nerve section are not in fact stages in map reassembly, but (abnormal) terminal configurations in themselves.

Gaze & Jacobson (1963) used this procedure to follow map reassembly after optic nerve section in adult Xenopus. These studies were then repeated and extended by Gaze & Keating (1970). Gaze & Jacobson distinguished four patterns in these regenerating animals: Pattern 1 maps attained complete tectal coverage from one or two restricted regions (usually the nasal or temporal pole), the regions themselves being internally disorganised; Pattern 2 maps were organised in the mediolateral but not the rostrocaudal tectal axis; Pattern 3 maps were essentially normal; Pattern 4 involved a partial or complete

restoration of the normal map, with in addition an abberent "ipsilateral" projection on the contralateral tectum and an abberent "contralateral" projection on the ipsilateral tectum. Patterns 1,2 and 3 appeared to form a temporal sequence in that the earliest time studied yielded Pattern 1 predominantly, with Pattern 2 increasing in frequency at intermediate times and Pattern 3 appearing last. The Pattern 4 maps which Gaze & Jacobson interpreted as a "super-regeneration", were shown by Gaze & Keating to occur only when the optic nerve was cut and not when it was crushed. They suggested that Pattern 4 was a result of optic fibres escaping from the optic sheath and failing to cross at the chiasma. On the assumption that these patterns do indeed represent a temporal sequence in regeneration, Gaze & Keating suggested that a competitive sorting-out process would account for the results. Patterns 1,2 and 3 indicate an initially diffuse map, becoming ordered in the mediolateral axis and then finally in both axes. The sorting out in the mediolateral axis would be expected to precede that in the rostrocaudal axis due to the mode of arrival of the optic fibres at the tectum, beginning at the rostral edge and sweeping caudally. Thus sorting out in a mediolateral axis would be possible in the advancing rostral strip of fibres.

Cronly-Dillon (1968) studied the time course of regeneration after optic nerve section in adult newts, recording at 5 and at 9 months after operation. It should be noted that the mode of regeneration in newts is different from that in Xenopus: in newts the entire retina undergoes degeneration after optic nerve section and is reconstituted, presumably from the pigment epithelium, though with contribution also from the ciliary margin (Levine & Cronly-Dillon 1974). At 5 months Cronly-Dillon found that central retina had

regained normal connections, but that peripheral retina had diffuse
tectal representation with enlarged multi-unit receptive fields.
By 9 months the normal map had been restored. He interpreted his
results as indicating that central retina was more highly specified
than peripheral retina and that while the central fibres homed in
directly on their tectal targets, the peripheral fibres gradually
sorted out by a competitive mechanism. However, since the retina
may well be regenerating almost entirely from central pigment
epithelium, the results may indicate no more than a centro-peripheral
gradient of neuronal maturation times.

B.2.   Studies on the Developing Map

The modes of growth of retina and tectum during development
are different. Whereas the retina grows in concentric rings at the
ciliary margin (Straznicky & Gaze 1971), the tectum grows, approximately
from front to back (Straznicky & Gaze 1972). This involves considerable
problems in maintaining a constant retinotopic projection during
growth. Straznicky & Gaze (1972) therefore predicted that there
would be a necessity for connections to slide during development.
Similar results and conclusions have been obtained from autoradiographic
analysis of the mode of growth of the goldfish retina and tectum
(Meyer 1974a).

Results consistent with this prediction have been obtained
from electrophysiological studies of the developing map in Xenopus
tadpoles (Gaze, Chung & Keating 1972; Gaze, Keating & Chung 1974).
The map was examined from stage 44 to 66. No ordering of the map
was apparent before stage 48. By stage 48 the map had achieved the

normal polarity of the adult map, though it still differed from the
adult map in two important respects: firstly, the multi-unit
receptive field sizes were considerably larger than those in the
adult (histological studies of Lazar & Scott - unpublished - indicate
that the terminal arborizations of young optic terminals may extend
for 300 - 500 microns); secondly, the map displayed considerable non-
linearity (that is to say equal electrode steps across the tectum
did not correspond to equal steps across the visual field), with
the caudal half of the tectum receiving a considerably expanded
representation of the temporal visual field. In addition the map
did not cover the entire tectum until stage 66. Prior to this stage
the caudomedial segment of the tectum is electrically silent. The
map thus shifts from an initial rostrolateral position caudally and
medially across the tectum, paralleling the direction of the wave
of mitosis and, presumably, differentiation in tectal development
(Straznicky & Gaze 1972). Anatomical studies of map genesis,
utilizing degeneration and autoradiography techniques, (Scott 1974)
are consistent with the electrophysiological studies and again show
a caudal and medial movement of the tectal zones receiving innervation.
Gaze et al. (1972, 1974) interpreted their results as indicating a
shifting of connections during development. As more temporal fibres
are formed at the retina and reach the tectum so they must displace
existing fibres caudally from the rostral pole in order to preserve
the polarity of the map. Again a competitive innervation mechanism
would seem to be implicated. The non-linearity of the pre-stage 66
map, however, indicates that the sliding of connections does not take
place equally across the extent of the tectum. Until stage 66, the
tectal extent occupied by the nasal half-field (temporal hemiretina)
does not enlarge to anything like the extent of the temporal half-

field (nasal hemiretina) which expands continuously into the newly maturing caudal extent of tectum. Thus there is a relative compression of the nasal half-field and a relative expansion of the temporal half-field, which is not attributable to the increased rostral curvature of the tadpole tectum (Gaze et al. 1974). This may mean that until metamorphosis the connections formed by the temporal field (nasal retina) do not shift appreciably . One is still left however with an apparent shifting of nasal field (temporal retina) fibres.

In order to critically asses these results it is necessary to examine alternative possibilities for their explanation. It may be that cell-to-cell connections remain constant and that the shifting connections that apparently underly the maps are in fact due to cell migration, either in the retina or the tectum. This latter mode, shifting of terminal sites carrying their optic connections with them has been observed in the development of the visual system of Daphnia (LoPresti, Macagno & Levinthal 1973; Macagno, LoPresti & Levinthal 1973). This possibility in Xenopus would seem to be excluded by the autoradiographic studies of Straznicky & Gaze (1972). With short duration labelling, no labelled cells were seen to migrate caudally from their site of origin. Labelling studies (Horder & Spitzer 1973; Hunt 1975) seem to argue against the other possibility: the migration and resorting of cells within the retina. Horder & Spitzer (1973) did not find translocation of labelled single cells from their site of (random) injection into the eye. Furthermore heavy labelling of growing retinae (Hunt 1975) yield regular and peripherally decreasing gradients of label, consistent with the supposition that cells remain more or less immobile at their locus of origin. The final possibility that the results are due to optical

abnormalities of the tadpole visual system is not so easy to overcome.
Histological (Scott 1974) and field potential analysis (Chung et al.
1974 a,b) indicate the presence of functioning synapses between optic
terminals and the tadpole tectum. Preliminary studies indicate that
tadpole optics are normal (Land: unpublished). Thus, the inference
that connections are sliding during the development of the <u>Xenopus</u> map,
would seem in the main justified, and a common competitive sorting
mechanism may govern the disposition of fibre terminals both in
development and in regeneration of the map.

B.3.   <u>Competitive Innervation Mechanisms</u>

The studies quoted on development and regeneration of the
retinotectal map indicate that suboptimal connections (in terms of
the final map configuration) can be formed between retinal and
tectal elements and that these suboptimal connections may be
competitively displaced by more appropriate fibre terminals. This
does not in itself allow one to infer whether the optimal connections
are or are not context invariant. Experiments in which the time
course of map compression was followed after half tectal ablation
in goldfish (Gaze & Sharma 1970; Yoon 1971, 1972 a) also show the
gradual displacement of connections by the deprived fibre complement.
Competitive phenomena of this nature are not solely restricted to
the retinotectal system. Experiments involving nerve section and/ or
cross-reinnervation in neuromuscular systems in salamanders and
teleosts also show competitive displacement of a foreign nerve by a
appropriate nerve (Sperry & Arora 1965; Mark 1965; Marotte & Mark
1970; Stirling 1973). While then competitive innervation mechanisms
would seem to be of some ubiquity, it is not possible to infer from

this that the specificity labels of retinal and tectal elements
are deployed in a context-sensitive manner in map assembly. The
competitive sorting-out evidenced above may reflect only the short-
term kinetic properties of map assembly. That is to say, "suboptimal"
connections may form transiently during the sorting-out of the map.
These connections need not reflect the overall thermodynamic contraints
involved in forming the long term equilibrium configuration of the
map. The dangers of inferring from short term kinetics to long term
equilibria have been thoroughly discussed by Steinberg (1970) in
relation to in vitro cell-sorting experiments. Thus while information
concerning the process of map assembly is of relevance, it cannot
decide for or against "Sperry's hypothesis", which is phrased simply
in terms of terminal equilibrium conditions.

C   CONTROL ASSAY SYSTEMS FOR RETINOTECTAL SPECIFICITIES

We have seen that after the creation of size disparities in
the Xenopus retina and after the creation of both retinal and tectal
size disparities in goldfish, the extent of tectal coverage cannot
be unequivocially taken as an assay of the retinal or tectal specificity
structures present. Either context-dependent spreading and compression
or regulation may account for complete tectal coverage from the
whole visual field. Two broadly similar attempts have been made
to build independent assays into the system by introducing a normal
eye, mapping in concert with the experimental eye to the same tectum
(Straznicky et al. 1971; Jacobson & Hunt 1973; Hunt & Jacobson 1973 c).

The technique introduced by Straznicky et al. was the post-
metamorphic uncrossing of the optic chiasma, resulting usually in

each tectum being dually innervated by both eyes. This assay was developed for the analysis of the tectal specificity structure contralateral to a compound eye. Thus after chiasma uncross a normal eye and the compound eye project to the same tectum. The ability of the normal eye to establish a complete map across the tectum contralateral to the compound eye was taken to indicate the normality of that tectum. No comment was offered by these authors regarding the specificity structure of the compound eye. Hunt & Jacobson introduced the "three eyed" assay. Here a normal eye, as standard, is implanted in the same orbit as the experimental eye, with a resulting dual projection to the contralateral tectum from both eyes. These authors were concerned to assay the specificity structure of the experimental retina. They reason that in such a "competitive innervation" assay the extent of overlap of the two projections gives a quantitative measure of the normality of the experimental retina. For example, in an eye fragment recombination experiment (Hunt & Jacobson 1973b) to be discussed in more detail in Chapter 3., an embryonically nasal fragment with an embryonically ventral fragment projected in register across the entire tectum with no regions of non-overlap of the projections. Although these eyes were not assayed by competitive innervation, the reasoning involved in the interpretation is the same and will serve to illustrate the implicit assumptions being made. They argued that the total overlap indicated that both fragments had altered their specificity structures, on the grounds that had the specificity structures remained unchanged the nasal fragment should have projected to the caudal half of the tectum, the ventral fragment to the medial half of the tectum leaving a rostro-lateral quadrant empty. This interpretation depends on an assumption which the authors do not explicitly state. On the hypothesis that the fragments conserve

their partial sets of specificity values, the prediction of partially overlapping projections rests on the assumption that the axon terminals of the two fragments will interact competitively on the basis of their specificity markers for tectal sites. If such interaction does not take place (i.e. if the axon terminals of the two fragments behave as independent populations) then context dependent spreading (as in compound eyes) will generate the same final map configuration.

It is important to notice therefore that in such competitive innervation assays, only projections with areas of non-overlap are interpretable; totally overlapping projections are compatible with a number of interpretations depending on the criteria of fibre population interactions. Thus the interpretability of these assays depend on a knowledge of the conditions under which fibre populations will interact at the tectum. The data on this point are ambiguous. There are two reported examples of interaction generating non-overlapping projections. Feldman & Gaze (1975 b) reconstituted normal eyes by removal and replacement of the temporal half of the eye. In contrast to the NN or TT compound eye (for which this experiment served as control) where both fragments project in register across the whole tectum these reconstituted normal eyes showed nasal retina mapping to caudal tectum and temporal retina to rostral tectum with no region of overlapping projection from the two fragments. The other example is that of small eyes produced after partial recovery from FUdR-induced growth arrest, mapped in a competitive innervation situation (Hunt: personal communication). These eyes interact with the normal eye to map only to the centre of the tectum, while similar microphthalmic eyes alone map across the entire tectum. These

results might be taken to indicate a vindication of competitive
innervation assays were it not for the existence of situations where
interactions clearly do not take place. Such a situation can be
obtained serendipitously from partially succesful uncrossing of
the optic chiasma (Gaze, Feldman & Keating: personal communication)
where the whole of one (normal) eye and a part of the other (normal)
eye both project in register across the entire tectum. Here the
specificities of the partial projection are known to be a partial
set (since they come from a normal eye) and optical abnormalities
in this eye (giving the appearance of responses only from a small
part of the visual field) can be ruled out by a complete visuotectal
map to the other tectum. Thus a known partial specificity set,
mapping in concert with a complete set, has projected to the entire
tectum, and interaction on the basis of specificity sets can be
excluded. It is not clear what are the reasons for the discrepancy
between these results and those obtained from the FUdR treated small
eyes. Among the possibilities may be:-

1) non-simultaneous _initial_ input of the two populations in
the chiasma uncross situation compared with simultaneous
initial input of the two populations in the FUdR assay.

2) later time of dual input (juvenile life as compared with
larval life) in the chiasma uncross situation.

3) dual input comes from different orbits in chiasma uncross
situation and from the same orbit in FUdR result.

4) effect of FUdR on ganglion cell properties other than
its specificity values.

Thus the interpretation of competitive innervation assays
remains problematic. Clearly, when areas of non-overlap of projections

are obtained, some sort of interaction has taken place. However doubts remain as to the situations in which interaction will and will not take place, and as to the precise nature of the interaction. Is interaction affected by conditions of timing, by site of input etc? Are the cellular properties involved in interaction those governing normal map assembly (specificity values) or are they some other set of properties related, for example, to retinal position or division history which are not normally involved in map assembly? Are the cell properties involved in interaction the same in different experimental situations? Similar doubts surround another promising assay system: the _in vitro_ cell aggregation assay of Barbera et al. (1973). Radiolabelled dorsal retinal suspensions incubated with dorsal and ventral half tecta yield a 2:1 ratio of adhesion with ventral tectum. Similarly ventral retina yields a 2:1 ratio of adhesion with dorsal as opposed to ventral tectum. It is not yet clear whether this specificity, which parallels that of map assembly, actually involves the identical cell properties.

In conclusion, in the retinotectal system, where the inference of cellular and molecular mechanisms lies at considerable distance from the experimental data, it is necessary to adopt a critical and tentative evaluation of much of the data on map assembly. In an experimental situation any, all or none of three parameters may be changed: retinal ganglion cell properties and their deployment, tectal cell properties and their deployment, the cell properties being deployed in map assembly. It is necessary, where possible, to introduce additional controls in order to be able to hold two of the three parameters constant. To infer directly from the experimentally introduced derangement and the final map to the mechanism operating

ignores the possibility that the same final configuration may be achieved from several starting configurations. While some additional control assays are being developed they also rest on the unexplored conditions of fibre population interaction. A comparison of results obtained with the chiasma uncross and "three eye" assays may allow of some conclusions as to the importance of timing factors and site of input of the populations in generating interaction. Finally, the possibility has to be recognized that the maps generated after experimental trauma may not involve the same cellular properties in their map assembly as are involved in the normal situation, or may involve additional properties. For example, we know as yet nothing of the constraints on maximum density of fibre packing at tectal synaptic sites. Possibly the discrepancy between size disparity experiments using Xenopus and goldfish tecta may be related to a difference in such constraints.

If then, the methodological problems associated with direct experiments on the rules of map assembly are numerous, we are not drawn to abandon all experimentation. While the retinotectal map does not assay specificity structure, it can be used for an analysis of the relative ordering or polarity of that structure. Hopefully, from analysis of the topography of the structure in a variety of experimental situations we may be able to piece together an understanding of the nature of the cellular properties being deployed, leading eventually to a more analytic understanding of map assembly. At the very least we can use the retinotectal map to assay retinal polarity and hence to analyse the nature of the developmental events involved in establishing and transmitting retinal pattern.

CHAPTER 3 :   THE ONTOGENESIS OF THE RETINAL AND TECTAL

PATTERNS : A REVIEW OF THE LITERATURE

A.   ONTOGENESIS OF THE RETINAL PATTERN

Sperry (1945) predicted, by analogy with the work of Harrison's school on developmental polarity of the limb and ear buds, that the retina would be polarized along the anteroposterior (AP) and dorsoventral (DV) axes of the embryo, with AP polarity being established before DV. This prediction has been confirmed several times in a number of amphibian species (Szekely 1954; Jacobson 1968a; Sharma & Hollyfield 1974a;Hunt & Piatt quoted in Hunt 1975). The technique involved in establishing this observation is that classically used by Harrison's school, rotation of the organ rudiment. Jacobson (1968a) found in Xenopus that axial polarization occurred during a critical 5 hour period in tailbud stages. Rotation of the eye anlage prior to Nieuwkoop & Faber stage 29/30 resulted in normal visuotectal maps. Rotation by 180 degrees at stage 29/30 resulted in AP reversed, DV normal maps. Thus at stage 29/30 the retina acquired an AP polarity from the surround and at stage 31 a DV polarity. Subsequent control experiments (Hunt & Jacobson: quoted in Hunt 1975) involving rotation of pre-stage 28 eye anlage through a variety of angles other than 180 degrees all yielded normal maps, while the same experiment with stage 31 and older anlage yielded map rotations corresponding to the degree of eye rotation. Thus it can be asserted that the realignment of axes that occur in pre-stage 28 anlage are indeed the result of a realignment with the major embryo rather than a simple reversal of axes intrinsic to the retinal rudiment.

These results open up a number of questions. What is the nature of these reference axes? What is the mechanism of their determination? How stable is this determination? How are the axes transmitted to progeny ganglion cells (99% of which have not yet been born at the stage of axial determination; Straznicky & Gaze 1971)?

A.1.   The Nature of the Unspecified State and the Events of
       Determination

Hunt & Jacobson (1972b) examined the nature of the unspecified

state by explantation of eye anlage to culture at various stages

from 22 to 28, followed by their retransplantation to final host

orbits when they had achieved the morphology characteristic of stage

39 retinae. It was found that these eyes formed retinotopic maps

with the tectum. The polarity of the map axes, when the eyes were

implanted in host orbits with rotation, was found to correspond with

those of the original orientation of the eye anlage in the donor

embryos. Thus these eye anlage possessed presumptive axes as far

back as stage 22 (optic bulge stage). In the absence of further

contact with surrounding tissue these axes were stable and became

irreversibly determined. In situ rotation from stages 22 to 28 or

transplantation with 180 rotation into stage 22 to 28 host orbits

resulted in normal maps, aligned with the host embryo axes. Thus

the presumptive stage 22 to 28 axes are stable but reversible. It

seems likely that presumptive axes will be present at any stage of

explantation, as part of the general neural plate axes.

The specificity characteristics and the spatial localization

of the external axial cues have been investigated in a number of

studies (Hunt & Jacobson 1972a; Hunt & Jacobson 1974c; Hunt & Piatt:

quoted in Hunt 1975). Hunt & Jacobson (1972a) showed that axial

replacement would occur on transplantation of pre-stage 28 eye anlage

to the flank. This demonstration that axial cues were not localized

solely in the periorbital tissues, but present throughout the flank,

was consistent with earlier observations that amphibian limb and ear

anlage could acquire polarity on heterotopic flank grafting (Harrison

1921; Swett 1937). Hunt & Jacobson (1974c) have obtained some
information on the time characteristics of the extrinsic axial cues.
The generation of inverted maps after in situ rotation of stage 23/24
eye anlage, followed by explantation to culture at stage 27 and final
implantation in normal orientation in the host, indicates the presence
of axial cues before the stage 28-31 period of determination. The
same experiment using transplantation to stage 32 and older hosts
indicates the persistence of axial cues past the determination period
(Hunt 1975 quotes cases of axial replacement of pre-stage 28 anlage
in stage 38 hosts). These authors also followed the time course of
axial replacement, by varying the time intervening between in situ
rotation of stage 24/25 anlage and explantation to culture. No
inversions of the map were found after 1.5 hours of rotation, but
an increasing incidence of rotation was found with 2, 4 and 6 hours
of rotation. Thus axial replacement can occur after as little as
2 hours. Furthermore many AP inverted DV normal maps were found, but
no AP normal DV inverted maps or partial inversions, suggesting that
axial replacement may be an all-or-none event exhibiting the same
AP before DV rule as normal axis polarization. Finally, these authors
found that ommiting the tissue culture step in the first experiment
(i.e. in situ rotation followed by transplantation in normal orientation
to final host orbit) resulted in a few cases of AP inverted DV normal
maps (0-10%) with the remainder normal. This indicates that the axial
replacement occurring after in situ rotation in the donor has the
same stable but reversible characteristics as were found for the
original presumptive axes (Hunt & Jacobson 1972 a,b). Hunt & Piatt
(quoted in Hunt 1975) examined the species specificity of the axial
cues by intermediate transplantations of stage 24 Xenopus anlage to
the orbits of stage 30 Ambystoma punctatum or stage 16 $\pm$ 1 Rana pipiens.

The occurrence of rotated but ordered maps in <u>Xenopus</u> final hosts indicates that the cues for axial specification are evolutionarily conserved across species lines.

The mechanism of the axial determination trigger has been examined by Hunt & Jacobson (1974a) and by Hunt, Bergey & Holtzer (1975). Hunt & Jacobson (1974a) asked the question: is the trigger for determination intrinsic or extrinsic to the retina? Early eye anlage (stage 22/23) were transplanted into stage 29 or 31 hosts in normal orientation and retransplanted into stage 27/28 final host orbits in 180 degree rotated orientation, when the eyes had reached stage 27. Had premature contact with the determination stage environment precipitated determination, the resulting maps should have been inverted. In fact, they were normal, indicating that the stage 27 eyes were still labile. In a parallel series of experiments early stage 28 eyes were transplanted in normal orientation to stage 23 intermediate hosts reached stage 27, the eye was, as before, tested for axial lability by rotated implantation in stage 27/28 final host orbits. The development of rotated maps indicated that contact with pre-stage 28 environment had not delayed determination. Thus the timing of the determination trigger relates to the stage of the eye and not to the stage of the environment. The authors concluded that the trigger for axial determination was inherent in the eye anlage.

Further analysis of this trigger mechanism was undertaken by Hunt et al. (1974) using 5-bromodeoxyuridine, which by an unknown mechanism tends to differentially suppress the expression of terminal cytodifferentiation without affecting cell maintenance or replication (Holtzer et al. 1972). It appears from work with a number of other

developing systems that BUdR exerts its effects only on mitotic cell
populations (i.e. those cells which have withdrawn from the mitotic
cycle in preparation for terminal cytodifferentiation are unaffected)
and that the effects are reversible by restoration of the tissue to
a normal medium. Eye anlage were treated in situ by injection of
BUdR at various stages, followed by rotated implantation in the
orbits of final hosts when the eyes reached stage 31/32. If the
BUdR treatment had blocked axial determination, axial replacement
should take place on the final host, resulting in normal maps.
BUdR treatment at stage 24 $\pm$ 1 completely blocked the cytodifferent-
iation of all neuronal types in the retina and normal maps developed
(indicating that determination had not taken place by stage 31/32.)
Treatment from stage 27- blocked cytodifferentiation of photoreceptors
and interneurones but permitted cytodifferentiation of ganglion cells
(which withdraw from the mitotic cycle 1 - 2 hours earlier than the
other neuronal precursors). Inverted maps developed from these eyes,
indicating that axial determination had occurred by stage 31/32.
Treatment from stage 29/30 failed to block cytodifferentiation of
any of the retinal neuronal populations and axial determination again
occurred normally in these eyes. Thus the tripping of the trigger
for axial determination would seem to occur in concert with the onset
of ganglion cell cytodiffernetiation. This conclusion is consistent
with the observations of Jacobson (1968b) on Xenopus and Crossland
et al. (1974) on chick that axial determination occurs at the stage
where the first cells of the retinal neuroepithelium (ganglion cell
precursors) are withdrawing from the mitotic cycle. The BUdR results
also have the virtue of disengaging cell division from the determination
trigger. As a result of BUdR differentiation blockade, the retinal
neuroepithelium becomes greatly enlarged. The results then allow

the conclusion that the determination trigger is not related to
cell division by a critical cell mass effect. It is, of course
possible, that the trigger is normally entrained to a mitotic
'countdown', with determination occurring as soon as possible after
the release from BUdR blockade in these experiments.

In summary the undetermined eye anlage has stable but reversible
axes. Reversal can take place either in the orbit or on the flank
and appears to be accomplished in an all-or-none fashion within a
matter of hours, with indications of a general AP-before-DV rule.
While the cues for axial determination are extrinsic (and operate
across species boundaries), the trigger for determination is intrinsic
to the eye, yolked to the initial stages of the ganglion cell
cytodifferentiation programme. The axial cues show a similar
temporal "safety margin" to that classically displayed by induction
system; that is to say they are present both before and after
passage through the critical stage 28-31 determination period.

A considerable concordance between these results and those
classically obtained by Harrison's school may be noted. The ability
of the retina to acquire polarity on the flank parallels that of the
limb bud in heterotopic grafts (Harrison 1921; Takaya 1941). The
finding that the dorsal and ventral midlines do not possess axial
cues (Swett 1938a) is again born out (Hunt & Jacobson 1973a). The
finding of an intrinsic ocular trigger for determination is anticipated
by similar studies on the limb bud (Swett 1937, 1938a). Furthermore
the finding of a stable but reversible polarity prior to determination
is common to both sets of work (Hunt & Jacobson 1972b; Swett 1938a).
Thus it seems likely that we are dealing with the same embryological

process in these diverse systems. We may expect to find confirmation in the retina of the finding (Nicholas 1922; Swett 1938b) that it is the immediate surround which confers polarity on the tissue, rather than the global surround. Nicholas (1922) found that rotation of the limb bud with a surrounding ring of tissue resulted in an inverted limb, rather than the axial replacement found when the limb bud alone was rotated. The interpretation of this finding is however complicated by the observations of Swett (1938b) transplanting the dorsal surround ventrally and vice versa. He found that the rotated limb bud repolarized when the ventral material was transplanted dorsally, but retained its inverted axial polarity when the dorsal material was transplanted ventrally. He further found that the orientation of the ventrally located graft was irrelevant. It seems likely, therefore, that the graft was not repolarizing the limb bud but rather blocking repolarizing vectors originating from the body wall, allowing the precocious determination of the presumptive axial orientation of the inverted bud. If this is the correct interpretation, we may plausibly conclude that the dorsoventral axis is propagated from the ventral region dorsally. Results (to be discussed in more detail below) obtained by Hunt (1975) indicate a dorsoventral different-ial of developmental "potency" in the retina, with ventral retina more "potent" than dorsal. There are, as yet, no data on the direction of propagation of AP cues.

Another finding, of an extremely tentative nature, which may be common to both the retina and the classical systems, is the non-independence of the AP and DV axes. Harrison (1936) found an increased proportion of twinned structures developing from ear buds after rotation of both AP and DV axes, as compared with rotation of only

one axis. He interpreted this as indicating a possible interactive effect of AP and DV axes. An interaction of the AP and DV axes, predicted on the phase shift model of Goodwin & Cohen (1969), has been invoked (Goodwin 1972) to account for the map distortions in double ventral compound eyes (Straznicky et al. 1974). It seems probable that some sort of interaction of the two axes occurs, but the nature of this interaction is not clear. This is especially pointed up by the finding (Hunt, personal communication) that AP organized, DV disorganized and AP disorganized, DV organized maps can be produced. Thus in some sense, whatever the possible interactions, it appears that AP and DV information can act as independent tissue labels.

The final point which deserves comment is the results of heterotopic flank grafting (Hunt & Jacobson 1972a; Harrison 1921). If the specification of axes in the tissue is thought to allow rank ordering of the cells in space by reading of positional information (Wolpert 1969, 1971) along the axes, the finding of normal pattern formation after heterotopic grafting allows of the following interpretations:-

a) cells in an array can read relative rather than absolute positional signals (since, assuming the positional signal to be a substance gradient, the absolute values of gradient concentration on the flank will be lower than in the orbit. Hence cells cannot be interpreting their positional inform-ation by means of differentiation triggers responding to critical threshold values of the gradient).

or b) the extrinsic polarity vectors induce the formation of an intrinsic organizer (or gradient source) within the anlage

and positional information is then propagated internally
within the anlage.

or c) the polarity vectors simply establish heteropolar axes
with no internal rank ordering along the axes (rather
like an arrow with a head and a tail, as compared with a
gradient).

Again, the observation that the presumptive axes of the pre-stage 28
anlage are stable in vitro (Hunt & Jacobson 1972b) indicates the
absence of a need for continual propagation of the extrinsic axial
cues across the retina. This in turn would seem to indicate either
the presence of an intrinsic ocular organizer to maintain the
presumptive axes, or a local homeostatic mechanism, whereby the
retinal cells remember and maintain their axial organization.

A.2.   Stability of the Determined State.

The determined axes are resistent to rotation (Jacobson 1968a),
in vitro culturing for several days (Hunt & Jacobson 1972b), transplant-
ation to the flank (Hunt & Jacobson 1972a) and serial backgrafting
to the pre-stage 28 environment combined with a delay in achieving
innervation (Hunt & Jacobson 1974a). Thus at least in the intact
eye anlage, after stage 31, the axes are stable, and resistant to
axial replacement challenges. The nature of the determination, however
remains unelucidated by these findings. Hunt & Jacobson (1973b) have
raised the question: is axial determination a property of the entire
retinal cell sheet or of the individual ganglion cells? A number
of experiments have been designed to obtain answers to this question
(Hunt & Jacobson 1973b, 1974b; Berman & Hunt 1975; Hunt 1975). These
experiments have involved disruption of retinal integrity by various

reconstructions of eyes, by midline transection of eyes, by fusion of eyes and by partial ablation of eyes.

Hunt & Jacobson (1973b) reconstructed eyes from nasal—right fragments and either temporal—left or ventral fragments, whose side of origin was not stated. Mirror—reduplicated projections, occupying in register the entire tectal extent were obtained. The authors argue that not only does this indicate an alteration of presumptive fate by the transplanted fragments (temporal or ventral) as assayed by their polarity, but also an extension of the set of specificity labels in both fragments, as assayed by the extension of their maps across the entire tectal extent. As argued in Chapter 2, extent of tectal coverage cannot be taken unequivocally as an assay of the set of specificity labels present, since the conditions of fibre interaction remain unknown. The polarity of the map obtained from the fragment is a more reliable indicator of the preservation or alteration of presumptive fate in these experiments. The map obtained from the temporal left fragment does indeed indicate an alteration of presumptive fate. Both AP and DV axes of the fragment have reversed yielding a total mirror—reduplicated map, analagous to the double nasal (NN) compound eye maps (Gaze et al 1963, 1965). The map shown in the authors' text figure for ventral fragments is not so unequivocally interpreted. The distortion of the map axes is such that the map may be interpreted as consistent with the ventral fragment having retained its polarity, if it is a ventral right fragment, but not if it is a ventral left fragment.

Hunt & Jacobson (1974b) have obtained evidence of mirror reduplication of the projection after surgical transection of stage

32 eyes along the horizontal or vertical midlines. Vertical midline transections resulted in 40% double nasal maps, 60% normal, while horizontal midline transections resulted in 40% double ventral, 60% normal maps. Double temporal and double dorsal maps were never obtained. Fusion of two eyes implanted into the same orbit also yielded double nasal and double ventral maps, but these results cannot be considered as corroborative evidence since the eyes were fused in NT-TN or DV-VD orientation and are thus equally consistent with retention of the original polarity.

The necessity for caution in interpreting these results is indicated by results obtained from eye fragments alone, made by partial ablations of the eye. (Berman & Hunt 1975; Feldman & Gaze 1975a) Nasal, temporal and ventral fragments (approximately half of the tissue mass) were made at stage 32 (Feldman & Gaze 1975a) and at stage 25/26, stage 31/32 and stage 38 $\pm$ 1 (Berman & Hunt 1975). All three types of fragments yielded a majority of normal maps, with axial orientation corresponding to the orientation of implantation (Hunt & Berman 1975). A few cases, however, yielded mirror-reduplicated maps which were specific for the type of fragment (NN maps from nasal fragments, TT from temporal fragments and VV from ventral fragments). Again map orientation corresponded to orientation of implantation and was unaffected by backgrafting to stage 27/28 hosts or 48 hours in vitro (Hunt & Bermman 1975). The incidence of reduplication was related to the stage of the tissue and to its site of origin: young fragments yielded fewer reduplications than older and temporal fragments fewer than nasal or ventral fragments. Some instances of partial reduplications were observed (Feldman & Gaze 1975a; Berman & Hunt 1975; Hunt & Berman 1975).

If, then, single eye fragments can yield mirror-reduplicated projections, it is possible that the results obtained in eye reconstructions and midline transections may result from graft elimination in the results of Hunt & Jacobson (1973b, 1974b). In order to sustain the interpretation offered by Hunt & Jacobson that reprogramming is occurring in the reconstructed eyes and midline transections, it is thus critical to know that the tissue fragment in which reprogramming is thought to have occurred is indeed the tissue fragment originally occupying that site and not one derived from the reprogramming tissue. This could be achieved by control experiments in which one of the fragments is cytologically tagged (radiolabelling or mutant tissue markers such as albino or anucleolate heterozygote). However Hunt (1975) has elegently demonstrated the possibility of genuine reprogramming. Nasal-right/temporal-left eyes were prepared in the right orbit (Hunt 1975) and after 12 or 30 hours of contact the N fragment was removed. In some of the 12 hour and nearly all of the 30 hour cases, the resulting maps were AP inverted, DV normal. In a few 30 hour cases and about 50% of 12 hour cases inversion of both axes was obtained, indicating a probable intermediate state in the reprogramming process which has the above AP inverted DV normal orientation as its final state (Hunt & Jacobson 1973b), (here again the AP-before-DV rule is in evidence). Finally a few 12 hour cases and all control cases (temporal-left fragments grafted alone into the enucleated right orbit) showed an AP normal DV inverted map corresponding to the original polarity. This work demonstrates unequivocally that reprogramming can take place but does not tell us in which of the other experimental series reprogramming is responsible for the results and in which graft loss and reconstitution of a mirror-reduplicated projection from the remaining fragment. Hunt (1975)

offers some circumstantial evidence in favour of the reprogramming
mechanism for the reconstructed eye results. Of these, the most
convincing is the observation of patchwork pigment patterns and
supernumary choroidal fissures, consistent with the retention of
the graft tissue. However the finding that mirror-reduplication
can be produced as the end result of what seem to be two different
processes (regeneration from an isolated fragment and reprogramming
of one fragment by another) illustrates the necessity for adequate
controls and cautions against inference from final map configuration
to initial starting point.

The evidence would thus seem to point to the possibility of
reprogramming and hence to the hypothesis that axial determination
may be a property of the entire retinal ganglion cell sheet, rather
than a mosaic property of individual ganglion cells. However, no
mechanism has been suggested for the reprogramming events and it is
difficult to detail precisely the situations in which reprogramming
maps occur and what rules it may follow.

A.3.   Growth and the Elaboration of the Pattern

A problem mentioned in connection with axial determination was
the elaboration of the retinal pattern with growth. Since, at the
stage of axial determination, only 1% of the adult total of retinal
ganglion cells are present (Straznicky & Gaze 1971) a major problem
remains in understanding how the other 99% of cells generated at the
ciliary margin are incorporated into the pattern. Finally this
question comes down to the understanding of how new ganglion cells
acquire their neurospecificity "labels". The mapping data, discussed

in Chapter 2, indicate that an orderly retinotopic map is present
on the tectum from tadpole stage 50 onwards (Gaze et al. 1972, 1974).
Thus the pattern is not specified de novo at metamorphosis when the
adult state is achieved. In seeking an understanding of this
continuous and sequential development of the pattern, we are again
faced with the inability to assay pattern values in the Xenopus system.
Only relative axial ordering can, for the present, be studied.

One can imagine three broad classes of mechanisms for programming
of new ganglion cells:-

1. Inheritance of pattern value commitments by ganglion cell
progeny from their mother cells – a clonal inheritance
model.

2. Continuous field–like respecification of positional
information throughout development – a "regulation" model.

3. Local interactions between new ganglion cells and their
nearest specified neighbours – a local cueing model.

The suggestion by several authors (Yoon 1971, 1972a, b, 1973; Meyer &
Sperry 1973) that the retinotectal map "regulates" in the face of
surgically introduced size disparity contain implicitly a regulation
model for normal growth. Such a model would also be compatible with
the reprogramming results which indicate some degree of developmental
lability at least until stage 32. Hunt's (1975) suggestion that
pattern elaboration occurs by new cells cueing on the older central
cells would also seem to be a variant of the regulation model.

At present, attempts to decide among the alternatives presented
above by inspection of the data are necessarily limited owing to the
striking poverty of relevant data. However, certain approaches may

be made. Not only will the behaviour of eye fragments and reconstruct-
ed eyes be relevant here, but also studies involving attempts to
disrupt the normal pattern of cell communication (Holtzer & Hunt 1974:
quoted in Hunt 1975) and studies of the normal pattern of communication
(Dixon & Cronly-Dillon 1972, 1974; Jacobson 1973).

Clonal Inheritance Models:

It is clear from the reprogramming results that the retinal
pattern after stage 32 is not a clonal mosaic. The differentiation
commitments of eye fragments can be altered to at least the extent
of reversal of the pattern axes. In this context however, two
criticisms may limit the possible use of such data. Firstly all
results reflect the outcome of operations at stage 32, only a matter
of hours after developmental lability in the intact eye primordium
has vanished. It is not yet known if such modifiability persists
into later developmental stages. The second limitation to the validity
of these experiments in the context of developmental elaboration of
the pattern is a general one concerning surgical intervention techniques
as a whole. One can never be entirely sure that the behaviour
produced after any given intervention (e.g. fragment recombination)
is representative of the normal mode of behaviour. That is one is
never sure to what extent a perturbed system behaves similarly to
the unperturbed one. Fortunately, however, the reprogramming
experiments are not the only ones which speak in favour of developmental
lability.

Holtzer & Hunt (quoted in Hunt 1975) attempted a test of the
cell lineage model by elimination of 60-90% of the ganglion cell

precursors at the ciliary margin. Growth was arrested by treatment
of stage 39/40 eyes with FUdR, an inhibitor of thymidylate synthetase.
After 3 weeks growth resumed and in most cases the treated eye had
reached the size of the untreated contralateral eye by juvenile
stages. Mapping of these eyes resulted in normal maps from those
eyes transplanted to final hosts with zero degree rotation and
inverted maps from those eyes transplanted with 180 degree rotation.
Prelabelling the eyes with ^{3}H thymidine prior to FUdR treatment
allowed autoradiographic tracking of the cell lineage of the final
map. Acute examination revealed that FUdR treatment had killed
most of the precursor cells by thymineless death: only a few heavily
labelled cells were seen in patches at the ciliary margin. At 2 weeks
the holes in the ciliary ring had been filled in by the progeny of
the survivor cells (as evidenced by the finding of circumferential
gradients of label). At 5 weeks a radial gradient of label was
found superimposed on the prior circumferential gradients, indicating
the resumption of normal radial growth. Thus the ciliary margin was
reconstituted by a circumferential spreading of cell lines which in
the normal eye would have extended radially. Had these cell lines
given rise to their presumptive specificity labels, the map should
have been partitioned among a few giant "pie-slice" sectors each
containing no internal order. The finding of normal maps indicated
the existence of some form of intercellular communication. Thus a
cell lineage model seems inadequate to account for the harmonious
meshing of the new ganglion cells into the axial pattern.

Some additional evidence against a cell lineage model is provided
by studies on the regeneration of Newt retinae (Levine & Cronly-Dillon
1974). They found that after retinal degeneration induced by optic

nerve section, the retina would regenerate both from the ciliary margin and, by metaplasia, from the central pigment epithelium. When the front of the eye (the limbus including the ciliary margin) was excised and replaced in 180 degree rotated orientation, the resulting maps were wholly or partially rotated. Thus the ciliary margin supplies cells carrying specificity labels and not simply an uncommitted cell population to be polarized subsequent to total reconstitution of the retina. However, the ciliary margin is producing cells located anatomically more central to itself and these cells carry specificity labels appropriate to their position. Thus the ciliary margin is giving rise to cells carrying labels to which these cells would not normally give rise. Here again some global cueing is indicated, although the situation in Xenopus and Triturus may not be strictly comparable in view of the different modes of retinal reconstitution.

The FUdR experiments described above may, however, have been prematurely interpreted. They do indeed seem to rule out a clonal model where the entire pattern of adult elements is present at the ciliary margin from stage 32 onwards. However the deployment of clonal commitments may be sequential rather than being expressed together at stage 32. That is to say, development of the pattern may proceed by the gradual compartmentation of an originally common pool of cells. Such gradual creation of clonal compartments has been observed in the development of Drosophila leg discs (Bryant & Schneiderman 1969) and wing discs (Garcia-Bellido et al. 1973). If the "determination" events were conceived of as partitioning the retina into nasal and temporal and then dorsal and ventral compartments (see Chapter 8.) the few clumps of viable ciliary margin ganglion

cell precursors after FUdR treatment would be sufficient to reconstitute these compartments. Provided further compartmentation steps did not occur until after stage 40 a normal map would be expected to result. It seems unlikely that the results of retinal regeneration in adult Triturus will admit of a similar explanation. However, not only is direct comparison with the Xenopus results problematic, as argued above, but it is not necessarily true that the centripetally growing cells derived from the ciliary margin themselves carry any information concerning pattern values (either in normal centrifugal or in regeneration centripetal growth). It may indeed by that such information is transmitted after the regeneration process is complete, from the (presumably) spatially intact pigment epithelial cells (see discussion below of local cueing models).

At present then, we are still restricted to the information from stage 32 recominants and transections concerning developmental lability.

Regulation Models:

Given the interpretation of the FUdR experiments offered by Hunt (i.e. that they preclude a clonal inheritance mechanism and require some form of cellular interaction), he has suggested (Hunt, 1975) that new ganglion cells are "specified" by the older central ganglion cells. He has found "scrambled" maps after surgical removal of the central cells at stage 39. However, the experimental data for this situation has not yet been published and the possibility remains that the disorder was due to optical abnormalities or disruption of the retinal tissue rather than a failure to acquire spcificity values.

If one again invokes the dubious comparison between Xenopus and Triturus results, it is clear that the centre and the periphery can act as independent domains as far as axial polarity is concerned (Levine & Cronly-Dillon 1974). Not only are peripherally rotated maps obtained but also totally rotated maps. Totally unrotated maps (as would be predicted on a central cueing model) are never found.

Finally, if Hunt's model is taken to imply that the entire pattern of specificity values are present at stage 32, regulating in a size invariant fashion with growth, contrary evidence is also to be found in the FUdR studies quoted above. While most eyes recover from the treatment and go on to generate normal sized eyes, in a small percentage of cases (Hunt 1975) recovery was incomplete resulting in microphthalmia. When these small eyes were mapped in concert with a normal eye in a competitive innervation assay (see Chapter 2) they were found to map only to the centre of the tectum. The difficulties in interpreting such an assay have already been mentioned in Chapter 2. If one tentatively accepts that competition between fibre populations in this situation does depend on the specificity labels of optic terminals, this result would indicate that the small eyes possess only a partial set of specificities. While none of these results alone is greatly convincing, together they do rather suggest that growth does not simply expand a pre-existing pattern but rather that more distal pattern values arise during growth.

A final contraindication to a regulation model is provided by the patterns resulting from eye fragment construction in which the cut edges of the fragment were apposed (Straznicky, Gaze & Keating: quoted in Feldman & Gaze 1975a). Although both normal and

appropriately mirror-reduplicated maps were reported here as for the simple fragment experiments, there was a third class of results. In these the rows of field positions showed a distinct curvature towards the zone of apposition, rather as if they had been "bent" round in the act of rounding up the fragment by apposition of the cut surfaces. Such a map configuration is hardly compatible with a regulation model. Instead it suggests that the new ganglion cells derived from the ciliary margin at the region of apposition inherited from their progenitors the curvature imposed in the mechanical deformation of the fragment.

Local Cueing Models

There is almost no evidence bearing on a local cueing mechanism. If one were able to find evidence of some sort of cellular interaction operating at later stages of development than stage 32, a local cueing mechanism would be indicated if a global regulation mechanism could be ruled out. Such evidence is clearly indirect and somewhat unsatisfactory. There is however evidence for a cell interaction process which can be spatially localized and which correlates with the events of axial determination. This is the finding of gap junctions between the retinal ganglion cells in an electron microscopic study of the Xenopus retina from stages 26-31 (Dixon & Cronly-Dillon 1972). Such specialized cell junctions are a consistent feature of many early embryonic tissues and are probably the structural counterparts of the electrotonic cell junctions demonstrated electrophysiologically (review: Furshpan & Potter 1968). That is to say these junctions probably allow the easy transport of ions and small molecules between cells.

As such, gap junctions are attractive candidates for the locus of cell interactions of developmental significance. There is indeed some evidence to relate such junctional communication to the presence of field characteristics. (Bennet & Trinkhaus 1970; Palmer & Slack 1970). Dixon & Cronly-Dillon found that all retinal ganglion cells were junctionally coupled from stages 26-31, but that at stage 31 junctions disappeared from the central cells, remaining only at the periphery. Jacobson & Loewenstein (quoted in Jacobson 1973) have found a corresponding functional effect. Fluorescent tracer molecules injected at one site in the pre-stage 31 retina rapidly spread to the other cells. After stage 31 communication is blocked. Thus the junctions are indeed transmitting junctions. Both is space and time the distribution of these junctions shows an intriguing correlation with the development of the retinal pattern. Before axial commitment and the onset of terminal cytodifferentiation (i.e. before stage 31) junctions are uniformly distributed. At stage 31, when the axial commitment is established (Jacobson 1968a) and the central ganglion cells complete their final DNA synthesis (Jacobson 1968b), junctions disappear from the central cells and remain at the actively dividing (and therefore presumably undifferentiated) margin. It is unfortunate that the distribution of gap junctions was not followed in later stages but their disappearance from the centre after stage 31 suggests an autonomy of cell interaction in the periphery. There is of course no conclusive proof that these junctions have any causal role to play in pattern formation. Indeed in some systems junctional communication has been demonstrated across developmental field boundaries (Sheridan 1968; Potter, Furshpan & Lennox 1966; Warner & Lawrence 1973; Caveny 1974).

Granted for the moment the assumption that junctional

communication is involved in pattern formation, the work of Dixon
and Cronly-Dillon contains a further suggestion. Not only are
gap junctions found between retinal ganglion cells but also between
ganglion cells and the pigment epithelium (Dixon & Cronly-Dillon
1974). Since in urodeles the retina may be derived by metaplasia
from the pigment epithelium (review: Stone 1959) and such a retina
posseses retinotopic order in its tectal projection (Levine & Cronly-
Dillon 1974), the pigment epithelium would also seem to possess a
specificity structure. Indeed there is no convincing evidence that
the neurospecificities expressed by the ganglion cell terminals at
the tectum are intrinsic properties of the ganglion cells themselves.
It may be that the development of pattern occurs in the pigment
epithelium, with an "induced" expression in the ganglion cells.
Evidence from a variety of pigment mutations in mammalian retinogeni-
culate projections (Guillery et al 1974) suggests that the pigment
epithelium may be involved in specifying axonal decussation at the
optic chiasma.

B   STUDIES OF TECTAL PATTERN

In comparison with the data accumulated on retinal patterning,
almost nothing is known of corresponding events in the tectum, for
the obvious reason that it is much less accessible and much less
manipulable from the point of view of embryological operation.
While Sperry's chemoaffinity hypothesis suggests that the retinotectal
map assembles by means of affinity between similarly labelled retinal
and tectal elements, in the case of the tectum this hypothesis is
at some considerable distance from the experimental data. It is
entirely possible on the basis of the data discussed so far that the

tectum, indeed, possesses no specificity structure whatever and that
the optic fibre population sorts out internally with respect only
to retinal specificity. There is however, some evidence that the
tectum also is polarized.

Attempts to analyse the time of tectal polarization by
classic rudiment rotation techniques proved unsuccessful. Crelin
(1952) excised the right tectal rudiment of <u>Amblystoma</u> between stages
23-46 and rotated the right tectal rudiment from stages 23-35. In
both series, after stage 30 there was decreasing ability to restore
a histologically normal tectum. Optic function was assayed by
optokinetic reflex. Again visual function appeared to decline after
stage 30. However, in no case was there evidence of definitive rotation
the connection between histological appearance and specification is
unknown and the optokinetic reflex is an extremely unsatisfactory
assay of visual function. Indeed there is evidence that this
reflex persists after tectal ablation (Mark & Feldman 1972).

More successful results have been obtained by rotation of
tectal segments in the adult animal. Sharma & Gaze (1971) rotated
tectal segments in adult goldfish by $90^{o}$and obtained, on electro-
physiologocial mapping, a normal projection to the unoperated area
of the tectum and an S-shaped ripple pattern within the graft
similar to that obtained in insect cuticle pattern after 90° rotation
(Locke 1967; Lawrence 1970). Essentially compatible, but more simply
interpretable results were obtained after 180° rotation of segments
of adult goldfish tectum (Yoon 1973). Here projection to the graft
was 180° rotated, 180° rotation of tectal segments in juvenile
<u>Xenopus</u> (Levine & Jacobson 1974) again yielded 180° rotated projection

patterns within the graft.

These experiments have been interpreted as confirmation of tectal specificity. However, at best, this interpretation cannot be considered as necessarily true. Firstly, the experiments involved in situ rotation rather than translocation up or down an axis. Therefore, they can indicate only polarity and not necessarily specificity as understood in Sperry's (1945) terms. Secondly, the experiments were all performed on adult organisms whose tecta had already been innervated. Therefore, the results may be due to an alteration in the tectal cells occurring after innervation, such as an induction of specificity by the optic terminals. Indeed, it may be that the results simply reflect retinal/retinal recognition due to residual presynaptic terminal membrane adhering to the tectal postsynaptic sites after the operation. The present data can therefore only be considered to provide weak evidence in favour of a tectal specificity.

CHAPTER 4 :   INTRODUCTION TO THE EXPERIMENTS

A   EXPERIMENTS WITH PARTIAL TECTA: A REEXAMINATION OF SYSTEMS
MATCHING IN THE AMPHIBIAN TECTUM

As summarized in Chapter 2, the results obtained with size-
disparity experiments in <u>Xenopus</u> do not show internal consistency
between eye and tectal operations. Compound eyes made at stage 32
"spread" to cover the whole tectum, whereas creating a size disparity
in the tectum by half tectal ablation does not result in "compression"
of the whole field either in late tadpole stages (Straznicky et
al. 1971) or in adult life (Straznicky 1973). A further disruption
of a consistent picture is to be found in the data from teleost
partial tectal ablation (Gaze & Sharma 1970; Yoon 1971; Sharma 1972)
where compression of the whole field onto the remaining tectum does
occur. Meyer & Sperry (1973) confirmed the lack of plasticity in
amphibian tecta, in experiments on partial tectal ablation in <u>Hyla</u>.
They argued that the apparent "spreading" and "compression" found
in other studies reflects a phenomenon of embryonic field regulation
in actively growing tissue (<u>Xenopus</u> embryonic eye and goldfish eye
and tectum) and that in tissues, such as the adult amphibian
tectum, where growth has ceased such regulation will not occur.
Hence the apparent rigid specificity of connections found in half
tectal amphibian experiments.

In view of the arguments presented in Chapter 2 against the
embryonic regulation hypothesis, it was decided to reinvestigate the
apparent discrepancy posed by tectal size disparities in <u>Xenopus</u>.
As in the experiments of Straznicky et al. (1971) half tectal
ablation (rostral or caudal) was performed in late tadpole life
(stages 54 - 59). To maximize the chance of competitive resorting

of fibres, two additional variables were included in the experiment-
al design. Firstly, the optic chiasma was cut after metamorphosis,
and secondly a longer time was allowed to elapse between tectal
ablation and electrophysiological mapping. The importance of
competitive reassortment is reflected in the results of Gaze &
Sharma (1970) with partial tectal ablation in goldfish. Significantly
better "compression" was obtained when the optic nerve was cut.
Generally, only partially compressed maps were obtained where
optic nerve section was not performed. The importance of regeneration
time is clear from previous experiments on goldfish partial tecta
(Gaze & Sharma 1970; Yoon 1971; Meyer 1974b; Hope & Gaze: personal
communication). With short intervening times between tectal
ablation and mapping, only the appropriate half field is found to
map to the half tectum. With longer elapsed time compression
becomes increasingly complete. This time course of compression
has been found to repeat itself when the optic nerve is resectioned
following complete compression (Cook & Horder 1974). Thus intervals
of 18 - 24 months were allowed to elapse from the time of tectal
ablation to the time of mapping, as compared with 4 - 7 months in
the case of Straznicky (1973).

B   THE STABILITY AND NATURE OF THE RETINAL PROGRAMME: REGULATION
        AND REGENERATION IN SURGICALLY PERTURBED EYES AND EYE FRAGMENTS

B.1.   Concerning the Stability of Polarity Vectors in Transected
        Embryonic Eyes.

        The problem of the basis of polarity remains one of the oldest
and most central questions in embryology. First noted by Gilbert in

the seventeenth century in plant grafting experiments (quoted in
Oppenheimer 1967), it was compared to the phenomenon of magnetism.
Its physical basis however remains elusive to the present time. As
a first step towards understanding this basis two broad classes of
mechanisms may be envisaged. Polarity may be seen either as a global
property of the whole field or as a local property of individual
regions or cells (Wolpert 1968). In a global mechanism models such
as gradient fields suggest themselves (Wolpert 1969; Lawrence, Crick
& Munro 1972) where polarity is assigned to cells by the slope of the
system gradient. In a local mechanism, polarity would represent
some inherent property of individual cells or subregions of the
field. Here the underlying basis might be conceived of as oriented
asymmetries of organelles such as microtubules or polarized transport
systems.

While the amphibian retinotectal system, as argued in Chapter
2 and 3, does not as yet allow of unequivocal assay of pattern, it
does provide a fairly refined analysis of polarity. As summarized
in Chapter 3, the post-stage 31 retinal axes are resistant to
perturbation induced by rotation, back-grafting and delay in achieving
tectal innervation. All these procedures involve use of the intact
eye rudiment and tell us little of the processes underlying axial
commitment and maintenance. An approach to further analysis has
been undertaken by Hunt & Jacobson (1973b). They reported the results
of a variety of eye recombination procedures (composite eyes formed
from nasal right and temporal left or ventral fragments). The
transplanted fragment (temporal or ventral) was found to reverse its
polarity in these cases to yield mirror-reduplicated, "double-nasal"
type maps. These authors argue that polarity can then not depend on

a local mechanism but must involve the global behaviour of the entire eye rudiment. Further experiments have been reported by these authors (Hunt & Jacobson 1974b) in which simple transection of the stage 32 eye rudiment along either the horizontal or vertical midline leads to 40% of the resulting maps being double-ventral and double-nasal (respectively) mirror reduplicants. Without arguing the case in detail, Hunt & Jacobson note that the finding of only two of the possible four classes of reduplication (double-temporal and double-dorsal maps were never found) is consistent with models, such as gradient models, in which one end of an axis (nasal or ventral) dominates the other (temporal or dorsal).

The latter paradigm, midline transection, has been employed in the present study towards a further characterisation of the global polarity of the retinal field. However, when pilot experiments failed to confirm the finding of mirror-reduplication, reported by Hunt & Jacobson (1974b), a search was initiated for variables which might explain the discrepancy of results. Two additional variables were introduced into the experimental design: stage of operation and ionic strength of the operating and rearing media. Stages between 29/30 and 37/38 were transected along either the horizontal or vertical midlines of the eye. This allowed control for possible variation in staging criteria and in addition allowed examination of the effect of tissue discontinuity prior to the stage of axial determination. Ionic strength of the operating medium was also considered as a possible variable, since to gross inspection, healing rate is proportional to ionic strength and since ionic strength may affect junctional coupling of developing cells (Loewenstein et al. 1967). Hunt & Jacobson operated in a solution of 25% Holtfreter's

5% Steinberg's (Hunt; personal communication), while most of the operations in the present experiments utilised 100% Niu-Twitty solution. Thus an additional control series was run in which stage 32 vertical midline transections were performed in a 20% Holtfreter's / 5% Steinberg's solution. Operated animals were left for twenty-four hours post operatively in the same ionic strength solution.

It was hoped, in this way, to clear up the discrepancy of results and, through an understanding of the reasons for the discrepancy, achieve a preliminary characterisation of the cell communication processes underlying polarity maintenance.

B.2. Concerning the Mechanism of Pattern Restoration in Eye Fragments: the Role of Central Retina and Tissue Compartments in Determining Mirror Reduplication.

Of the several abberations of pattern formation found in nature and in experimental situations, that of mirror-reduplication is among the most intriguing. The finding that abberant reduplication of pattern or parts of a pattern exhibited regular characteristics of mirror symmetry was first noted by Bateson (1894) and was regarded by him as an important clue to the mechanism of evolution. In the light of current knowledge such hopes may seem more than a little anachronistically grandiose, nevertheless the phylogenetic ubiquity of the phenomenon suggests that it may throw light on a somewhat general mechanism of pattern formation.

The experimental study of pattern restoration following tissue

removal in both developing and regenerating systems has thrown further light on the phenomenon and its degree of generality. In both insect (Bryant 1971; Schubiger 1971; Postlethwait & Schneiderman 1974; Gehring & Nothiger 1973) and amphibian (Butler 1955) systems removal of tissue comprising part of a pattern or presumptive pattern leads to two alternative and mutually exclusive pathways of pattern reconstitution: either restoration of the entire pattern or mirror reduplication of the remaining pattern elements. Such behaviour can be formulated as a "rule of distal transformation" (Rose 1962; Wolpert 1971). The rule indicates that pattern reconsitution from a cut surface can only result in the formation of pattern elements which normally lie distal to that surface. Figure 2 shows the operation of the rule to produce the two alternate pathways. An idealized pattern is shown as consisting of a set of six monotonically varying gradient values (Wolpert 1969) with the pattern transected through element 3. Regeneration from the cut surfaces of fragments A and B will, according to the rule, form the more distal pattern elements 4, 5 and 6. Thus fragment A will regenerate the complete pattern, while fragment B will form a mirror regenerate of elements 4,5 and 6.

Studies of chick limb development (Saunders 1948; Summerbell, Lewis & Wolpert 1973) indicates a similar distilization of pattern value during development and the work of Summerbell et al. suggests a possible mechanism. These authors suggest that a developmental clock operates within a region, the "progress zone", immediately subjacent to the apical ectodermal ridge. Within the progress zone, and only within it, can cells change their presumptive pattern value, as a function of the time they or their progenitors have spent within

FIGURE 2. The "Rule of Distal Transformation". The top diagram
shows an idealized representation of a pattern consisting of
6 discrete elements (labelled 1 - 6). Corresponding to each
pattern element is a pattern value, represented by six levels
of a gradient. Transection between elements 3 and 4 produces
two fragments, A and B. Cells at the cut edge produce the
remaining three distal elements (4 - 6). These distal
regenerates are represented in dotted lines. Fragment A
reconstitutes the complete pattern while fragment B produces a
mirror image of itself.
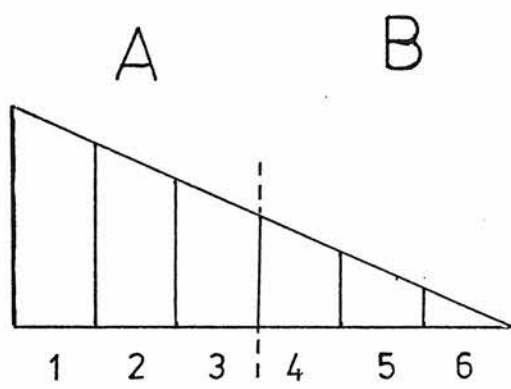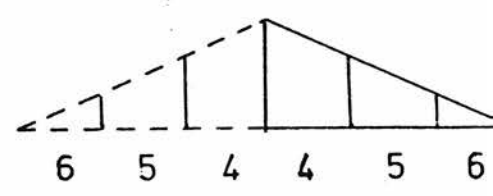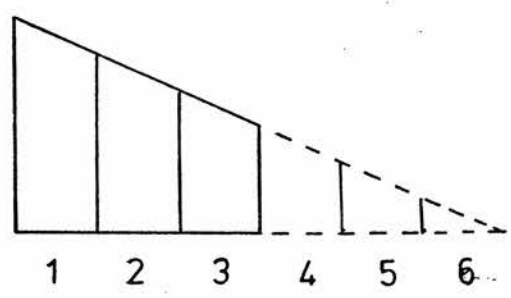
the zone. Thus the longer a cell spends within the zone the
more distal its pattern value. Such a "clock" mechanism would
immediately explain the distal transformation of pattern value
at a cut edge in regeneration situations. In contrast with this
mechanism, systems (such as Hydra, where pattern formation appears
to be under the control of a positional "map" : Wolpert et al. 1971,
1974) which display classic regulative "field" properties, do
not require growth for pattern reconstitution (Hicklin & Wolpert
1973; Cooke 1973) and never show mirror reduplication after simple
transection or tissue removal. Rather the gradient regulates to
restore the entire pattern harmoniously over both fragments. It
is tempting therefore to offer the generalization that distal
transformation applies only to epimorphic systems, while morphallactic
systems show field regulation.

It has recently been reported that the Xenopus neural retina,
after removal of half the eye anlage, forms either normal or mirror-
reduplicated retinotectal maps (Gaze 1970; Feldman & Gaze 1975a;
Berman & Hunt 1975). In the light of the foregoing discussion it
seemed worth investigating this phenomenon further, in order both
to probe the mechanism of pattern formation in the retina and to
assess the possible generality of this mechanism by comparison with
the other systems discussed. Wolpert (1971) has detailed the
predictions from the distal transformation rule applied to the
conventional picture of pattern formation in the amphibian retina.
The retina is thought to be polarized along two independent and
mutually orthogonal axes, one in the AP and the other in the DV
orientation (Sperry 1945; Szekely 1954; Jacobson 1968a). If this
is the case, after formation of half eyes, either nasal or temporal

fragments should mirror reduplicate <u>but</u> <u>not</u> <u>both</u>, while the complementary half should yield normal maps (see Figure 2). However Feldman & Gaze (1975a) and Berman & Hunt (1975) report two findings at variance with these predictions. Firstly, both nasal and temporal fragments will give rise to mirror reduplicated projections; and secondly normal and mirrored maps are not found with equal frequency – the mirrored maps form a minority class of results. If however, the centre rather than either of the peripheries is regarded as the "organizing" region of the retinal pattern, both these findings can be accommodated. Proximo-distal elaboration of pattern will then correspond to a centro-peripheral anatomical organisation. Since the retina grows in concentric rings (Straznicky & Gaze 1971) such a hypothesis suggests a similar connection between pattern formation and growth to that proposed for the chick limb. The hypothesis predicts that eye fragments lacking the centre (corresponding to distal fragment B in Figure 2) will mirror reduplicate, while eye fragments in which the centre is present (corresponding to proximal fragment A in Figure 2) will yield normal maps. Thus both nasal and temporal fragments would be expected to give rise to a proportion of mirrored maps as a minority class in experiments aimed at the construction of "half" eyes.

The hypothesis has been tested systematically in two series of operations. In the Series 1 the role of the centre is examined by the construction of eye fragments known to either contain or exclude the centre. In this series the plane of section in creation of the fragments was parallel to the major embryo axes. Thus the plane of section ran either parallel to the nasotemporal or dorso-ventral axes. In Series 11 the proposition was tested that the

properties of the retinal pattern are disposed in a radially
symmetrical fashion about the centre. Fragments known to
contain or lack the centre were again made, but in this case
the plane of ablation was oblique (approximate angle of 45°)
to the major axes. Thus nasoventral and termporodorsal fragments
were constructed.

B.3. The Retinotectal Map from Eye Primordia Explanted to
Culture at Early Neurula Stages: Further Tests of a
Hypothesis

As suggested in the previous section of this Chapter,
the finding of mirror reduplication as a resultant map from
both nasal and temporal fragments, suggests the possibility.
that the properties of the pattern field (which will incorporate
new ganglion cells into the map) are disposed about the centre
in a radially symmetrical fashion. The simplest means by
which this could occur is found in a positional coordinate
system with radial rather than Cartesian coordinates. That
is to say, a positional field in which cells differentiated
as a result of receiving information about their distance
from the centre ("r" component) and about displacement from
a boundary zone about the circumference ("θ" component). It will
be seen that such a model is formally identical with that proposed
for chick limb pattern formation (Summerbell et al 1973; Smith
et al. 1974; Summerbell 1974). In the progress zone model for
chick limb formation distal elaboration of pattern is linked to
a developmental clock. The authors suggest that the clock might

register some such variable as the number of cell cycles spent in
the "progress zone" at the distal tip of the growing bud. Preliminary
results (Cook 1975: in press) would seem to suggest a similar mechanism
in the elaboration of somites in the tail bud of <u>Xenopus</u> larvae.
A radial component ("r") in the developing retina might similarly
be specified by cells registering their mitotic history up to the
point where they leave the growth zone at the ciliary margin
(Straznicky & Gaze 1971).

Since a variety of types of coordinate systems might, in
principle, underly the normal map, inspection of the normal map or
its mode of development does not in itself allow such a hypothesis
to be tested. It is necessary to intervene in the establishment of
the coordinate system, to disclose the underlying components. According
to the above hypothesis the "r" component would be patterned directly
in the growth process intself and as such would be relatively stable.
The "θ" component would however be established by the axial determination
events of stage 28 - 31. If it were possible to disrupt these events,
preventing the establishment of "θ", the model predicts a map in which
field positions are ordered in a centroperipheral axis but not in an
angular or circumferential axis.

An attempt to create such a disruption was therefore made
by explanting small fragments of the anterior embryo (containing the
eye primordia) at early neurula stages. It was hoped that in so doing
the eye primordia might continue to develop in the absence of the
global embryonic axial cues (Hunt & Jacobson 1972a) and so generate
a map, after transplantation to normal host orbits, which displayed
only an "r" component.

C   THE ACQUISITION OF DEVELOPMENTAL AXES: EXPERIMENTS ON THE

SPATIAL DISPOSITION OF AXIAL CUES USING CHEMICALLY PREPARED

CYCLOPEAN EYES

The results of grafting axially undetermined organ rudiments

to heterotopic sites on the flank (Harrison 1921; Hunt & Jacobson

1972a), summarized in Chapter 3, have indicated the existence of

global axial cues within the head and flank. If this is indeed the

case, nodal points of bilateral symmetry should occur where the

dorsoventral axes of each side meet (dorsal and ventral midlines)

and where the anteroposterior axes of each side meet (at the centres

of the head and tail). Nicholas (1924) attempted to test this by

transplanting Amblystoma limb buds to the dorsal and ventral midlines.

Despite poor growth in these locations, some indication of doubling

was obtained.

It was decided to attempt a comparable experiment for the

anteroposterior axis by causing the development of an eye in the

centre of the head. If there are indeed embryo-wide axes meeting at,

or propagating from, this point the resulting map should be reduplicated

with a posterior pole at each extremity and an anterior pole centrally.

In other words a double temporal projection should result. In view

of the difficulty of placing a graft in the precise centre of the

putative embryo field, it was decided to accomplish this by causing

cyclopean development of the embryo. This can be accomplished by

brief treatment of amphibian eggs at blastula stages with Lithium

ions (Adelmann 1934, 1936; Hall 1942; Backstrom 1953). For reasons

which remain largely unknown (though the effect may be due to

increased protoplasmic viscosity: Ranzi & Citterio 1954), Li^+ prevents

correct development of the anterior portion of the embry axes,

including failure of separation and lateralization of the two eye

primordia. Instead a single centrally located cyclopean eye

develops. It was hoped that such cyclopean eyes, transplanted to

normal hosts after they had passed the stage of axial determination

would successfully form connections with the tectum and thus allow

study of the spatial disposition of the global axial cues.

CHAPTER 5 :   MATERIALS AND METHODS

A.   TECHNIQUE AND THEORY OF ELECTROPHYSIOLOGICAL MAPPING

A.1.   Preparation of animals

        Post-metamorphic adults were prepared for mapping as follows:
approximately 2 ml. d-tubocurarine (0.1%) in distilled water were
injected into the dorsal lymph sac to achieve paralysis; anaesthesia
was then produced with ether vapour; the optic tecta were surgically
exposed by removal of the cranial skin, the skull (using a dental
drill) and the meninges and the animal was decerebrated; paraffin
oil was applied to the tecta throughout exposure and subsequent
mapping as needed to prevent drying. The optic tecta were then
photographed with a superimposed rectilinear grid (with an internode
spacing of 100-200 microns) to facilitate placing of the electrode.

        For the majority of experiments to be described here pre-
metamorphic tadpoles (Nieuwkoop & Faber stages 53-61) were used.
Tadpoles were prepared by reflection of cranial skin, cartilage
(or precartilaginous membrane) and meninges to expose the optic
tecta under 1:2,000 MS 222 (tricaine methane sulphonate: Sandoz)
anaesthesia. The mapping was conducted with the tadpoles immersed
in oxygenated full strength Niu-Twitty solution inside a perspex
dome with sufficient MS 222 to maintain a light level of anaesthesia.
Two types of dome were used: either a hemispherical dome or a
spherical dome with the animal centrally on a raised platform,
(when it was desired to map positions lying below the horizontal
meridian of the visual field. Electrode placing was accomplished
by means of a micromanipulator array which allowed controlled
movement in the anteroposterior and mediolateral axes of the tectum

when appropriately aligned. After the conclusion of the mapping, the cardinal points of the tectum were read off the micromanipulator micrometers (rostral, caudal and lateral poles and rostral and caudal midlines) to allow diagrammatic reconstruction of the tectum and of electrode positions within it.

A.2. Mapping Procedure

The animal was set up with the desired eye centred on an Aimark perimeter. Electrodes were placed on the tectum using the micromanipulator under visual guidance through a swing arm Zeiss dissecting microscope which viewed the tectum from above and rostrally. Sequential electrode positions were generally 100-200 microns apart. In the case of tadpole mapping glass electrodes filled with Woods metal and tipped with gold and platinum electrolytically (tip diameter 1-5 microns) were used. In the case of adults laquered tungsten electrodes (tip diameter 1-5 microns) were used.

At each electrode penetration the approximate depth of maximal response was crudely located using the microscope light as stimulus. The microscope light was then switched off. The effective stimulus area in the visual field was then localized by movement of small black discs (subtending 2-5°) against the perimeter. The position of the stimulus area was then transposed to a visual field chart from the coordinates of the perimeter. Responses to stimulation were action potentials from presynaptic single or multiple units. The responses were monitored visually on an oscilloscope or aurally through a loudspeaker.

A.3.   Theory of the Mapping Procedure

The technique described above allows analysis of retino-
tectal map at fairly high resolution (4-6 ganglion cell diameters
in the retina; 5-10 cell diameters in the tectum: Hunt & Jacobson
1973c). It is worth discussing however, precisely what it is that
the mapping technique is analysing. The available evidence suggests
that the electrode is picking up from optic nerve terminals. The
inference that recording is from a terminal is fairly direct: since
fairly small electrode steps across the tectum (20-50 microns)
results in a corresponding shift in the visual field positions it
seems unlikely that the electrode can be recording from fibres of
passage, travelling past its tip. Had it been the case that fibres
of passage were recordable, the same visual field locus should have
been detectable at several tectal loci in the direction of passage
of the fibre. The argument that the recorded potentials are pre-
synaptic is more indirect: firstly, single unit responses recordable
in superficial tectal layers have the same receptive field character-
istics as those recordable in the optic nerve while single units
deeper in the tectum have receptive field characteristics different
from those in the optic nerve (Gaze 1970); secondly, high frequency
electrical stimulation of the retina are recordable with unchanged
waveshape in the superficial layers of the tectum (Buser & Dussardier
1953). This would seem to indicate the visually driven superficial
tectal units are presynaptic. Since recordings are made in the
superficial layers, it is likely that most or all of the units recorded
are presynaptic optic axon terminals.

An important criticism of the mapping technique has been

raised by Hunt & Jacobson (1973e). They point out that the technique
assays visuotectal maps not retinotectal connections. Visuotectal
rather than retinotectal because optical abberations or other
factors may supervene between the topography of the visual field
and its conversion into retinotopically organized impulse trains.
Since direct retinal stimulation is not used the map is visuotectal.
Further, in most cases, it is a presynaptic map which is assayed rather
than a pattern of connections. To establish a connectivity map,
recordings must be made postsynaptically. While it remains an
assumption that the map as standardly assayed represents the pattern
of connectivity, the argument that the electrode is recording from
axon terminals renders this assumption reasonable. This criticism
was levelled particularly damningly at maps elicited from tadpoles.
Hunt & Jacobson argued that there was no evidence for functioning
synapses at the stages mapped. However, recent anatomical (Scott
1974) and electrophysiological (Chung et al 1974) has confirmed the
presence of functionally active synapses in the tadpole.

The final criticism to be made of the technique has already
been stated in a previous chapter (Ch.2.). That is, that since the
rules governing assembly of the map are only hazily known, no
unequivocal inference can be made from the map to the specificity
identities of the ganglion cell population. Only relative ordering
or polarity can be directly assayed. That is to say, reversals of
an axis or part of an axis can be assayed. Deletions of part of an
axis will not necessarily be picked up. For cases of translocation
or tandem duplication of parts of an axis, the resolving ability of
the technique is, as yet unclear.

B.   EXPERIMENTS WITH PARTIAL TECTAL ABLATION AND CHIASMA UNCROSS

B.1.   Tectal Ablation

Under MS 222 anaesthesia tadpoles at stages 54 - 59 had
their optic tectal exposed as described in section A.1 of this
chapter. Half of one tectum (either rostral or caudal half) was
excised as follows: the area to be ablated was cut free with tungsten
needles, bent to allow easy undercutting of the connection with
deeper mesencephalic levels. The area was then removed by aspiration.
Operated animals were not fed for the week following operation, but
no other precautions were found to be necessary against infection
while the wound healed.

B.2.   Chiasma Uncross

Juvenile animals approximately 6 months post-metamorphic
were anaesthetized by immersion in a 1:1,500 solution of MS 222.
The animals were then pinned out on a cork board with the mouth
pinned open and the optic chiasma sectioned through the roof of the
mouth under a Zeiss dissecting microscope. (Fig.3.) Operated animals
were not fed for the week following operation. During the period of
ensuing blindness it was necessary to hand-feed the animals. Animals
were fed either tubifex or chopped liver. Vision returned at
individually variable periods over a matter of several months.

B.3.   Types of Operations

Three classes of operated animals were produced in this series:
those with tectal ablation alone; those with chiasma uncross alone;
and those with both tectal ablation and chiasma uncross.

FIGURE 3. Diagramatic representation of the chiasma uncross
operation. The head of a post—metamorphic froglet is shown
in ventral view. The mouth has been pinned open at the
points designated by the crosses. The cartilage area shown
by dotted lines is removed to expose the optic chiasma as
illustrated in the left—hand diagram. The chiasma is then
divided completely as shown in the right—hand diagram.
U.J. — upper jaw; n.c. — nasal cavity; E — eye; b.v. —
blood vessel; O.C. — optic chiasma; L.J. — lower jaw;
X — location of pin.

U.J.

n.c.

E

b.v.

O.C.

L.J.
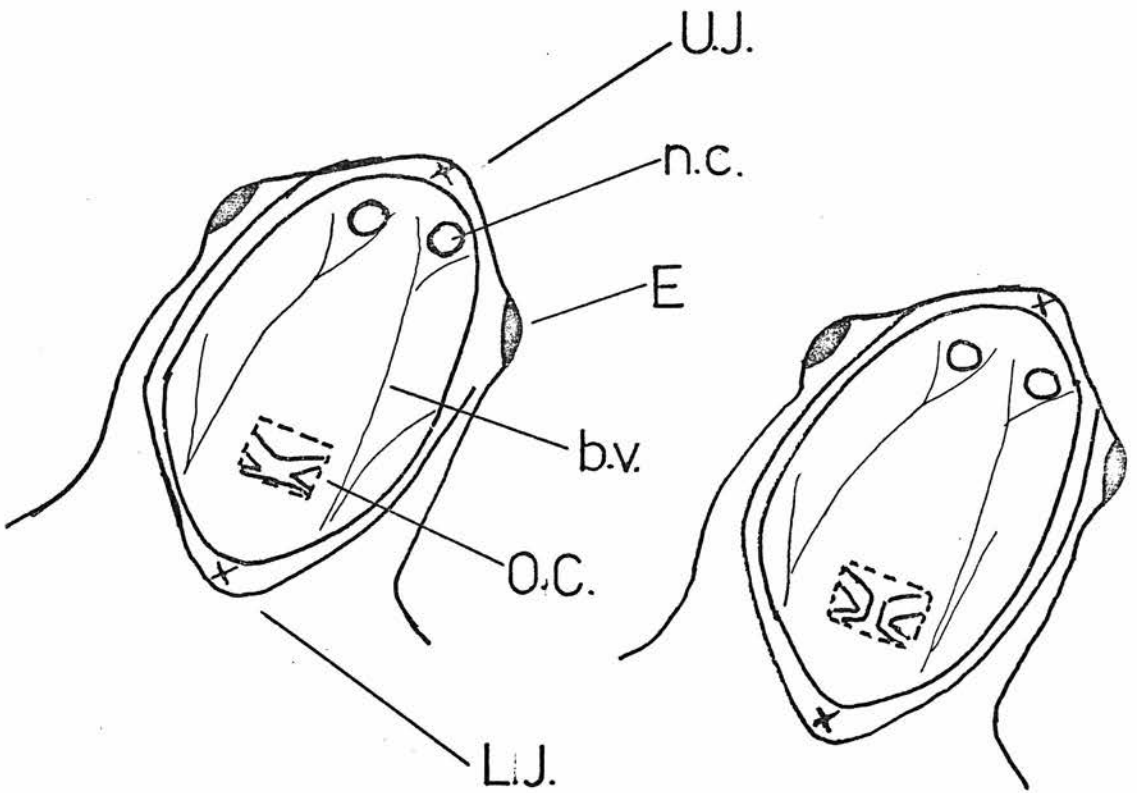
C.   EMBRYONIC OPERATIONS

C.1. General Technique

Xenopus laevis embryos were obtained from breeding pairs
in the standard manner by injection of chorionic gonadotrophin,
the male receiving 400 IU and the female 600 IU.   Embryos were
selected and staged according to the normal table of Nieuwkoop
& Faber (1956).   Operations were performed on a bed of 70%
beeswax and 30% paraffin wax under MS 222 (tricaine methane
sulphonate: Sandoz) anaesthesia in either full strength Niu-
Twitty solution or 50% Holtfreter 5% Steinberg solution
(unless otherwise stated).   Operating instruments were fine
micropipettes and electrolytically sharpened tungsten needles.
Operations were performed using a Zeiss dissecting microscope
at x25 or x50 magnification.   Following operation the embryos
were transferred to the operating solution (minus MS 222) at
50% strength for a 24 hour healing period prior to transfer to
stock rearing solution.   Operated embryos were reared in perspex
boxes (5 - 8 animals per box) in 20% solution (either 20% Stearns
or 20% Holtfreters) and fed either on strained baby soup (Heinz)
or strained nettle powder until the time of mapping.

C.2. Eye Fragments

Orthogonal fragments

The aim of the experiment was, as described in Chapter 4
to create eye fragments which either lacked or contained the

central portion of the retina. Fragments containing the centre

will be referred to as central (c) and those lacking it as

peripheral (p). In this series fragments were made in which the

plane of ablation was parallel either with the anteroposterior

axis (dorsal and ventral fragments) or with the dorsoventral

axis (nasal and temporal fragments). Figure 4a shows the position

of ablation planes in nasal central and nasal peripheral fragments.

Figure 4b shows the plane of ablation for ventral central and

ventral peripheral fragments. The following notation will be used

to describe the operations: nasal central fragments will be

referred to as $\frac{1}{2}$N(c) and nasal peripheral as $\frac{1}{2}$N(p); temporal central

fragments as $\frac{1}{2}$T(c) and peripheral as $\frac{1}{2}$T(p); ventral central and

peripheral as $\frac{1}{2}$V(c) and $\frac{1}{2}$V(p) respectively; similarly dorsal

fragments will be referred to as $\frac{1}{2}$D(c) and $\frac{1}{2}$D(p).

The majority of the operations were performed at stage 32 .

However, a small proportion of nasal, temporal and ventral fragments

were prepared at stages 35/36 and 37/38. The ectoderm over the

left eye rudiment was gently dissected away from the portion of the

rudiment to be excised with tungsten needles. The desired area

was then cut free of the remainder of the rudiment and surrounding

tissue and removed by gentle suction with a fine micropipette. In

the case of nasal and temporal fragments it was possible to use

the ventral fissure as a guideline for the location of the centre

in determining the positioning of the cuts for central and peripheral

fragments. For dorsal and ventral fragments the extent of tissue

removed had to be assessed by eye.

The majority of the operated eye rudiments had rounded up to

FIGURE 4. The construction of central and peripheral orthogonal
eye fragments (a) nasal peripheral and nasal central fragments.
(b) ventral peripheral and ventral central fragments. The
poles of the eye are shown (N,T,D and V) and the centre of
the eye is represented by a point. The fragment retained in
situ and later mapped is indicated by shading. The dashed
lines indicate the position of the plane of ablation.

FIGURE 5. The construction of central and peripheral oblique
fragments. The poles of the eye and the centre are indicated
as in Figure 4. The fragment retained (nasoventral peripheral
and central fragments are illustrated) is again indicated by
shading and the plane of ablation by the dashed lines.
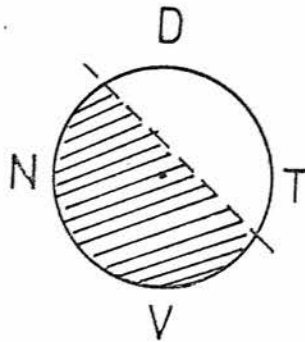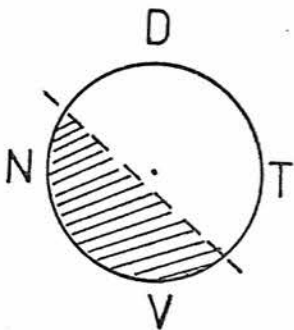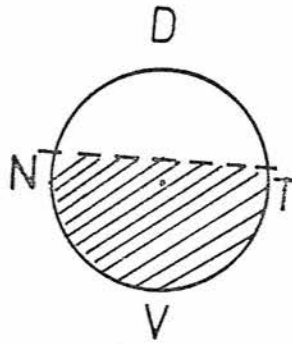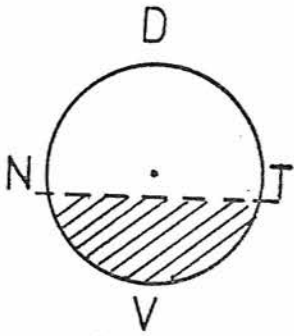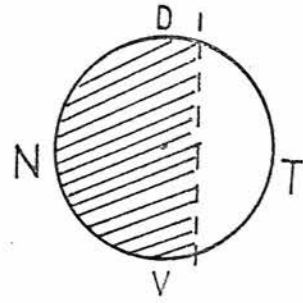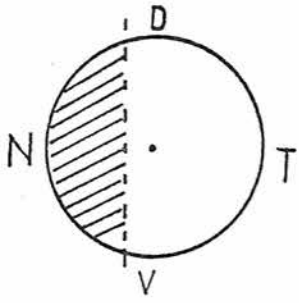
assume a morphologically normal but diminutive shape by 3-4 days of operation. By the time of mapping all the central fragments and many of the peripheral fragments were the same size as the unoperated right eye.

Oblique fragments

The operative procedure in this series was identical to that described above except that the plane of ablation ran obliquely to the main (AP and DV) embryo axes. With the same notation for central and peripheral fragments the following types of eye fragment were constructed: $\frac{1}{2}NV(c)$, $\frac{1}{2}NV(p)$ and $\frac{1}{2}TD(p)$. Figure 5 shows the plane of ablation for the $\frac{1}{2}NV(c)$ and $\frac{1}{2}NV(p)$ fragments. It will be seen that an attempt was made to retain some tissue both from the nasodorsal and from the temporoventral quadrants in the $\frac{1}{2}NV(p)$ series. In order to facilitate this control of tissue inclusion operations were all performed at stage 35/36 rather than stage 32. Rounding up of the fragments again occurred within 3-4 days of operation.

Cinemicrophotography

The initial stages of the "rounding up" of sample eye fragments were followed using time-lapse cinemicrophotography. A Bolex 16mm. camera was attached to a Wild dissecting microscope at a magnification of x 50. Operated fragments at stage 35/36 were filmed using a frame interval of 1 minute and a shutter speed of 5 seconds.

C.3.   Midline Transections

Series I & II (VMRL & HMRL)

Embryos were selected at the following stages: 29/30, 31,32, 35/36 and 37/38. Under anaesthesia, as described above, the left eye rudiment was transected along either the vertical (VMRL) or horizontal (HMRL) midlines using tungsten needles. To superficial inspection the halves healed together within 12 – 24 hours of the operation in 50% healing solution at room temperature. It was found to be necessary to keep the operated larvae under inspection during the first 24 hours due to occasional loss of part or whole of one of the fragments, due probably to excessive operative trauma. Only those animals in which the two fragments fused well by 24 hours were retained.

Series III (VMRL SC)

It was felt necessary in view of the conflict of results obtained by Hunt & Jacobson (1974b) and by myself, to control for possible effects of the ionic strength of the operating and post-operative solutions on healing times. A series of vertical midline transections were therefore made at stage 32. Animals were operated and allowed to heal in a 25% solution, (20% Holtfreters/5% Steinbergs).

Observations on Healing Rate.

The gross healing rate in Series I and Series III transections was examined by serial observation from the time of operation to 24 hours post-operation. Three animals in each group were photo-

graphed at 30 minute intervals during this period, thus building up a healing time series.

C.4, Cultured Eye Rudiment Transplants

The anterior fragments of early neurulae (stage 12/13) containing the optic and forebrain rudiments were excised from whole embryos using tungsten needles. In two cases the eye primordia transplanted were rendered semisynopthalmic by extirpation of the prechordal plate. They were divided into left and right halves and cultured in a medium composed of 95% Niu-Twitty and 5% calf serum (Flow Labs).

When synchronous normal host embryos had reached stage 37/38, the hosts were anaesthetized in MS 222 and the single eye developing in each fragment excised. The fragments were found to have self differentiated according to their presumptive fates and contained anterior ectodermal and mesodermal derivatives including cement gland, eye, forebrain and some midbrain tissue. The eyes were not synchronous in their development with those of the host embryos but lagged some 4-5 stages behind and were somewhat imperfectly formed with respect to choroid fissure closure and development of the pigment epithelium. Nevertheless they were fairly normal eyes. For left fragments the right eye of the host was excised using tungsten needles and replaced with the explant eye. Similarly right explant eyes were transplanted in place of the left eye of the host. The reason for this inter-change was to allow assessment at mapping of the origin of any. of the nasotemporal axes of the transplant eyes. An inverted

nasotemporal axis would indicate that the axis was acquired by the eye in the explanted fragment; while a normal axis would indicate that it was acquired from the host. The transplants were held in place by glass bridges for the 30-60 minutes after operation and then to 50% Niu-Twitty for 24 hours.

C.5. Cyclopean Eye Transplants

Cyclopean eyes were produced chemically using LiCl solution (1%). Batches of eggs were collected and dejellied in 10% Niu-Twitty solution. At late blastula (stage 9-) or early gastrula (stage 10-) they were pipetted into a wash solution of 1% LiCl in glass distilled water to remove Na^+ ions and then repipetted into the treatment solution (1% LiCl in glass distilled water) for 25 minutes. Eggs were then transferred through two wash solutions of 10% Niu-Twitty to remove excess Li^+ ions and stored in 10% Niu-Twitty solution. When synchronous untreated control embryos had reached stage 35/36, successfully treated cyclopean animals were selected from the experimental batch and anaesthetized along with normal host embryos in MS 222. The eye was dissected out of the cyclopean animals and assessed for true cyclopea (as opposed to minor degrees of synophthalmia). Only those eyes which appeared genuinely cyclopean were used. The left eye rudiment of host animals was then excised and the cyclopean eye transplanted in its place with normal orientation. Due to increased size of the cyclopean eyes it was frequently necessary to prepare a bed larger than the size of the extirpated host eye to receive the transplant. Glass bridges, sterilized in 70% alcohol, were used to hold the transplant in place during the

first 30 minutes to 1 hour of healing. Further healing in 50% solution for 24 hours was then allowed before transfer to rearing solution.

D.   HISTOLOGY

Routinely after electrophysiological mapping was completed animals were fixed in Susa and the structure of the optic system examined in 15 micron sections stained with Holmes silver.

CHAPTER 6 :   EXPERIMENTS WITH PARTIAL TECTA: A RE-EXAMINATION OF
              SYSTEMS MATCHING IN THE AMPHIBIAN TECTUM.

A.   HALF TECTAL ABLATION WITH CHIASMA UNCROSS

Of ten animals prepared in this series, six survived the sequence of operations and were available for electrophysiological mapping. The results obtained from these animals are shown in Table 1. Since uncrossing the optic chiasma results in each eye sending a projection to both tecta, a total of 24 maps was obtained.

A.1.   Histological Reconstruction

After mapping, animals were prepared for histology as described in Chapter 5. The relative sizes of the operated (right) and unoperated (left) tecta were determined by three dimensional reconstruction from 15 micron transverse sections. The sections were drawn out, using a camera lucida attachment to a Zeiss microscope, on graph paper, and the surface area of both tecta calculated. In the right hand column of Table 1, the surface area of the right tectum for each animal is expressed as a percentage of that of the left tectum. The hemitectal ablation was successful in all cases with the possible exception of animal $\frac{1}{2}$ T.Ch.C. 1 (right: left = 82%). As a standard for comparison a similar reconstruction was made of the tecta of five normal Xenopus. The right: left ratio for normals was found to average 94.1%, varying from 91.6% to 97.3%.

Thus, in the present experimental series, cases $\frac{1}{2}$ T.Ch.C. 2 (right: left = 44%) and $\frac{1}{2}$ T.Ch.C. 4 (right: left= 57%) clearly show successful hemitectal ablation without marked compensatory growth following the operation. Cases $\frac{1}{2}$ T.Ch.C. 6 (right: left =67%),

TABLE 1   RESULTS OF THE HALF TECTUM/CHIASMA UNCROSS EXPERIMENT

CODE	HALF ABLATED	L. EYE MAP ON R.TECTUM	L. EYE MAP ON L. TECTUM	R. EYE MAP ON R. TECTUM	R. EYE MAP ON L. TECTUM	TIME FROM CH.C. TO MAPPING	SURFACE AREA RIGHT/LEFT TECTUM (%)
½ T.Ch.C. 1	rostral	?	W.F.[a]	?	?	551 d.	82%
½ T.Ch.C. 2	caudal	W.F.	W.F.[a,b]	?	W.F.[c]	557 d.	44%
½ T.Ch.C. 3	caudal	W.F.[a]	W.F.[b]	C.F.(indirect)	W.F.[c]	442 d.	73%
½ T.Ch.C. 4	rostral	T.F.	T.F.	W.F.[a]	T.F.	570 d.	57%
½ T.Ch.C. 5	rostral	W.F.[a]	C.F.	C.F.[c]	W.F.[c,d]	593 d.	63%
½ T.Ch.C. 6	caudal	W.F.[a]	W.F.	C.F.[c]	W.F.[c]	510 d.	67%

Except for case ½ T.Ch.C. 4, all maps were obtained with the left eye centred. Thus exact maps are obtainable only through the left eye.

W.F. - whole field; T.F. - temporal field only; N.F. - nasal field only; C.F.-central field only;

? - inconclusive result due to paucity of localizable positions; a - whole field minus the most nasodorsal field positions; b - nasal extension of map probably due to indirect input; c - estimate (mapped with other eye centred); d - temporal extremity missing.

85.

$\frac{1}{2}$ T.Ch.C. 5 (right: left = 63%) and $\frac{1}{2}$ T.Ch.C. 3 (right: left = 73%)
fall outwith the normal range of size variation for unoperated
animals and also represent successful partial tectal ablation.

A.2.   Electrophysiological Mapping

In all cases, except $\frac{1}{2}$ T.Ch.C. 4, the animals were mapped
with the left eye centred on the perimeter. This meant that,
while maps were also obtained for the right eye, their axes were
somewhat skewed from normal (see Figures 7b, 8b, 9b, 10b and 11b).
The nasal pole of the right eye field is represented at the
centre of the perimeter, while the temporal pole is represented
beyond the edge of the perimeter at which the nasal pole of the
left field is represented. This meant that the extent of field
coverage in the right eye maps could only be roughly estimated.
These comments do not apply to right eye maps in case $\frac{1}{2}$ T.Ch.C. 4.
In this case the animal was mapped twice, once with the left eye
and again with the right eye centred.

Despite the disadvantage of mapping both eyes with respect
to the visual field of the left eye, this orientation allows the
left and right eye maps to be compared directly, since they both
represent the same visual space. This becomes important in
distinguishing the contributions made to the maps by direct and
indirect projections. In the normal animal as well as the direct,
completely crossed, projection of the whole field to the whole
contralateral tectum, there is an indirect projection from nasal
field to rostral ipsilateral tectum. This projection is thought to
be mediated by an intertectal linkage such that part of the contra-

lateral projection is mapped onto the ipsilateral tectum (Keating & Gaze 1970b). In the present experiments, uncrossing the chiasma will result in the formation of <u>direct</u> ipsilateral projections. Thus the eye of origin of a projection or partial projection cannot be used, as it could in the normal map, to determine whether or not it is mediated by the direct or the indirect pathway. However, there is evidence to suggest that the intertectal synapses are established under functional control, unlike the case for the direct projection, such that the intertectal relay links points on the two tecta which are receiving input from the same region of visual space (Keating 1968, Keating & Gaze 1970a). With both eyes centred on the same visual space points in both maps projecting to the same tectal site can be directly compared. Where the points are located in the appropriate part of the visual field for the visuotopy of the map, they are considered to be mediated by the direct projection. Conversely, where they are located in the same part of visual space, with consequent violation of visuotopy, they are considered to be mediated by the indirect projection. For example, in Figures 9a and b, consider positions 8. Position 8 in the left eye map (Fig. 9a) shows visuotopy as does the more temporal of the two positions 8 in the right eye map (Fig. 9b). However the position 8 near the centre of Fig. 9b does not show visuotopy and is located in a position congruent with position 8 in Figure 9a. This position is considered to be an indirect input.

Of the twelve maps obtained on operated tecta, three were inconclusive due to a paucity of localizable field positions; 3 showed only central field; one showed only temporal field; one

showed full field representation; and 4 showed a characteristic
map in which the entire field was represented minus a varying
amount of the nasodorsal quadrant. Of the 12 maps to the unoperated
tectum, one was inconclusive; one showed central field only;
2 had representation of temporal field only; 6 had full field
representation; and 2 showed the characteristic partially complete
map with nasodorsal retina absent.

Operations in all cases were performed on the right tectum
only. Either the rostral or the caudal half tectum was ablated.
Were connections to be formed with the residual half tectum in
the manner typical of the normal map, a residual rostral half
tectum should receive input only from the nasal half of the field
and a residual caudal half tectum only from the temporal half of
the field. It was the aim of the present experiments to investigate
how far such "appropriate" map connections were in fact established.

Maps on the Operated Tectum.

As summarised above, there was considerable variability
in the results obtained. Only a single "appropriate" map was
found. The map from the left eye to the residual caudal half
tectum in case $\frac{1}{2}$ T.Ch.C. 4 shows temporal field representation
only (Fig. 6a). At the other extreme, only a single case of full
field coverage was found. In case $\frac{1}{2}$ T.Ch.C. 2 the left eye to
the residual caudal half tectum (Fig. 7a), not only is the entire
temporal field represented but also the entire nasal field. The
map is retinotopically organized without obvious distortions or
unequal compression of the "inappropriate" nasal half field.

FIGURE 6. Visuotectal maps to a caudal half tectum (right)
after uncrossing the otpic chiasma in case $\frac{1}{2}$T.Ch.C.4 (a) left
field (centred.) map showing temporal field representation
(b) right field (centred.) map showing a characteristically
partially complete map. The poles of the visual field are
represented as follows: nasal (N), temporal (T), superior
(S) and inferior (I). The rostral midline of the tectum is
indicated by the arrow. The numbers in the visual field are
those at which stimulation evoked maximal response at the
similarly numbered electrode position on the tectum. Filled
circles on the tectum indicate electrode positions at which
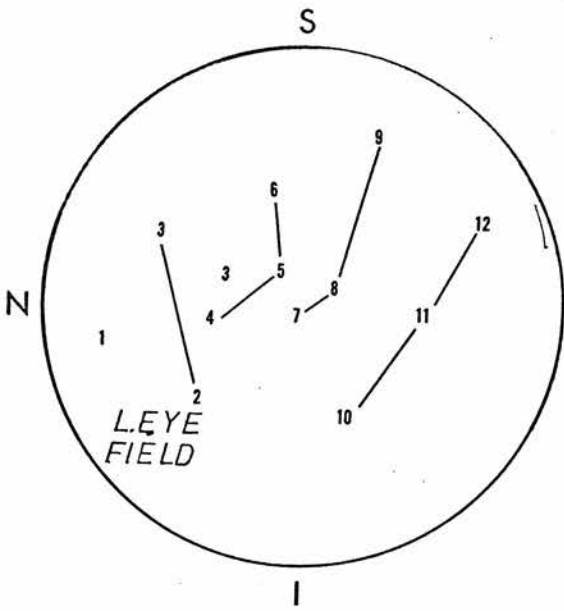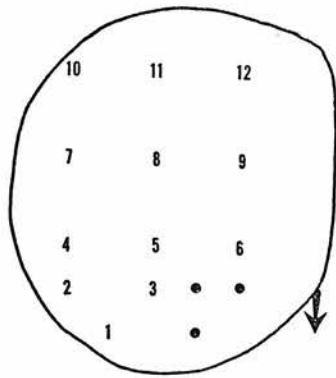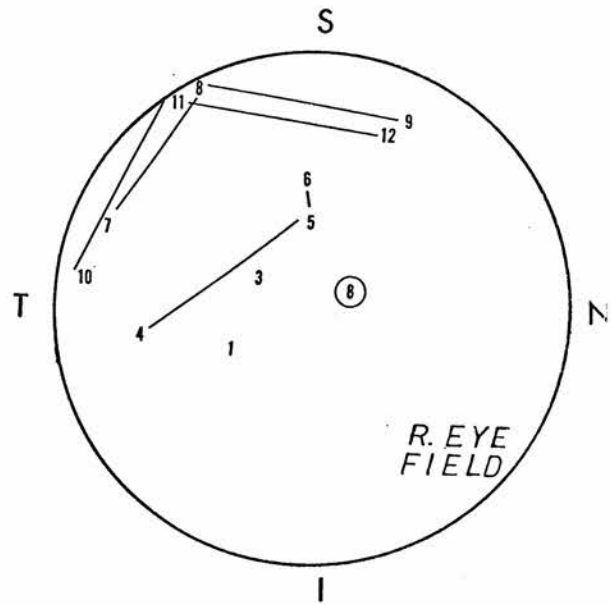no visual field position was localizable.
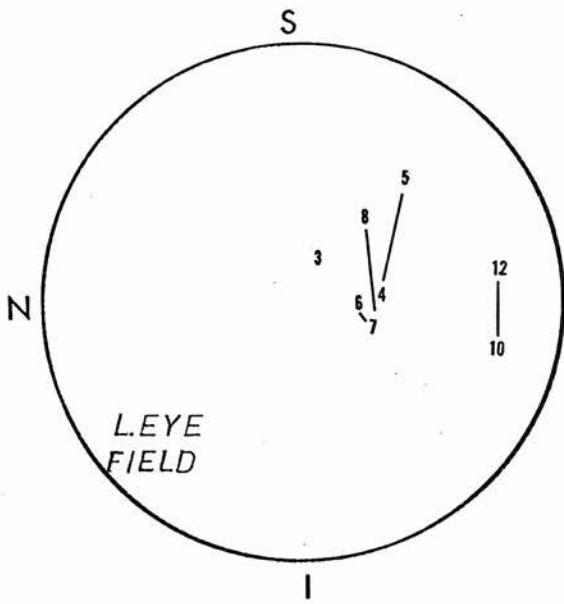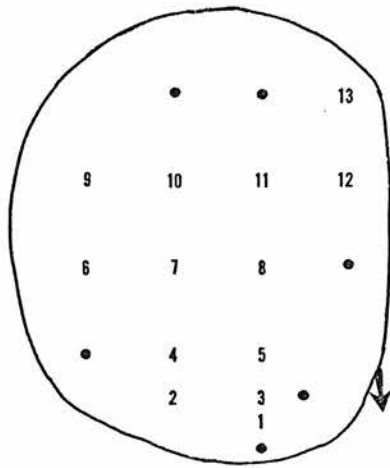
6 (a)
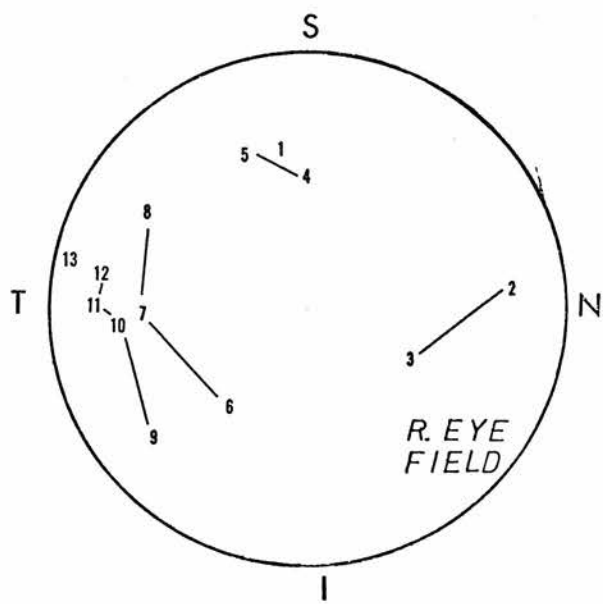
6(b)

FIGURE 7. Visuotectal maps to a rostral half tectum (right) after uncrossing the optic chiasma in case $\frac{1}{2}$T.Ch.C. 2 (a) left field (centred) map showing full field representation (b) right field (left eye centred) map showing paucity of field positions and poor ordering. Field positions identified as projecting via the indirect pathway are circled. Other conventions as in Figure 6.
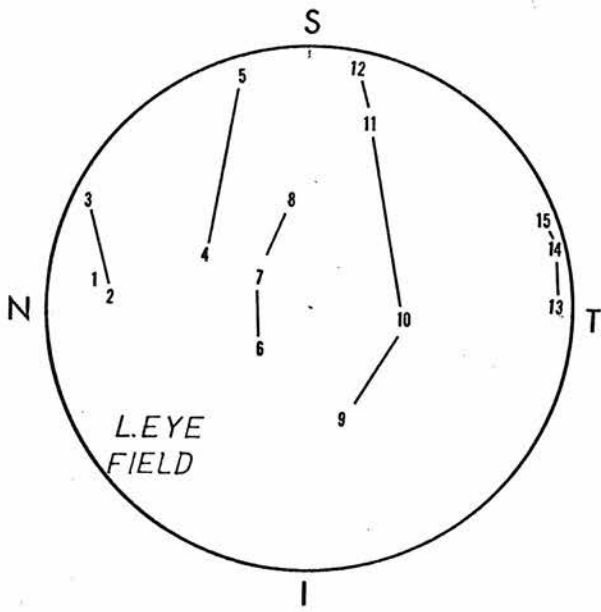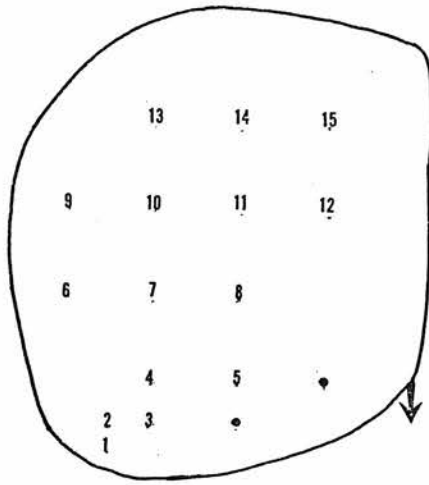
7(a)
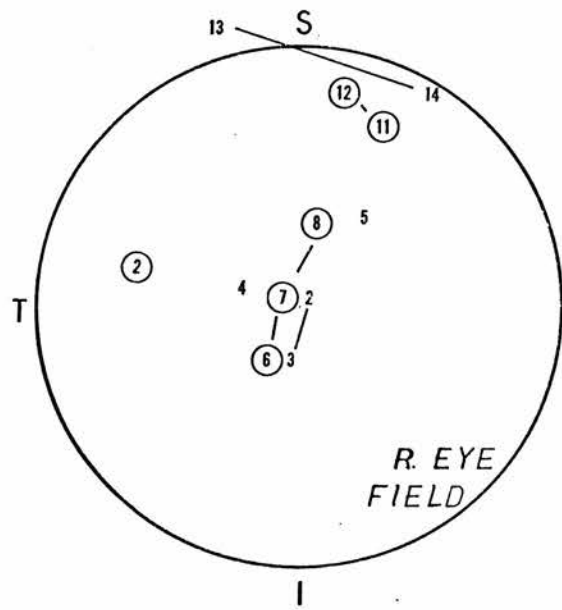
7(b)

However, an additional four maps (see Figures 9a, 10a and 6b) also showed a degree of field representation in excess of that appropriate to the residual half tectum. These maps are classed together in that they appeared to show temporal field representation with the most ventral aspect of the nasal half field also present. The severity of the nasodorsal field deficit was variable. This characteristic partially complete map was found both after rostral ($\frac{1}{2}$ T.Ch.C. 4 and $\frac{1}{2}$ T.Ch.C. 5) and after caudal ($\frac{1}{2}$T.Ch.C. 3 and $\frac{1}{2}$ T.Ch.C. 6) hemitectal ablation. There was no indication of a complementary map configuration in which some other field deficit reproducibly occurred.

An additional 3 maps showed representation of only the central field along the vertical midline. This configuration occurred in the maps from the right eye in cases $\frac{1}{2}$ T.Ch.C. 3 (Fig. 10b), $\frac{1}{2}$ T.Ch.C. 5 (Fig. 9b) and $\frac{1}{2}$ T.Ch.C. 6. Comparing Figures 10a and 10b, it will be seen that the right eye map (Fig. 10b) is produced almost entirely by the indirect ipsilateral pathway. This is indicated by the congruence of complementary points in the two maps and the curvature of the field rows in Fig. 10b which parallels that of the left eye map (Fig. 10a) rather than following the contours expected for a right eye mapped with the left eye centred (c.f. Figure 8b). The right eye to right tectum map in Figure 9b shows a single position which appears to be mediated via the indirect pathway. This is position 8 in the most nasal part of the field as discussed above. This point is clearly abberent with respect to the rest of the map, and can be identified as an indirect projection by virtue of its congruence with position 10 in the map from the left eye (Fig. 9a).

FIGURE 8. Visuotectal maps to a normal (left) tectum after
uncrossing the optic chiasma in case $\frac{1}{2}$T.Ch.C. 2. (a) left
field (centred) map showing a temporal field representation
via the direct projection and a projection from nasoventral
field via the indirect pathway (b) right field (left eye
centred) map showing full field representation. Conventions
as in Figures 6 and 7.
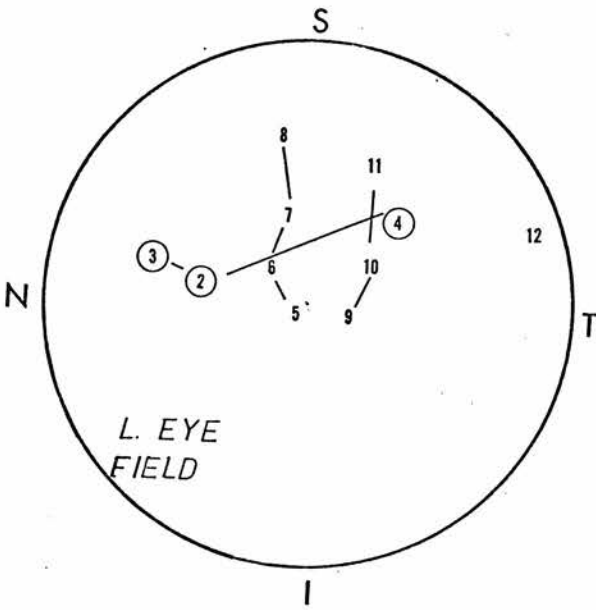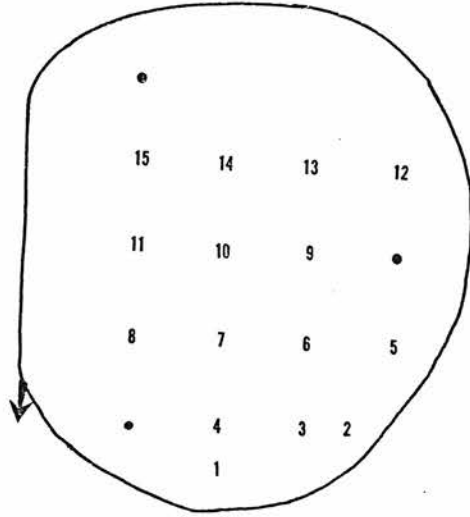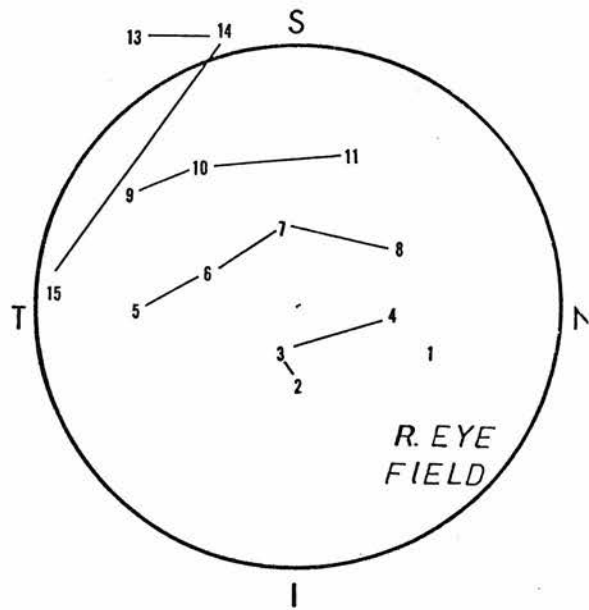
8(a) 8(b)

FIGURE 9. Visuotectal maps to a caudal half tectum (right) after uncrossing the optic chiasma in case $\frac{1}{2}$T.Ch.C. 5. (a) left field (centred) showing a characteristically partially complete map (b) right field (left eye centred) map showing a representation of central field via the direct projection. Conventions as in Figures 6 and 7.

9(a)   9(b)

FIGURE 10. Visuotectal maps to a rostral half tectum (right) after uncrossing the optic chiasma in case $\frac{1}{2}$T.Ch.C. 3. (a) left field (centred) map showing characteristic partially complete projection (b) right field (left eye centred) map composed entirely of indirect input. Conventions as in Figures 6 and 7.
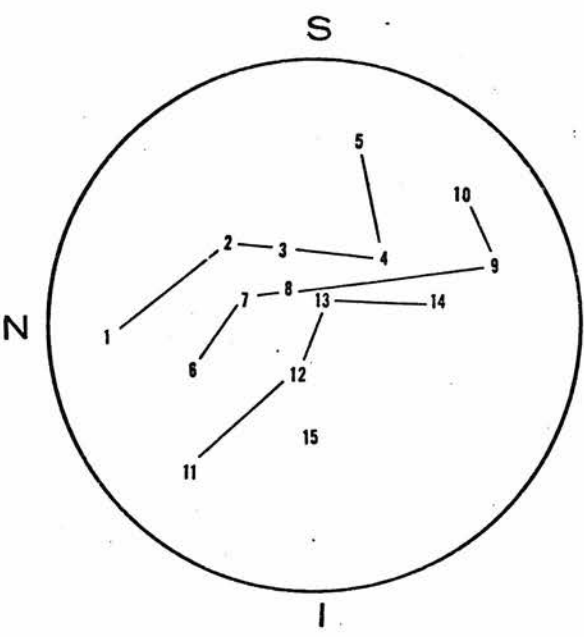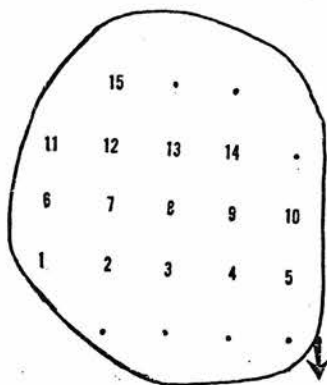
10(a)
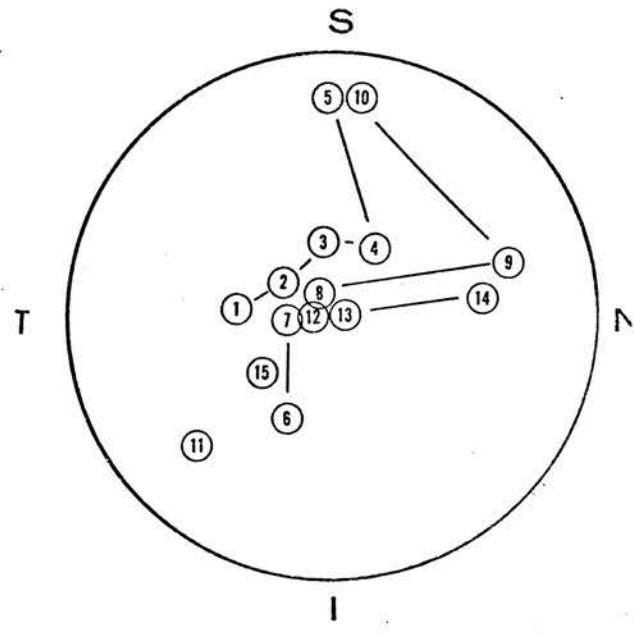
10(b)

Maps on the Unoperated Tectum.

The "appropriate" map from an unoperated eye to an unoperated tectum is, obviously, full field representation. Of the 11 interpretable cases in this group, 4 yielded such normal maps. Figures 8b and 11b illustrate this situation. However, the majority of maps in this group again showed varying degrees of completeness. Both left and right eyes in case $\frac{1}{2}$ T.Ch.C. 4 sent only temporal field projections to the left tectum. Two further cases ($\frac{1}{2}$ T.Ch.C. 2 and $\frac{1}{2}$ T.Ch.C. 3) would also appear to fall into this class. The most nasal field positions in the left eye to left tectum maps in these cases (Figs. 8a and 11a) are identified as ipsilateral (indirect) projections by the criteria outlined above (non-visuotopy and congruence with corresponding positions in the right eye maps). The most nasal positions in Figure 11a are, like position 8 in Figure 9b, reduplications projecting more caudally in the tectum than is normal for the direct input from the nasal field. Thus the direct map in Figures 8a and 11a is simply a temporal field represenation. In case $\frac{1}{2}$ T.Ch.C. 5 both left and right eye maps to the left tectum are incomplete. The left eye map shows a representation of the central field along the vertical midline and the right eye map a whole field minus the temporal extremity.

Direct and Indirect Projections

As discussed above, several of the maps obtained in the present studies were composites of direct (visuotopic) and indirect (non-visuotopic and congruent) inputs. The indirect inputs were in all cases located in the nasal field and tended to project

FIGURE 11. Visuotectal maps to a normal (left) tectum after uncrossing the optic chiasma in case $\frac{1}{2}$T.Ch.C. 3. (a) left field (centred) map showing temporal half field representation via the direct and nasal half field representation via the indirect pathway (b) right field (left eye centred) map showing full field representation with additional indirect inputs from nasal field. Conventions as in Figures 6 and 7.
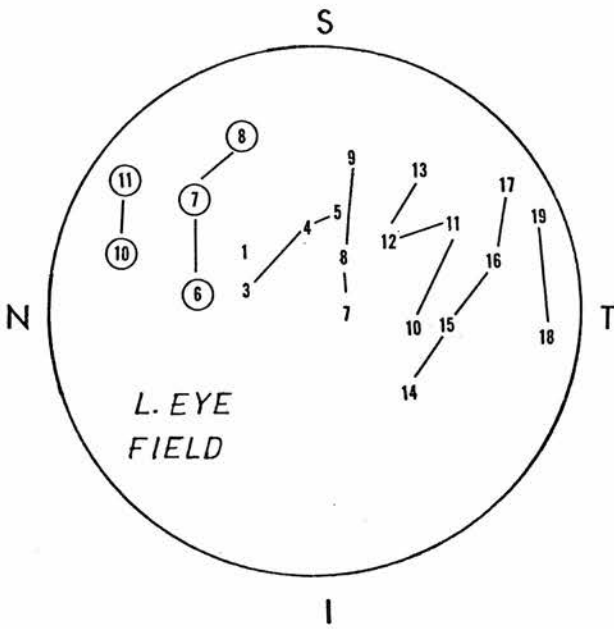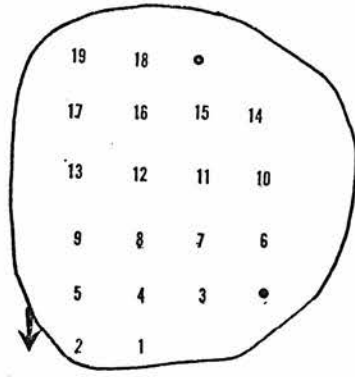
11(a)
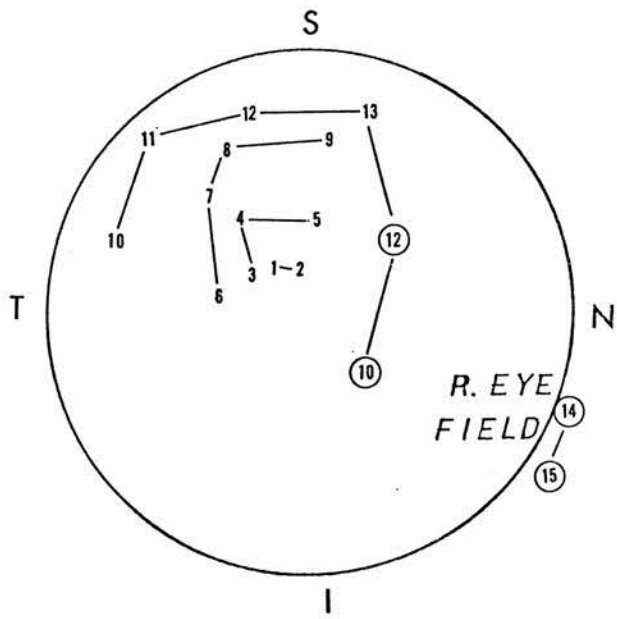
11(b)

rostrally in the tectum. Congruent points located in the
temporal field and/or projecting caudally in the tectum were
never found. Direct inputs could be found in all regions of
the visual field and projected to appropriate regions of the
tectum. This organization of direct and indirect inputs is
similar to that found in the normal map, where only nasal field
contributes to the indirect input (this being the only region
of the field which falls within the overlapping visual space
of both eyes).

B.   CHIASMA UNCROSS ALONE

The survival rate in this series was rather low. Of
19 animals prepared, 11 succumbed to infection and 2 died during
the preparation for mapping. The results obtained from the
remaining 6 animals are shown in Table 2. Maps of varying degrees
of completeness were obtained, the variability in this series
being if anything, somewhat greater than in the previous series.
In total the series comprised 22 maps. Of these 15 were made with
the relevent eye centred, thus allowing full analysis of the map.
One of these contained too few points to allow of analysis and
the remaining 14 broke down as follows: 2 whole field maps; 2 maps
showing the characteristically partially complete configuration
(nasodorsal field absent) found in the previous series; 1 with
a similar configuration but with corresponding non-responsive
sites in rostro-medial tectum; 5 temporal field maps; 1 temporal
field map with electrically silent regions in rostral tectum
corresponding to the field deficit; 1 with only central field
represented; 1 with only vertical midline representation; 1 with

TABLE 2   RESULTS OF THE CHIASMA UNCROSS EXPERIMENT

CODE	L. EYE MAP ON		R. EYE MAP ON		TIME FROM CH.C.
	R. TECTUM	L. TECTUM	R. TECTUM	L. TECTUM	TO MAPPING
Ch.C. 2.1	C.F.	T.F.	W.F.	T.F.	399 d.
Ch.C. 1.1	?	W.F.[a]	W.F.[a]	D.F. absent[c]	405 d.
Ch.C. 1.2	W.F.[f]	N.F.[f]	T.F.[b]	W.F.[a,d]	419 d.
Ch.C. 1.3	T.F.	?	N.F.[f]	vert. midl.[e]	529 d.
Ch.C. 1.4	N.F.[f]	T.F.	W.F.	N.F.(indirect)	531 d.
Ch.C. 1.5	n.o.	T.F.	n.o.	?	580 d.

W.F. – whole field; T.F. – temporal field only; N.F. – nasal field only; D.F. – dorsal field; ? – inconclusive result due to paucity of positions localizable; vert. midl. – vertical midline of the field; n.o. – not obtained.

a – nasodorsal field positions missing; b – no responses localizable from rostral tectum; c – no responses localizable from rostral and medial tectum; d – no responses localizable from most rostro-medial tectum; e – no responses localizable from caudal tectum; f – estimate (eye mapped with other eye centred).

the most dorsal field missing and corresponding electrically
silent regions in rostral and medial tectum. Seven maps were
obtained where the animal was centred on the opposite eye. Of
these 2 contained too few points for analysis and the remaining
5 break down as follows: 1 apparently whole field map; 2
apparently nasal field maps; 1 nasal field map which is probably
an indirect input and 1 clearly indirect input.

The primary object of this series was to serve as a control
for the previous one: to allow of evaluation of the effects of
chiasma uncross alone. As such, it is significant that the
present results contain all the major map configurations found
in the previous series.

The "appropriate" map for this paradigm (whole eye to
whole tectum) was found in only two cases. These are illustrated
in Figures 12b and 14b. It will readily be seen that, the right
eye to right tectum map of case Ch.C. 2.1 (Fig. 12b) is in fact
a composite of direct and indirect inputs. The most nasal
positions in Figure 12b do not preserve visuotopy in their tectal
representation and are thus likely to be subserved by the indirect
pathway. The criterion of congruence cannot be applied in this
case as the left eye map (Fig. 12a) was made with the left eye
centred and the right eye map (Fig. 12b) with the right eye centred.
Thus whole field representation via the direct pathway occurs in
only one case (Fig. 14b) in the right eye to right tectum map of
case Ch.C. 1.4

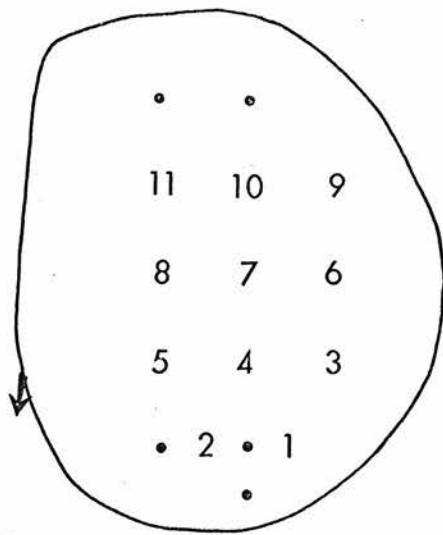The characteristic partially complete map is illustrated

FIGURE 12. Visuotectal maps to a normal (right) tectum
after uncrossing the optic chiasma in case Ch.C. 2.1
(a) left field (centred) map showing temporal field
representation (b) right field (centred) map showing full
field representation with most nasal field projecting via
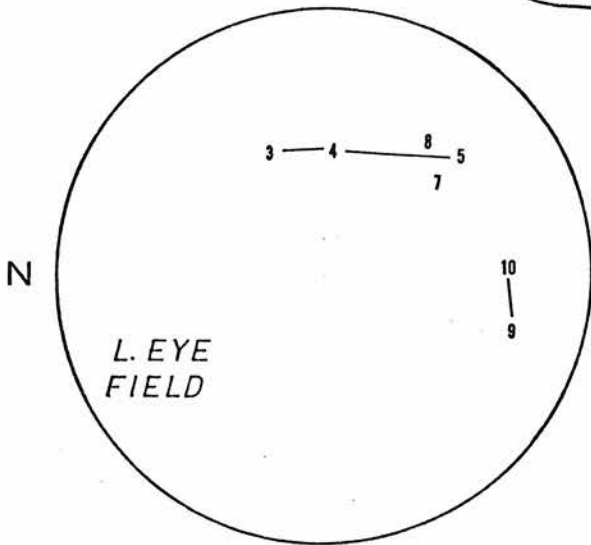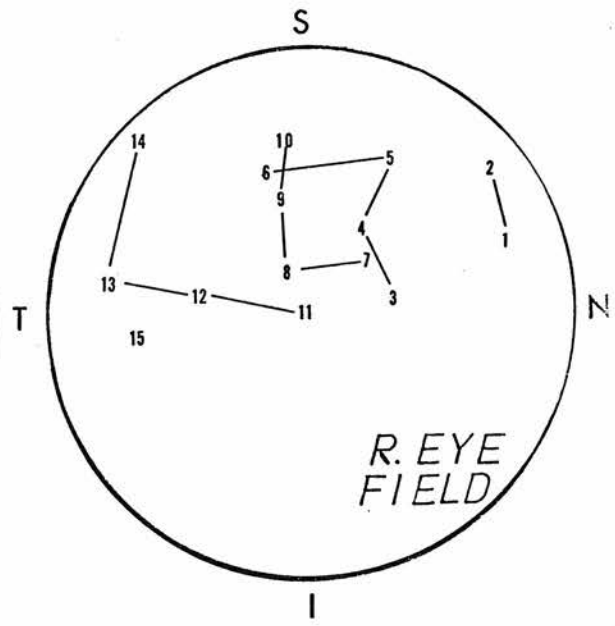the indirect pathway. Conventions as in Figures 6 and 7.
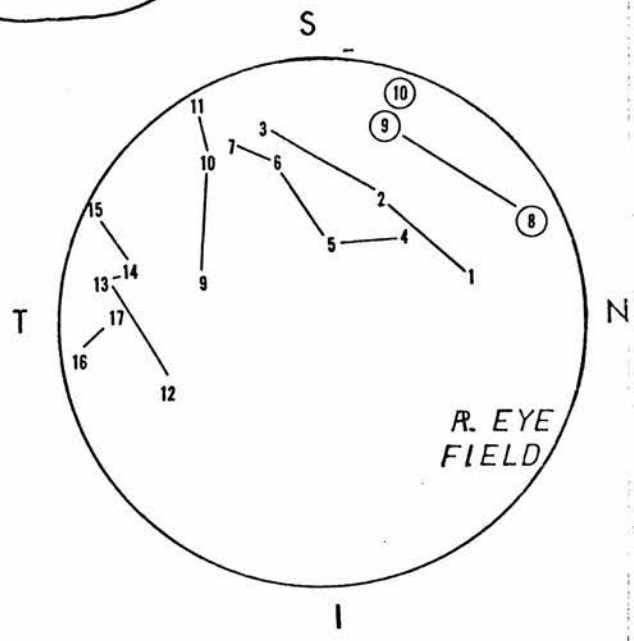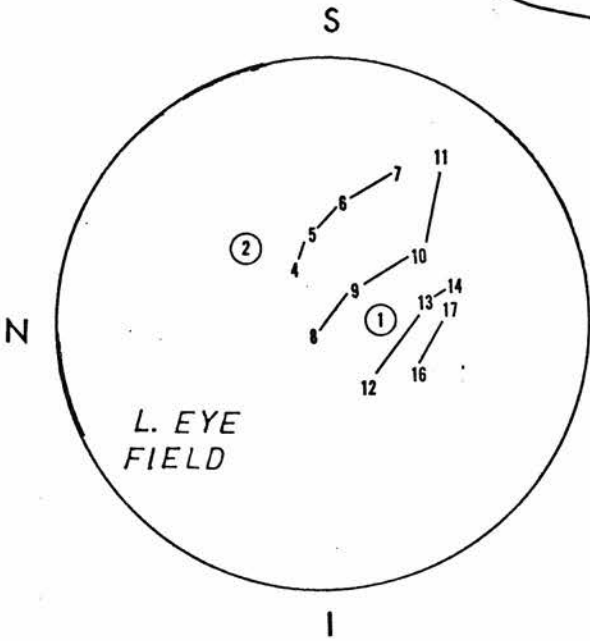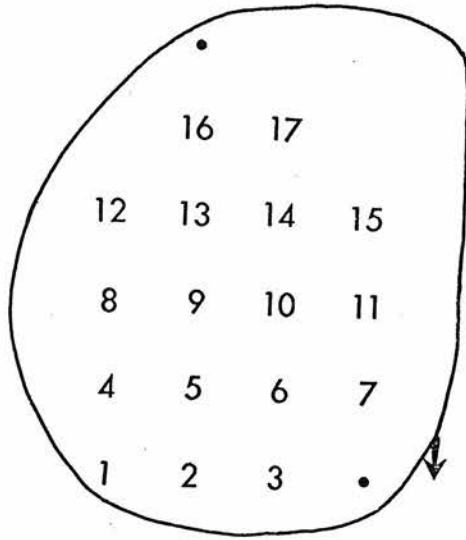
12(a) 12(b)

FIGURE 13. Visuotectal maps to a normal (left) tectum
after uncrossing the optic chiasma in case Ch.C. 2.1
(a) left field (centred) map showing temporal field
representation (b) right field (centred) map showing
temporal field representation. Conventions as in
Figures 6 and 7.

S   S

N   T   N

L. EYE FIELD   R. EYE FIELD

I   I
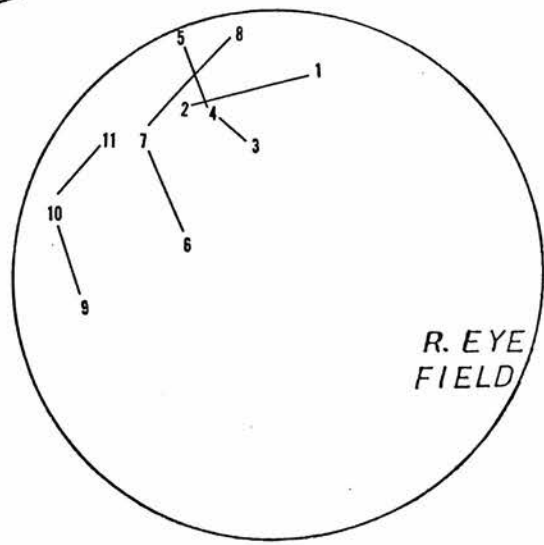
13(a)   13(b)

FIGURE 14. Visuotectal maps to a normal (right) tectum after uncrossing the optic chiasma in case Ch.C. 1.4 (a) left field (right eye centred) map composed entirely of indirect inputs (b) right field (centred) map showing full field representation. Conventions as in Figures 6 and 7.
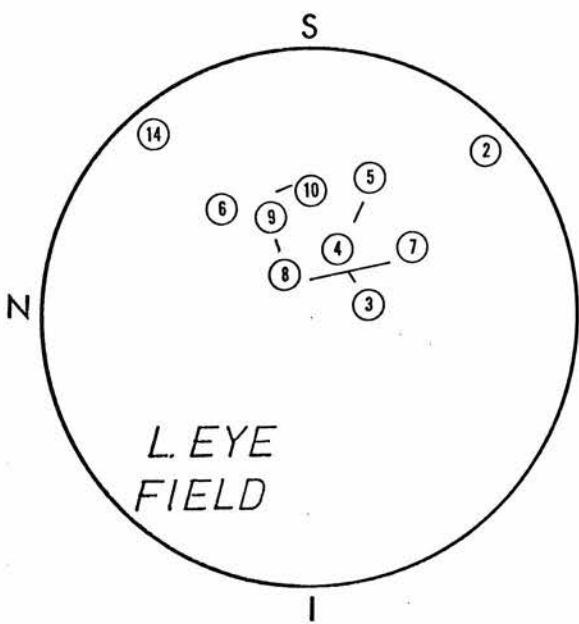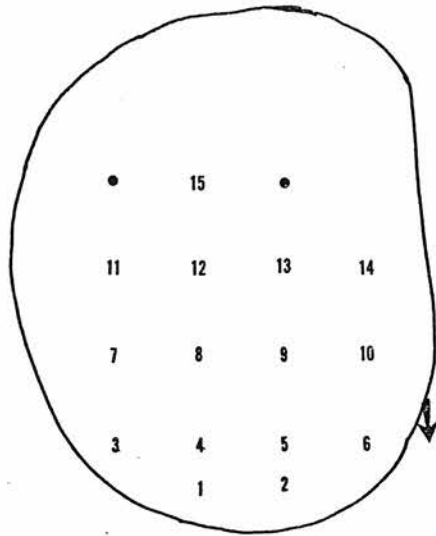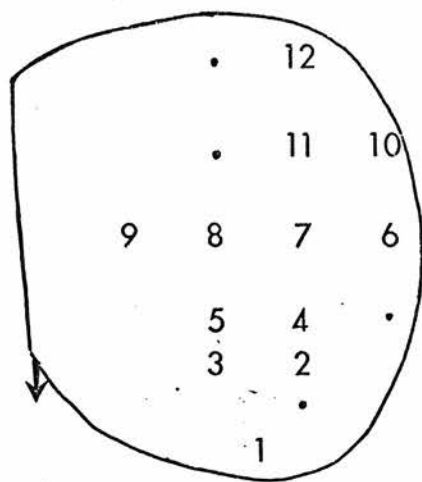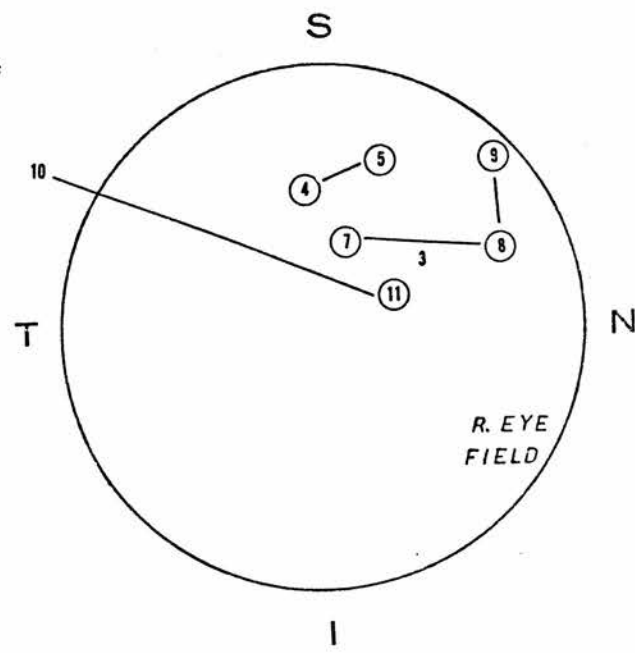
14(a)

14(b)

FIGURE 14. Visuotectal maps to a normal (left)
tectum after uncrossing the optic chiasma in case
Ch.C. 1.4. (c) left field (centre) map showing
temporal field representation (d) right field
(left eye centred) map showing nasal field
representation via the indirect pathway.
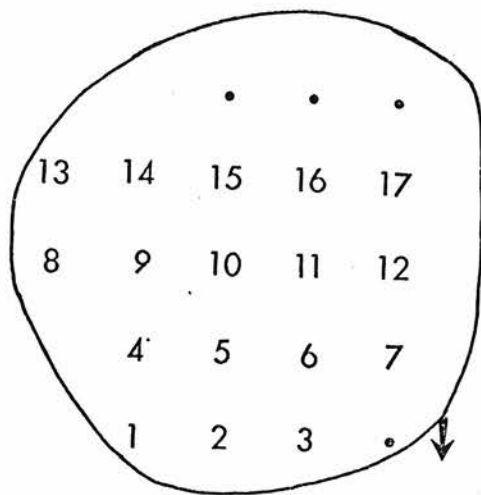Conventions as in Figures 6 and 7.
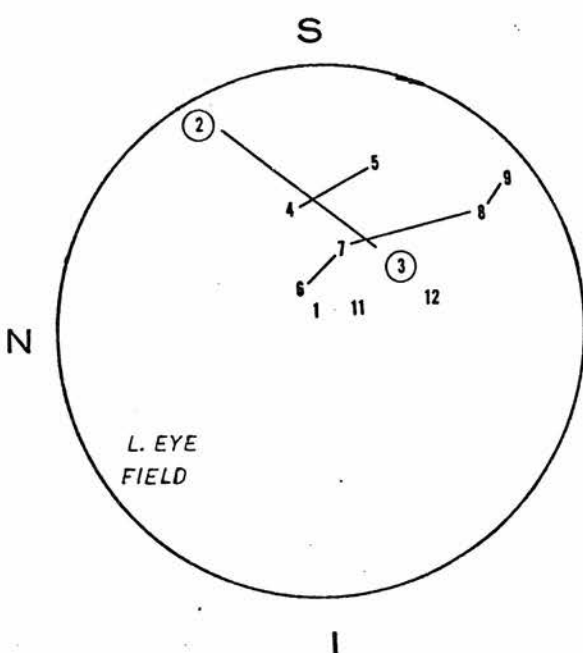
14(c)

14(d)

in Figures 15b and 16a while Figure 17b illustrates the case of
the right eye to left tectum map in animal Ch.C. 1.2 where this
map configuration was accompanied by corresponding electrically
silent regions of rostromedial tectum.

As in the previous series, temporal field maps formed the
majority category of the class of half field maps. Examples
are shown in Figures 12a, 13a, 13b. The map shown in Figure 16b
also represents a temporal half field direct projection with an
additional nasal extension subserved by the indirect pathway.
The two possible nasal half field projections were found with
the non-mapped eye centred and hence their identification is
equivocal. An example is shown in Figure 17a. A further minority
class of incomplete map is shown in Figure 14a. The absence of
visuotopy and the congruence of all points with the right eye
map (Fig. 14b) indicate that this entire map is an indirect
projection.
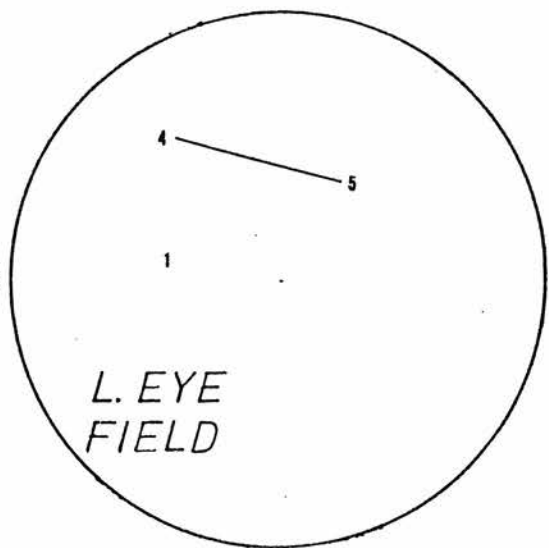
Direct and Indirect Projections

Examination of the maps obtained in the present series
reveals that, as in the previous series, several of the maps
were composites of direct and indirect inputs. Again the indirect
inputs were restricted to nasal field and rostral or mid-tectum.
Thus again it can be concluded that those retinal regions programmed
to produce the indirect projection in the normal situation are
the only ones doing so after chiasma uncross.

FIGURE 15. Visuotectal maps to a normal (right) tectum after
uncrossing the optic chiasma in case Ch.C. 1.1 (a) left field
(centred) map exhibiting too few points for analysis (b)
right field (centred) map showing the characteristic partially
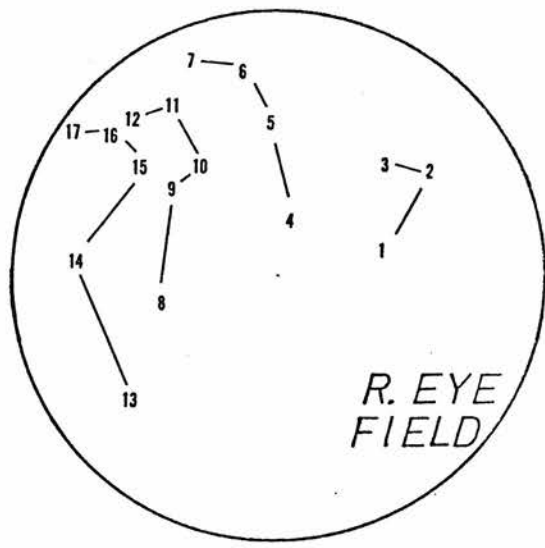complete map. Conventions as in Figures 6 and 7.

13 14   15   16   17

8   9   10   11   12

4   5   6   7

1   2   3

S   S

4
      5

1

N   T   N

L. EYE
FIELD   R. EYE
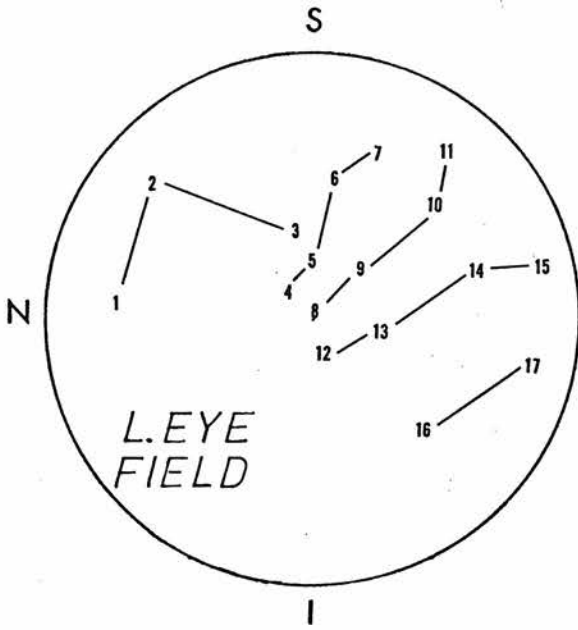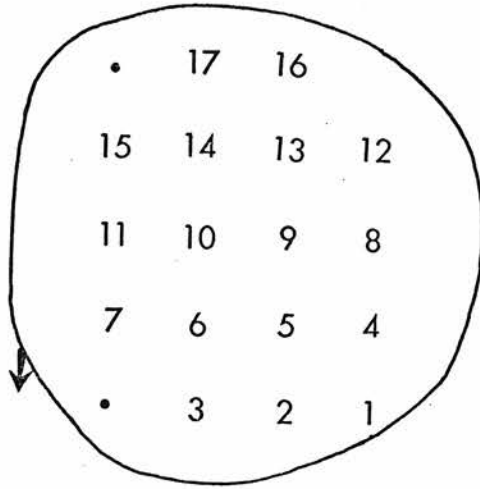   FIELD

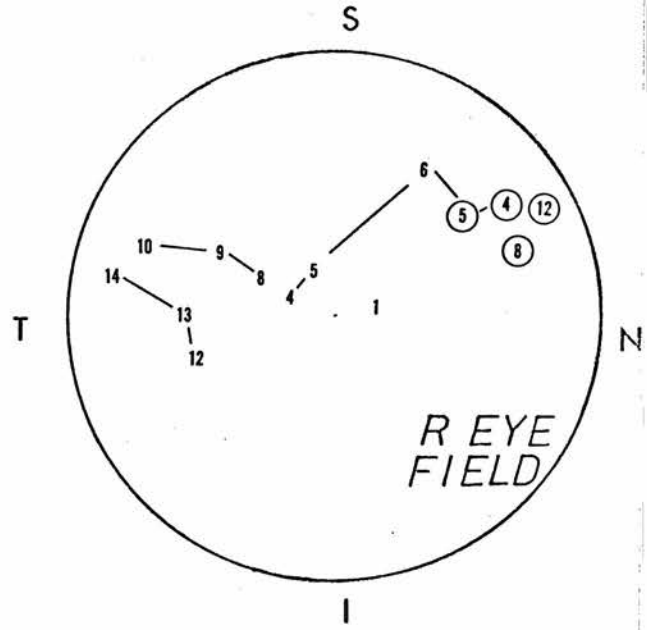I   I

15(a)   15(b)

FIGURE 16. Visuotectal maps to a normal (left) tectum after uncrossing the optic chiasma in case Ch.C. 1.1 (a) left field (centred) map showing the characteristic partially complete map (b) right field (centred) map showing an absence of representation of the most dorsal field. Conventions as in Figures 6 and 7.
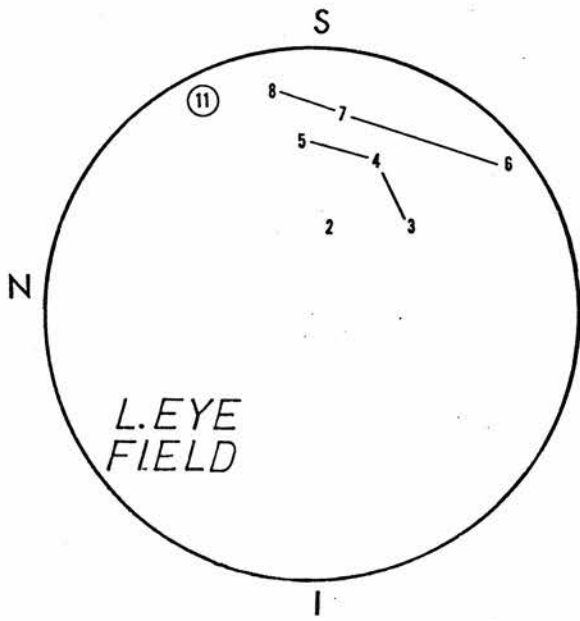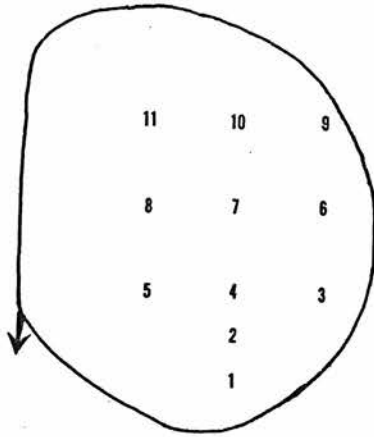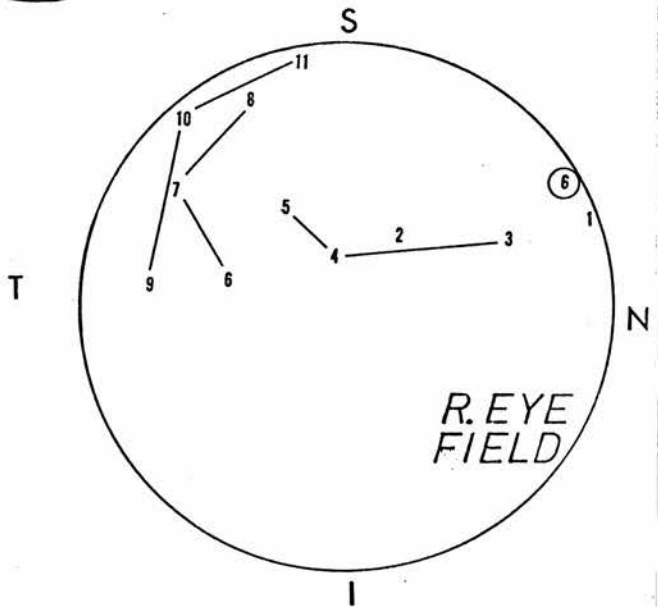
16(a)

16(b)

FIGURE 17. Visuotectal maps to a normal (left) tectum after
uncrossing the optic chiasma in case Ch.C. 1.2 (a) left field
(right eye centred) map showing nasal field representation
(b) right field (centred) map showing a characteristic
partially complete map.

17(a)

17(b)

Connectivity Mismatch

In addition to this series acting as a control for the
half tectal/chiasma uncross studies it was hoped that it might
serendipitously provide a result encountered in a previous
experiment utilizing chiasma uncross: that where the whole of
a known normal eye and part of another known normal eye project
in register across the entire extent of a tectum (Gaze, Keating
& Feldman: unpublished; discussed in Chapter 2.) Ideally such
a result might involve both eyes mapping completely to one
tectum (thus establishing their normality) and the whole of
one eye and part of the other eye mapping to the other tectum.
Such a result was not however obtained. In the present series
only two whole field maps were obtained (Figs. 12b and 14b).
Both these cases (Ch.C. 2.1. and 1.4) fail to achieve this ideal
configuration. However in both cases the right eye yields a full
field projection on one tectum and a partial projection across
the whole of the other tectum. The significance of this result
will be dealt with more fully in the discussion that follows.

C.   DISCUSSION OF EXPERIMENTS WITH PARTIAL TECTA AND UNCROSSED
       CHIASMA

C.1.   Field Compression in Anuran Partial Tecta

In the experiments with half tectal ablation plus chiasma
uncross a variety of map configurations was found with a range
of completeness of field representation. Undoubtedly the most
significant result however is that shown in Figure 7a. Here a

whole field representation is achieved on a residual rostral half tectum whose surface area at the time of mapping was 44% that of the unoperated tectum (Table 1.). In this case, at least, full field compression has occurred onto a partial tectum in the manner described in teleost experiments.

At first sight, the class of results designated as characteristic partially complete maps also represent a compression. Since the whole field is represented with the exception of the nasodorsal field positions this configuration exhibits a field representation in excess of that predicted for the formation of "appropriate" connections on a half tectum. However, before advancing this conclusion it is necessary to eliminate the alternative possibilities. The major alternative interpretation is that these results derived from partially successful half tectal ablations. Since it is difficult to determine the precise position of the lateral edge, it is possible that the map configuration might be accounted for by an attempted rostral hemitectal ablation which spared the rostrolateral aspect of the tectum. Four arguments tell against this possibility.

a)   No evidence of residual lateral tectum was found in the histological reconstructions.

b)   This map configuration was found on the operated tectum in 2 cases of caudal hemitectal ablation ($\frac{1}{2}$ T.Ch.C. 3 and 6: see Figure 10a) as well as two cases of rostral hemitectal ablation ($\frac{1}{2}$ T.Ch.C. 4 and 5: Figs. 6b and 9a). In the two cases of caudal hemitectal ablation the temporal half field is well represented.

c)   The result is also obtained on intact tecta in both the half tectum/chiasma uncross situation ($\frac{1}{2}$ T.Ch.C. 1 and 2: Fig. 8a) and in the chiasma uncross alone situation (Ch.C. 1.1 and 1.2 : Fig. 15b and 17b). Thus its occurrence is not dependent on tectal operation.

d)   In several of the cases where this result is obtained there are silent tectal regions corresponding to the nasodorsal field deficit ($\frac{1}{2}$ T.Ch.C. 3 and 4: Figs. 6b; Ch.C. 1.1 and 1.2: Figs. 15b and 17b). This argues a failure to establish functional connections rather than an absence of the appropriate tectal sites.

It would seem, therefore, that the characteristic partially complete map cannot be attributed to an incomplete hemitectal ablation sparing the lateral edge. Indeed, it cannot be attributed to any feature of tectal operation since it occurs also on intact tecta. Thus it would appear to reflect the contingencies of map reassembly after chiasma uncross.

In order to substantiate the conclusion that compression is occurring in the case of full field representation and the cases of partially complete field representation, a final alternative explanation must be dealt with. It might be suggested that the apparent compression was in fact due to retrograde degeneration of those retinal ganglion cells deprived of "appropriate" target sites after half tectal ablation. Thus the full field represent-ation in case $\frac{1}{2}$ T.Ch.C. 2 might in fact be produced by a retina composed solely of fibres giving rise to the projection appropriate to the residual rostral half tectum (i.e. temporal ganglion cells).

Two arguments tell against this possibility:

a) The map from the eye normally ipsilateral to the operated tectum onto that tectum should be a half field map if this were the case. However animal $\frac{1}{2}$ T.Ch.C. 4 (Fig.6b) shows the whole field minus the nasodorsal quadrant of the right eye mapping to the right (Caudal) half tectum.

b) Again, this hypothesis predicts that the eye normally contralateral to the operated tectum should form a map across only half the extent of the ipsilateral tectum. Yet case $\frac{1}{2}$ T.Ch.C. 6 shows the left eye forming a complete map across the whole of the left tectum.

Meyer & Sperry (1973) put forward their hypothesis of embryonic regulation to account for and unify two types of data (see Chapter 2): first the data suggesting a lability of connections formed in size disparity experiments with amphibian partial retinae (Gaze et al. 1963, 1965; Straznicky et al. 1971) goldfish partial retinae (Horder 1971; Yoon 1972a) and goldfish partial tecta (Gaze & Sharma 1970; Yoon 1971, 1972a; Meyer 1974b); and secondly the disparate data obtained from amphibian partial tecta (Straznicky 1973; Meyer & Sperry 1973). Their hypothesis allowed them to interpret the data as consistent with a context-invariant point-to-point rule of mapping (Sperry 1963, 1965). Counterposed to this rule is the suggestion (Gaze et al. 1963, 1965; Gaze & Keating 1972) that mapping is on a system-to-system basis governed by the polarity of the retinal and tectal elements and the available extent of the two elements.

While the data presented here cannot decide between
a system-to-system and a point-to-point mode of map assembly,
they do remove a central prop from the argument of Meyer & Sperry.
They show that reassembly of a map from the whole retina is
possible on a half tectum. This result is clearly predicted by
a systems matching hypothesis. To sustain a context-invariant
mapping rule, it would be necessary to postulate that the embryonic
regulative ability, invoked by Meyer & Sperry, continues beyond
metamorphosis. It is also worth noting that in a recent report
Meyer (1974b) has withdrawn the "regulation" interpretation
of teleost size disparity experiments.

C.2.   The Causes of Compression

As discussed above, compression has been found on anuran
half tecta. In contrast with previous size disparity experiments
with anuran tecta, two variables were altered. Firstly a greater
time was allowed from surgery to mapping and secondly, the optic
chiasma was cut. In the series discussed above either or both
of these contingencies might be necessary for compression to
occur. To decide which of these is responsible it is necessary
to hold one of the variables constant while altering the other.
I have attempted to do this by performing a series of experiments
in which a half tectum was ablated and the animals left for a
comparable period to those used above, without the introduction
of the uncrossed chiasma. However only two results were
obtained from this series and in these cases the half tectal
ablation appeared not to have been successful. Thus it is not
possible to assess the relative contribution of the two variables

to the compression phenomenon. It seems, however, more likely that compression results from the longer time elapsed between surgery and mapping in this series than in previous studies. Straznicky(1973) cut the optic nerve in his half tectal ablation series, to favour complete field restoration, and failed to obtain compression. While uncrossing the optic chiasma is not a strictly comparable operation, it is difficult to imagine how it might facilitate the compression phenomenon in a way which optic nerve section fails to do. The importance of regeneration time has, on the other hand, emerged as an important factor in facilitating lability of connections in size disparity experiments (Horder 1971; Yoon 1971, 1972a,b; Gaze 1974; Cook & Horder 1974).

It would seem likely then that greater elapsed regeneration time determines the finding of compression in the present study as opposed to "appropriate" formation of connections in previous studies. This then removes the discrepancy in behaviour after tectal size disparity experiments, previously inferred from comparison of anuran and teleost experiments. A species difference still remains between anurans and teleosts, but in the present interpretation this would now be seen as a quantitative difference, the anurans requiring a longer time (greater than 9 months) for compression than teleosts (4 to 5 months). It is not yet clear what the reason for this difference might be.

C.3.   Spreading or Regulation?

As discussed in the section B above, the variation in completeness of map re-establishment found in the chiasma uncross

paradigm, provided the possibility of comparing the mapping
of whole and partial retinal populations to the same tectum. The
map from the right eye in cases Ch.C. 2.1 and 1.4 offers the
conditions for such an analysis.   In case Ch.C. 2.1 the right
eye sends a whole field map to the right tectum (Fig. 12b) and
a temporal half field map to the left tectum (Fig. 13b). In
case Ch.C. 1.4 the right eye yields a complete projection on
the right tectum (Fig. 14b) and a nasal half field projection
on the left tectum (Fig. 14d). At first sight these results would
appear to suggest that a whole projection and a partial projection
from a known normal eye can achieve complete coverage of normal
tecta and thus that "appropriate" connections are not the only
ones to be formed. However, there are problems with this inter-
pretation. The nasal half field map on the left tectum in case
Ch.C. 1.4 (Fig. 14d) is in fact an indirect projection as is the
nasal extension of the right tectum map of case Ch.C. 2.1 (Fig.12b).
It is not clear whether maps obtained by separate pathways can
be compared in the manner desired. On the assumption that such
a comparison is possible the maps in Figures 12b and 14b assay
for the normality of the eyes which sends partial projections to
whole tecta (Figs. 13b and 14d). These results therefore provide
tentative support for a "spreading" rather than a "regulation"
mechanism and are complementary to a similar finding recently
reported by Feldman et al. (1975).

C.4.   Mode of Map Reassembly

The maps obtained in both the series discussed here show
considerable variability, ranging from the "appropriate" map to

whole field compression with varying other degrees of incomplete-
ness. None of the map configurations would appear to represent
determinate control of map assembly by either retina or tectum.
A given retina may produce projections of differing degrees of
completeness on the two tecta and a single tectum may receive
maps of differing degrees of completeness from either eye. Thus
the incomplete map configurations would seem to represent the
hazards of map reassembly. Neither can these configurations
be attributed to a property of partial tecta, as the same classes
of map configurations are found on both operated and normal tecta.
Finally the degree of map completeness does not correlate with
elapsed time from operation (Tables 1 and 2).

A somewhat consistent pattern of map reassembly appears
on re-examination of the present data in concert with previous
studies (Gaze & Jacobson 1963; Gaze & Keating 1970; Straznicky
et al. 1971). A majority class of the group of incomplete maps
in the two present series is constituted by nasally incomplete
maps (i.e. temporal half field and partially complete maps).
These constitute 10 out of the 15 incomplete maps in the first
series and 9 out of 16 incomplete maps in the second series.
Nasal field incompleteness is also evident in a number of the
maps reported after chiasma uncross by Straznicky et al. (1971).
This incompleteness in the present studies was often accompanied
by a nasal extension of the field subserved by the indirect
pathway. It may be that the greater hazard encountered in the
reassembly of this pathway and a possible mutually inhibitory
competition with the direct nasal pathway results in a frequent
nasal field deficit. A similar tendency to restore the nasal

field by the indirect pathway is evident in the "pattern 4"
regeneration maps from frogs with cut optic nerves (Gaze &
Jacobson 1963; Gaze & Keating 1970). In this situation an
aberrant indirect input is found on the contralateral tectum
superimposed on, and sometimes partially replacing, the direct
input. Since the temporal half field can only be restored by
the direct pathway regeneration errors and competitive exclusion
are diminished relative to the nasal input. On this basis
it is possible to understand the preponderance of temporal half
field maps and to see these and the partially complete maps as
two outcomes of a single process.

CHAPTER 7 : EXPERIMENTS ON PATTERN FORMATION IN THE
            EMBRYONIC RETINA

A — THE RESTORATION OF PATTERN IN RETINAL FRAGMENTS

A.1. Orthogonal Fragments

In this series eye fragments were prepared such that the centre was systematically either included (central fragments) or excluded (peripheral fragments). The plane of ablation was parallel to the major axes of the eye, producing nasal or temporal and dorsal or ventral fragments. The results of 72 such operations are presented in Tables 3a,b,c and d. In addition to showing the map configuration obtained, the Tables show the morphology of the eye at the time of mapping.

Morphological Observations:

After surgery the eye fragments underwent a gradual geometrical transformation over a period of 3–5 days, producing an increasingly rounded profile. By the end of this period the fragments had completely transformed into normally shaped but miniature eyes. A priori this rounding up might involve physical processes such as tension between the fragment or active processes such as cell migration and compensatory cell growth at the cut edge. Preliminary time lapse studies indicated a two stage process: the first being a rapid but incomplete approximation of the two halves of the cut edge (dorsal and ventral halves in a nasal or temporal fragment; nasal and temporal halves in a ventral fragment), which takes place within 24–36 hours. A more gradual rounding up follows. (Fig.18) It may then be that both physical and cell division processes are involved in restoring normal morphology. The fragment remains markedly smaller

Table 3a: Results of Orthogonal Nasal Fragment Experiments

CODE	STAGE AT OPERATION	MAP	EYE SIZE	PIGMENTATION	FISSURE
$\frac{1}{2}$N(p) 1	32	NN	normal	normal	V
$\frac{1}{2}$N(p) 2	32	NN	normal	normal	V
$\frac{1}{2}$N(p) 3	32	NN	normal	normal	V
$\frac{1}{2}$N(p) 4	32	NN	normal	normal	V
$\frac{1}{2}$N(p) 5	32	normal	normal	normal	V
$\frac{1}{2}$N(p) 6	32	?	small	normal	N
$\frac{1}{2}$N(p) 7	32	NN	normal	normal	V
$\frac{1}{2}$N(p) 8	32	normal	normal	normal	V
$\frac{1}{2}$N(p) 9	32	NN	normal	normal	TV & NV
$\frac{1}{2}$N(c) 1	32	normal	normal	normal	V
$\frac{1}{2}$N(c) 2	32	normal	normal	normal	V
$\frac{1}{2}$N(c) 3	32	normal	normal	normal	V
$\frac{1}{2}$N(c) 4	32	normal	normal	normal	V
$\frac{1}{2}$N(c) 5	32	normal	normal	normal	V
$\frac{1}{2}$N(c) 6	32	normal	normal	normal	V
$\frac{1}{2}$N(c) 7	32	normal	normal	normal	V

? = unanalysable

Table 3b: Results of Orthogonal Temporal Fragment Experiments

CODE	STAGE AT OPERATION	MAP	EYE SIZE	PIGMENTATION	FISSURE
$\frac{1}{2}$T(p) 1	35/36	?	smaller	normal	V
$\frac{1}{2}$T(p) 2	35/36	TT	normal	normal	V
$\frac{1}{2}$T(p) 3	37/38	TT	normal	normal	N
$\frac{1}{2}$T(p) 4	37/38	absent	normal	normal	N
$\frac{1}{2}$T(p) 5	37/38	absent	small	normal	V
$\frac{1}{2}$T(p) 6	32	?	normal	normal	V
$\frac{1}{2}$T(p) 7	32	normal	small	normal	N
$\frac{1}{2}$T(p) 8	32	normal	normal	normal	V
$\frac{1}{2}$T(p) 9	32	?	normal	normal	NV
$\frac{1}{2}$T(p) 10	32	TT	small	normal	N & T
$\frac{1}{2}$T(p) 11	32	normal	normal	normal	V
$\frac{1}{2}$T(p) 12	32	TT[a]	normal	normal	TV
$\frac{1}{2}$T(p) 13	32	TT[b]	small	normal	V
$\frac{1}{2}$T(p) 14	32	normal	normal	normal	V
$\frac{1}{2}$T(p) 15	32	TT	normal	normal	V
$\frac{1}{2}$T(p) 16	32	?	small	normal	ND & TD
$\frac{1}{2}$T(p) 17	32	TT	normal	normal	V
$\frac{1}{2}$T(c) 1	33/34	normal	normal	normal	V
$\frac{1}{2}$T(c) 2	33/34	normal	normal	normal	V
$\frac{1}{2}$T(c) 3	35/36	normal	normal	normal	V
$\frac{1}{2}$T(c) 4	35/36	normal	normal	normal	V
$\frac{1}{2}$T(c) 5	35/36	normal	normal	normal	V
$\frac{1}{2}$T(c) 6	35/36	normal	normal	normal	V
$\frac{1}{2}$T(c) 7	35/36	normal	normal	normal	NV & TV
$\frac{1}{2}$T(c) 8	32	normal	normal	normal	V
$\frac{1}{2}$T(c) 9	32	normal	normal	normal	V
$\frac{1}{2}$T(c) 10	35/36	normal	normal	normal	V
$\frac{1}{2}$T(c) 11	35/36	normal	normal	normal	V
$\frac{1}{2}$T(c) 12	35/36	normal	normal	normal	V

a = map rotated approximately 60° clockwise
b = map rotated approximately 15° clockwise

Table 3c: Results from Orthogonal Ventral Fragment Experiments

CODE	STAGE AT OPERATION	MAP	EYE SIZE	PIGMENTATION	FISSURE
$\frac{1}{2}$V(p) 1	35/36	VV	small	normal	V
$\frac{1}{2}$V(p) 2	35/36	VV	small	normal	V
$\frac{1}{2}$V(p) 3	35/36	regen.*	small	normal	V
$\frac{1}{2}$V(p) 4	32	normal	normal	silver	V
$\frac{1}{2}$V(p) 5	32	VV	normal	silver	NV & TV
$\frac{1}{2}$V(p) 6	32	normal	smaller	silver	V
$\frac{1}{2}$V(p) 7	32	VV	small	silver	V
$\frac{1}{2}$V(p) 8	32	?	normal	normal	V
$\frac{1}{2}$V(p) 9	32	normal	normal	silver	V
$\frac{1}{2}$V(p) 10	32	VV	normal	silver	V
$\frac{1}{2}$V(c) 1	35/36	regen.*	normal	normal	V
$\frac{1}{2}$V(c) 2	35/36	normal	normal	normal	(V
$\frac{1}{2}$V(c) 3	35/36	normal	normal	normal	V
$\frac{1}{2}$V(c) 4	32	normal	normal	normal	V
$\frac{1}{2}$V(c) 5	32	normal	normal	normal	V
$\frac{1}{2}$V(c) 6	32	normal	normal	normal	V
$\frac{1}{2}$V(c) 7	32	normal	normal	normal	V

*   =   disordered regeneration map.

Table 3d: Results from Orthogonal Dorsal Fragment Experiments

CODE	STAGE AT OPERATION	MAP	EYE SIZE	PIGMENTATION	FISSURE
$\frac{1}{2}$D(p) 1	32	normal	normal	normal	V
$\frac{1}{2}$D(p) 2	32	regen.*	small	normal	T
$\frac{1}{2}$D(p) 3	32	absent	normal	normal	V
$\frac{1}{2}$D(p) 4	32	absent	small	normal	V
$\frac{1}{2}$D(p) 5	32	absent	normal	normal	V
$\frac{1}{2}$D(c) 1	32	regen.*	normal	normal	V
$\frac{1}{2}$D(c) 2	32	normal	normal	normal	V
$\frac{1}{2}$D(c) 3	32	normal	normal	normal	V
$\frac{1}{2}$D(c) 4	32	normal	normal	normal	V
$\frac{1}{2}$D(c) 5	32	normal	normal	normal	V

* = disordered regeneration map

FIGURE 18. The healing of a temporal peripheral fragment
made in the left eye at stage 35/36. The nasal two-thirds
of the eye rudiment were removed (a) as described in Chapter
8.   The embryo was maintained in full strength Niu-Twitty
solution under 1: 30,000 MS 222 anaesthesia for 65 hours
and the healing of the fragment followed with cine-
microphotography. Photographs are stills taken from this
film (1 exposure per minute). The head of the embryo is
shown, the cement gland pointing to the lower right hand
corner. (a) embryo at operation (b) 1 hour after operation
(c) $2\frac{1}{2}$ hours after operation. Note the rounding of the
fragment (d) 8 hours after operation. Further rounding is
evident (e) $15\frac{1}{2}$ hours after operation. Rounding up is
proceeding more slowly (f) 31 hours after operation (g)
$53\frac{1}{2}$ hours after operation. Edges of fragment almost
completely apposed (h) 62 hours after operation (i) Summary
of time series shown in (a) - (h). Time since operation is
graphed against distance between the edges of the fragment
(as a measure of rounding up) and against the size of the
fragment, as measured from the photograph. A crude indication
is thereby provided of the initial rapid rounding up and the
slower initiation of growth. Open circles represent distance
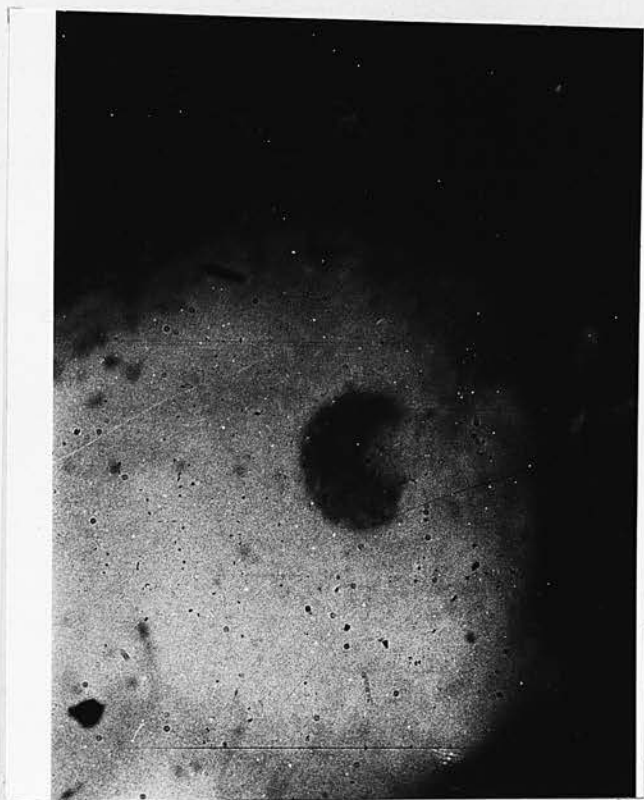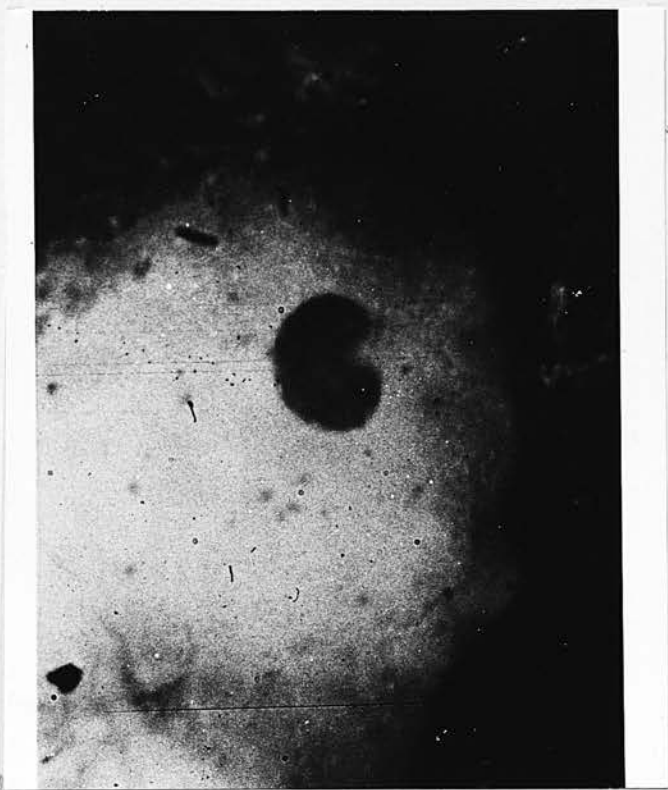between the edges (closure) and filled circles, the size of the
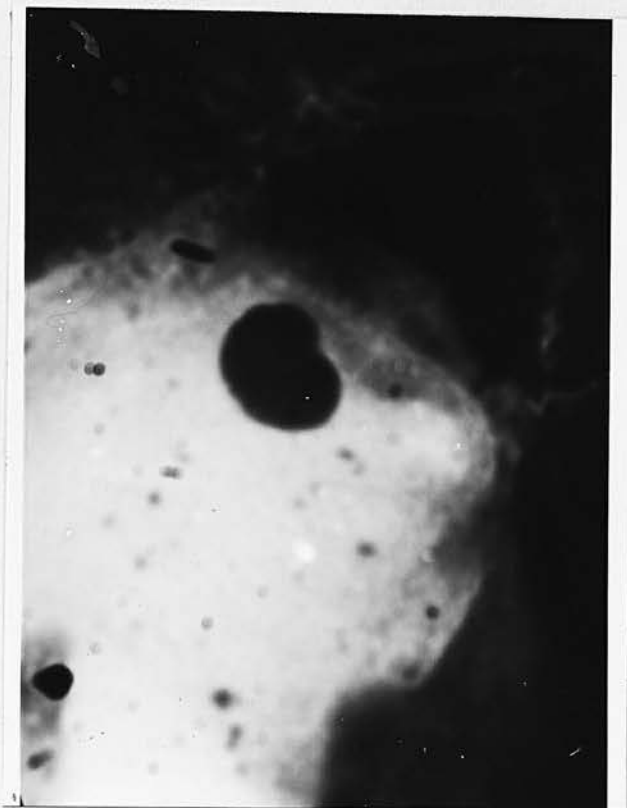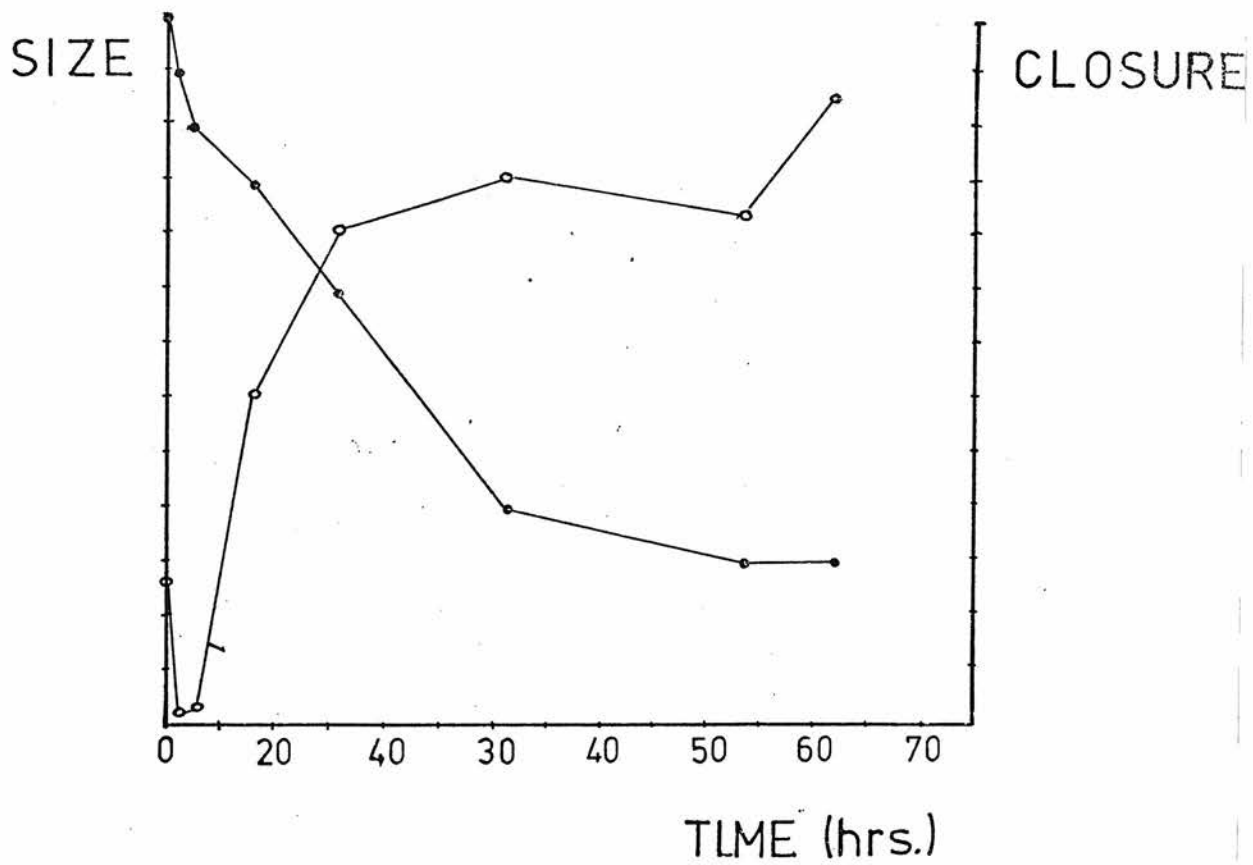fragment.

18 a

18 b

18 c

18 d

18 e

18 f

18 g

18 h

SIZE

CLOSURE

TLME (hrs.)

18(i)

than the unoperated eye for several weeks. Indeed in the first weeks after operation the size discrepancy between the two eyes becomes more marked. This might be due to an inhibition of growth processes in the operated eye (see Feldman & Gaze 1975a) or to the larger number of precursor cells present in the ciliary margin of the unoperated eye. Size parity between operated and unoperated eyes was not always restored by the stage of mapping (some 6-10 weeks after operation). However no correlation was apparent between the type of map and the achievement or failure of achievement of normal size (see Table 3).

The morphological pattern within the normal eye may be used to assess the morphological normality of the fragments. Two morphological markers are readily apparent: pigmentation pattern and the choroid fissure. In the normal eye a dorsoventral anisotropy of pigmentation is evident, the dorsal aspect of the eye being black and the ventral aspect silver, with a sharp boundary between the two. The choroid or ventral fissure is the scar left indicating the site of fusion of the two halves of the eye rudiment after invagination of the optic vesicle to form the optic cup. It is through this fissure that the fibres of the optic nerve are conducted out of the eye. In the normal animal the position of this fissure is ventral and slightly posterior. Since all peripheral fragments with the exception of ventral will lack the ventral fissure it was expected that this marker might show considerable variability in peripheral fragments.

In the majority of cases (85% : 61 out of 72) the fissure was formed ventrally. The 15% (11 out of 72) cases showing

misplaced fissure formation (Figure 19) were, with a single exception, restricted to the peripheral fragments. Of the 41 peripheral fragments 24% (10 cases) and of the 31 central fragments 3% (1case) thus showed aberrant fissure formation. Of the 11 cases of fissure aberration 2 occurred in nasal fragments, 5 in temporal fragments, 1 in ventral fragments and 1 in dorsal fragments. Seven of the 11 cases involved misplaced fissure formation, 3 involved a doubled fissure ventrally and 2 involved a doubled fissure nasally and temporally. In no case (Table 3) did fissure misplacement impart a corresponding rotation to the map. Nor were the fissure aberrations correlated with abnormalities of the map.

Pigmentation pattern was relatively normal in all nasal and temporal fragments (as might be expected since they contain a full dorsoventral extent of eye tissue). In some cases the boundary between black and silver pigment was less sharp than normal, but this was the only difference evident here. In contrast the ventral fragments displayed a characteristic abnormality of the pigment pattern. Ventral central fragments were all normal but a majority of ventral peripheral fragments formed eyes whose external pigmentation was entirely silver. That is the dorsal aspect of the eye showed a pigmentation pattern characteristic of the ventral aspect. However the presence of ventrally reduplicated morphology was not in itself an indicator of ventrally reduplicated maps. It is, however, interesting that Berman & Hunt (1975) found no such morphological reduplication in their ventral fragments.

FIGURE 19. Abnormally placed choroid fissure in case $\frac{1}{2}T(p)$ 3. The pointer, entering the photograph dorsal and anterior indicates the fissure which has reformed nasally. This fragment went on to generate a temporally reduplicated visuotectal map.
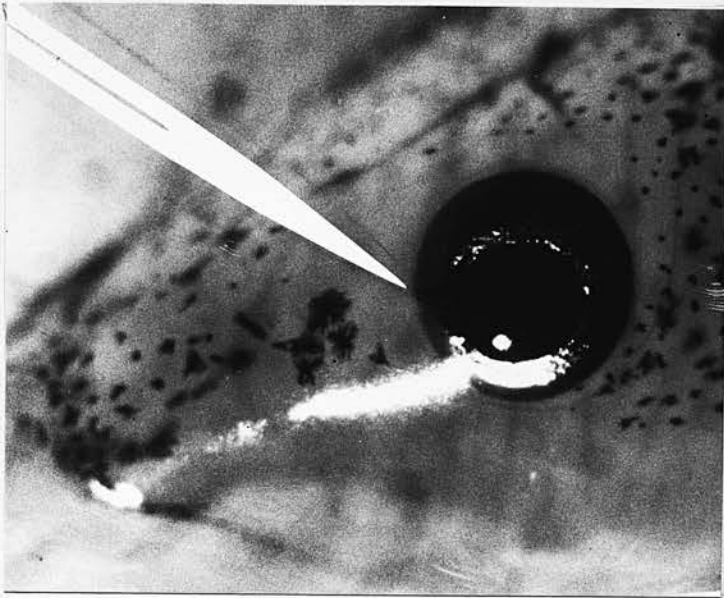
19

Electrophysiological Mapping

Maps were classified as normal or reduplicated and uninterpretable, absent or regenerated. Reduplicated maps show a complete reduplication of field positions in mirror symmetry about the appropriate midline of the field. No cases of partial reduplication (as reported by Feldman & Gaze 1975 and Berman & Hunt 1975) were found in the present study. Since all maps in this series were obtained from tadpole stages it is not possible to assess accurately the degree of tectal coverage. However, with the intrinsic variability in degree of tectal coverage shown by samples of normal tadpoles, the maps obtained from reduplication cases do not appear to be restricted to a noticeably smaller extent of tectum. Normal maps showed a complete lack of mirror-reduplication and were indistinguishable from maps obtained from normal tadpoles at corresponding stages. Where the map was classified as absent there was either a total failure to establish connections between the experimental eye and the tectum, or such connections as were formed were unlocalizable with the present mapping technique. Maps were classified as uninterpretable either when too few points were localized to allow analysis of the polarity of the map or when frank abnormalities of the optical apparatus (lens and /or cornea) were apparent. Disordered projection from one or a few visual field regions to the whole tectum were classified as regeneration maps. In all cases the type of reduplication found was appropriate to the nature of the original fragment. Thus where nasal fragments gave rise to reduplicated maps they were always "double nasal" maps; similarly for temporal fragments, "double temporal" maps and for ventral fragments, "double ventral" maps.

It will be seen from Table 3 that central (c) fragments always yielded only normal maps (29 out of 29 interpretable cases). Contrary to the hypothesis presented in Chapter 4 however the results from peripheral (p) fragments were not entirely restricted to the reduplicated class. A certain incidence of normal maps was found within each group of peripheral fragments.

Two maps only were obtained from the dorsal peripheral $(\frac{1}{2}D(p))$ series of which one may have been reduplicated. Mortality and failure of map assembly were high in this group.

Sample maps for the series are shown in Figures 20 - 31. Figure 20 illustrates a map obtained from a ventral central fragment. The characteristic curvature of field position rows in the temporal field and the non-linearity of the map, both characteristic of tadpole stage maps of normal animals are evident. Also characteristic of the normal tadpole map is the electrically silent region in the most caudomedial tectum. The polarity of the map is homogeneous and normal, with field position rows running nasotemporally for rostrocaudal tectal rows and dorsoventrally for mediolateral tectal rows. Maps from the other three groups of central fragments being identical in their organization are not shown. Figure 21 shows a normal map obtained from a nasal peripheral fragment. It is clearly

FIGURE 20. Visuotectal map from left field to right tectum
obtained from a ventral central fragment created at stage
32 and mapped in tadpole life.   This map illustrates the
features characteristic of the normal tadpole map.   Note the
curvature of field rows in the temporal field and the electric-
ally silent regions of tectum medially and caudally.   The
rostral midline of the tectum is indicated by the arrow.
The poles of the field are indicated as follows: Nasal(N),
temporal (T), superior (S) and inferior (I).   Numbers in the
visual field indicate the position at which stimulation evoked
maximal response at correspondingly numbered tectal electrode
positions. Filled circles on the tectum indicate electrode
positions from which no response was localizable.   Alphabetical
subscripts to field positions (e.g. 1a, 4a and 8a) indicate
deep penetration by the electrode at the correspondingly
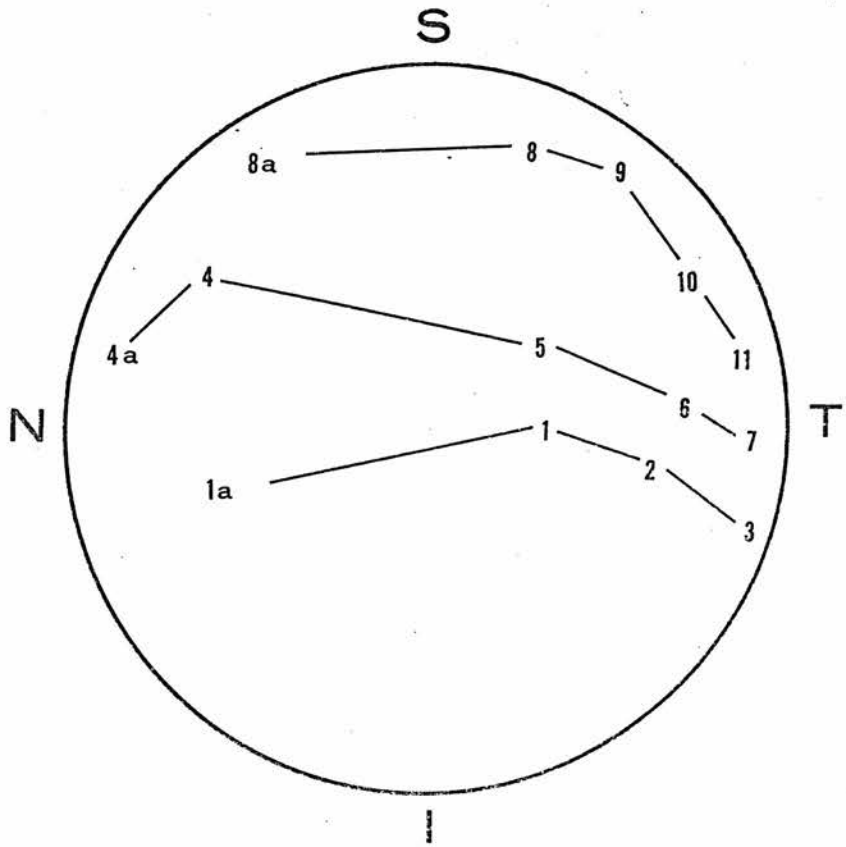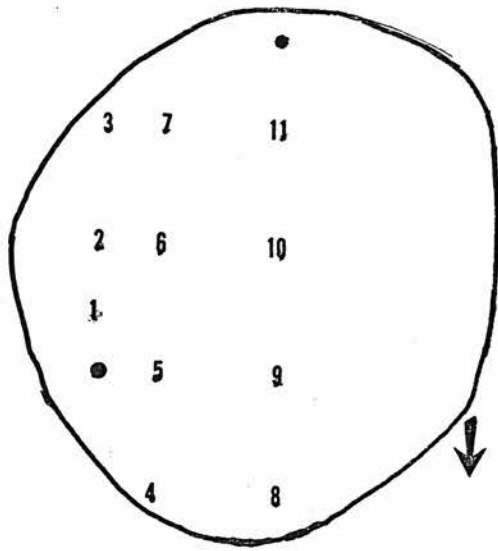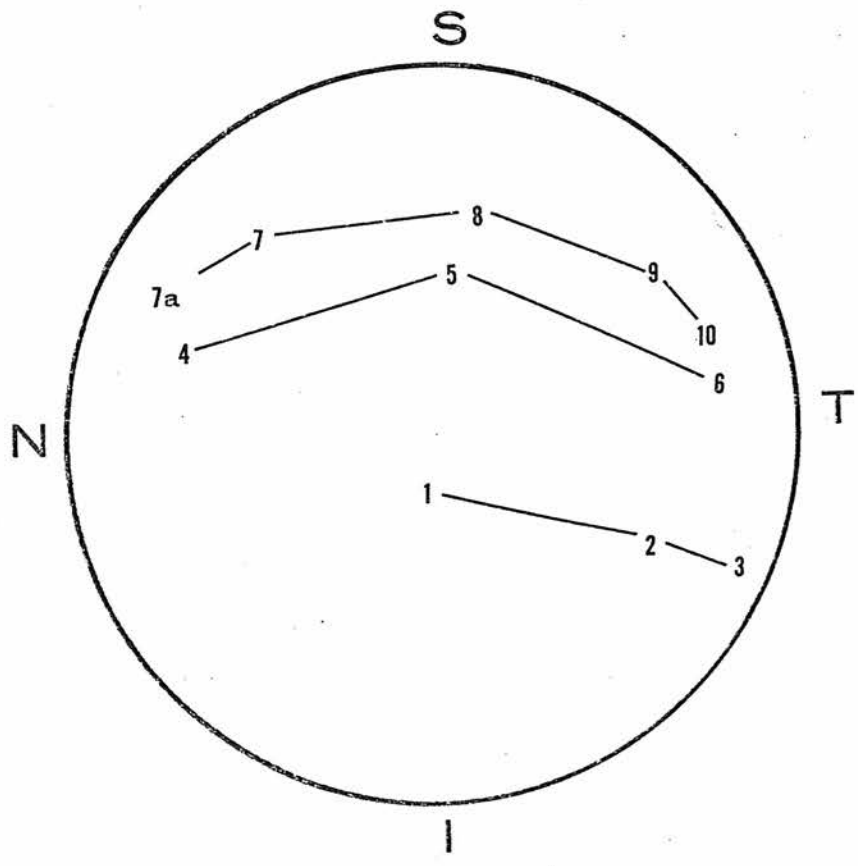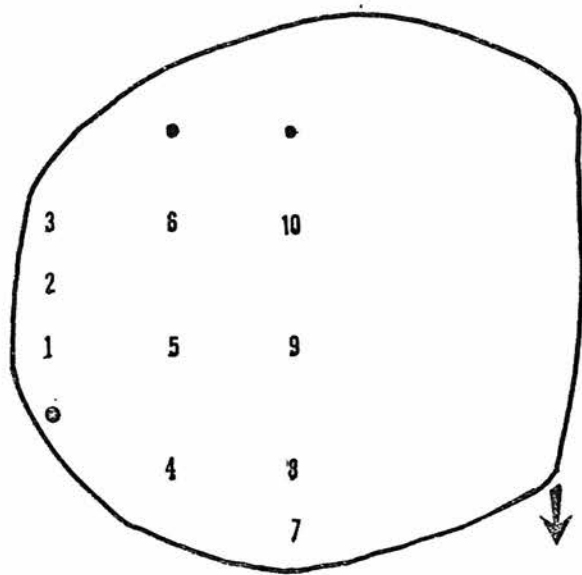numbered tectal positions.

FIGURE 21. Normal visuotectal map from left field to right
tectum obtained from a nasal peripheral fragment created
at stage 32 and mapped in tadpole life. Conventions as
in Figure 20

identical in its mode of organization to Figure 20 . Figures 22
and 23 show double-nasal type mirror reduplicants obtained from
nasal peripheral fragments. Here the mode of organization is
clearly different from that in Figures 20 and 21. Two identical
maps, disposed in mirror symmetry about the vertical midline of
the eye, project in register to the tectum. Thus each tectal
point receives input from two, symmetrical, visual field positions.
The polarity of the maps is similar to that obtained from
double nasal compound eyes (Gaze et al. 1963, 1965). That is to
say, most rostral tectum receives input from the vertical midline
of the field instead of the nasal extremity of the field as in
normal maps; the most caudal responsive tectum receives input from
both nasal and temporal poles of the field instead of from the
caudal pole as in normal maps. The dorsoventral ordering of these
maps is normal. Assuming no translocation of cells between the
operation and mapping, the polarity of the original fragment
(nasal half of the eye or temporal half of the field) has remained
as it was in the intact eye, while the polarity in the complementary
regenerate (temporal pole of the eye or nasal pole of the field)
has formed as a mirror image of the original fragment. It will
also be seen that the characteristic field row curvature and
expanded tectal representation found in the temporal field of
the normal map is now found at both nasal and temporal poles, both
of which, in this map, have acquired the mapping characteristics
of the temporal field.

The reduplication pattern obtained from temporal fragments
(illustrated in Figures 24 and 25) is markedly different from that
obtained from nasal fragments. Each tectal locus again receives

FIGURE 22. Reduplicated visuotectal map from left field
to right tectum obtained from a nasal peripheral fragment
created at stage 32 and mapped in tadpole life.   The
axis of symmetry lies along the vertical midline.   Conventions
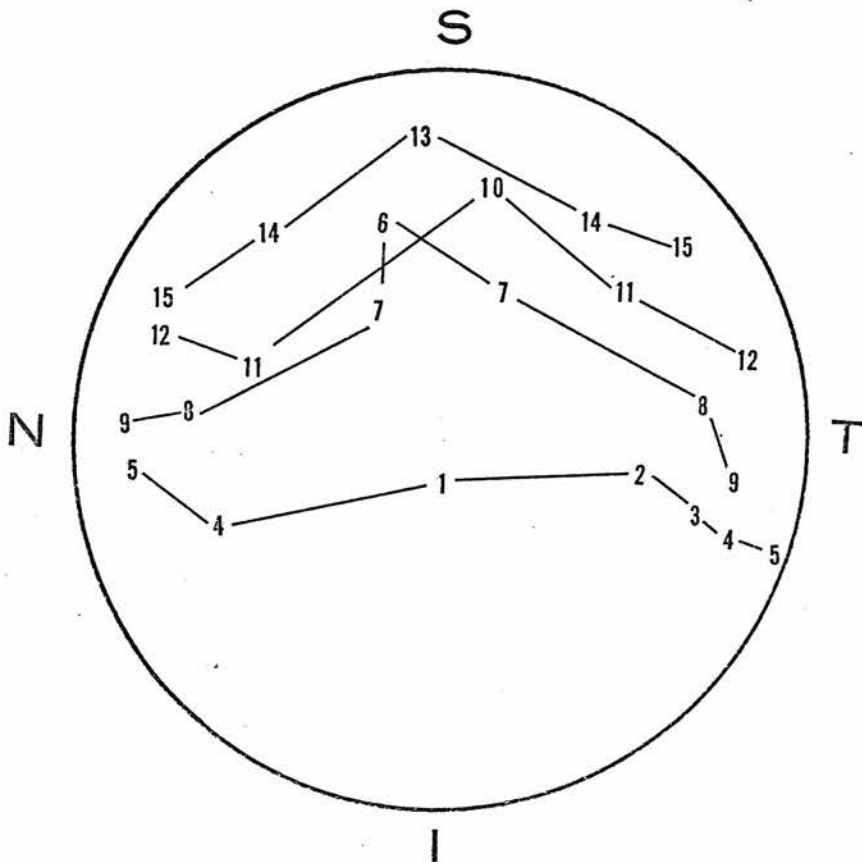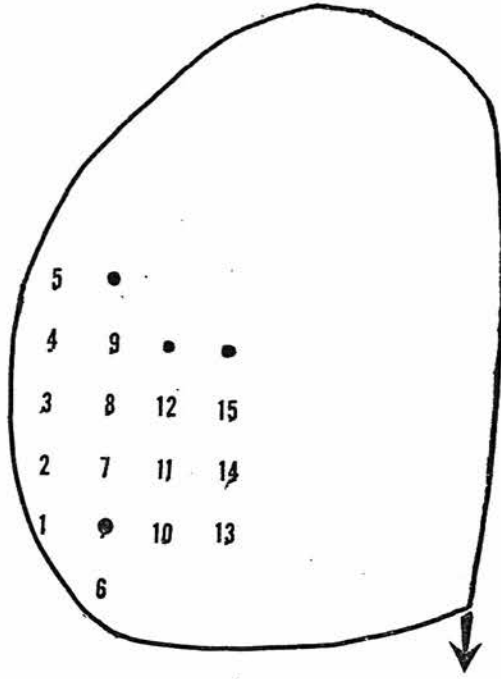as in Figure 20.

FIGURE 23. Reduplicated visuotectal map from left field to
right tectum obtained from a nasal peripheral fragment created
at stage 32 and mapped in tadpole life. The axis of symmetry
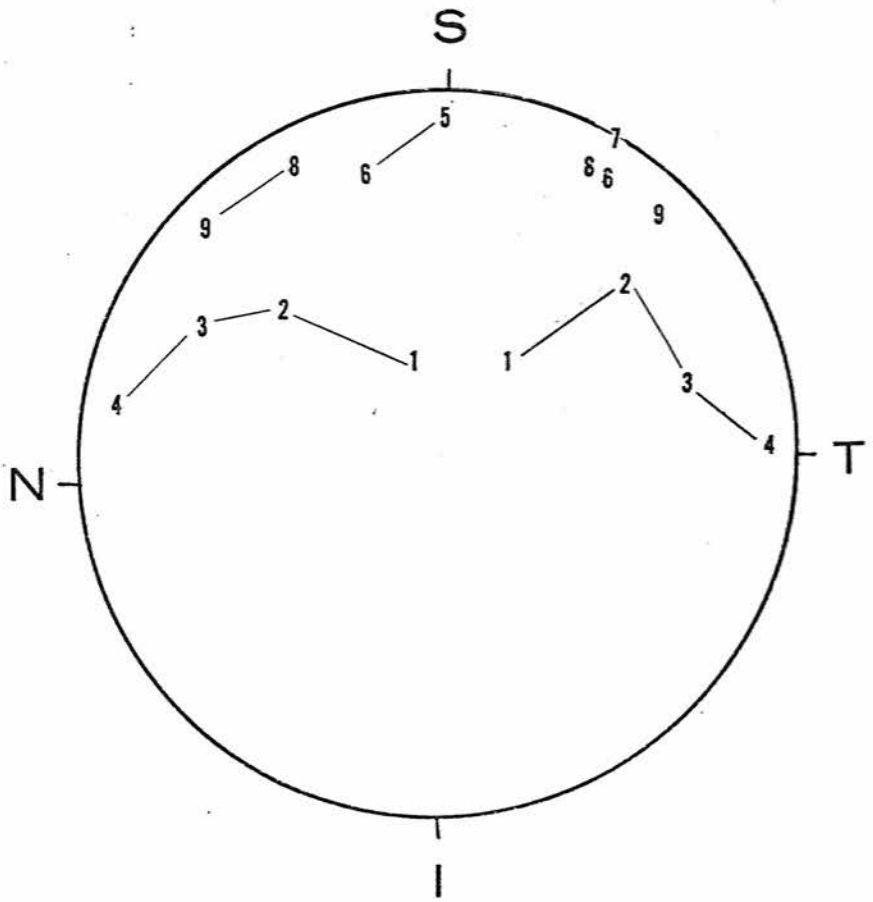lies along the vertical midline. Conventions as in Figure 20.

S

N

T

I

FIGURE 24. Reduplicated visuotectal map from left field to right tectum obtained from a temporal peripheral fragment created at stage 32 and mapped in tadpole life. The axis of symmetry lies along the vertical midline. Conventions as in Figure 20.
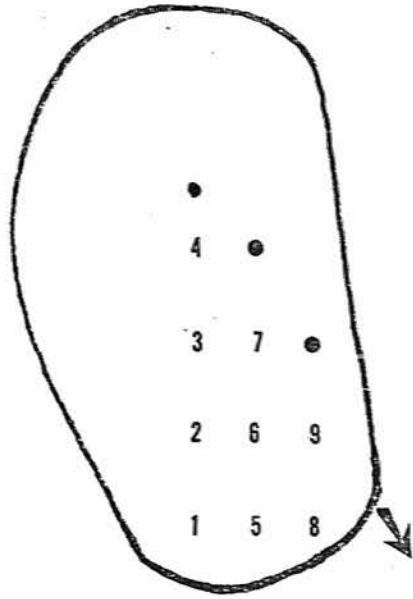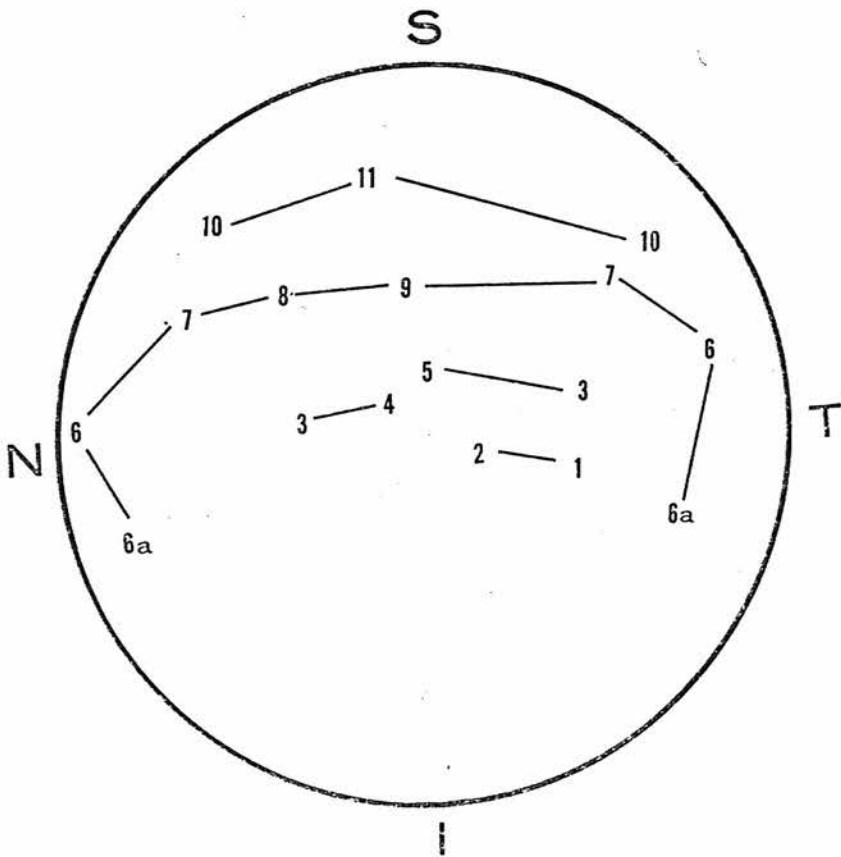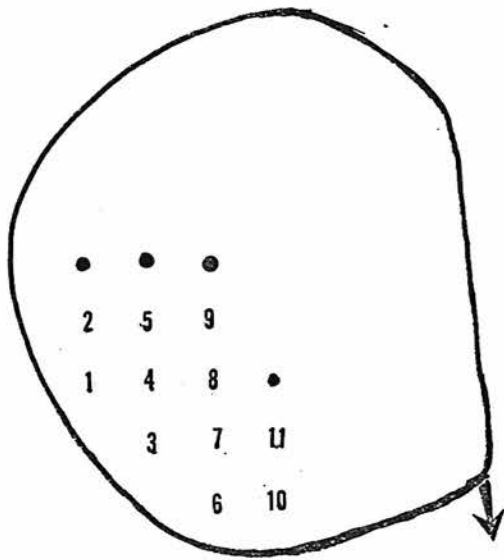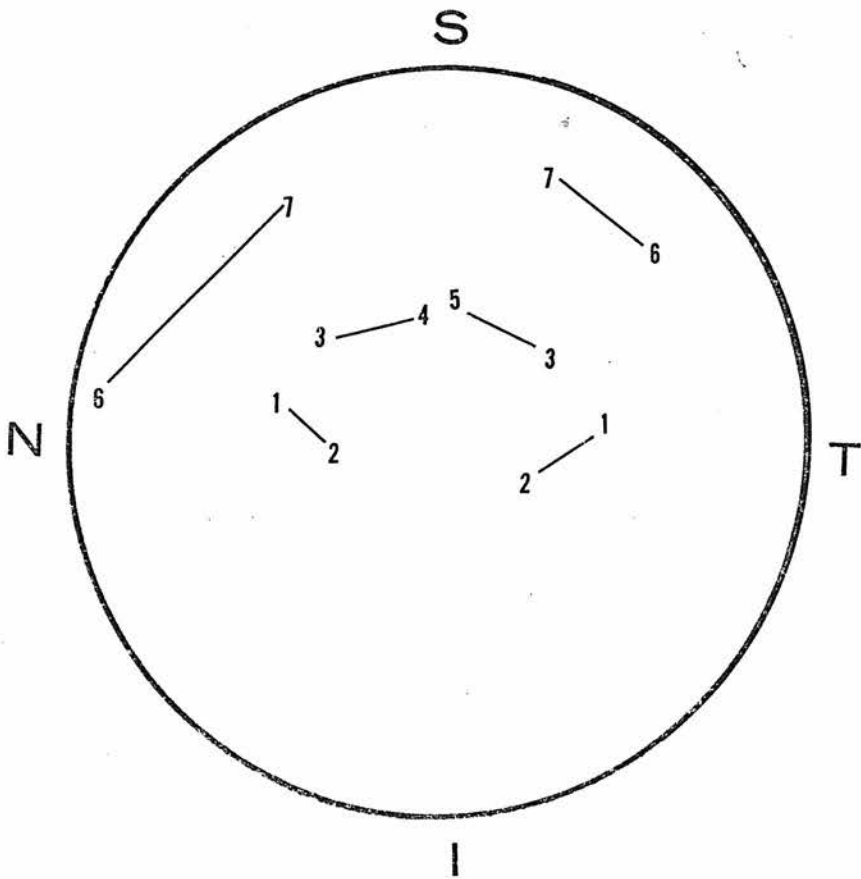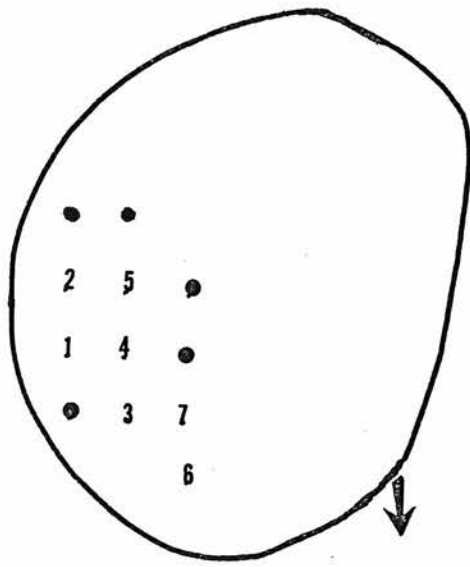
FIGURE 25. Reduplicated visuotectal map from left field to right tectum obtained from a temporal peripheral fragment created at stage 35/36 and mapped in tadpole life. The axis of symmetry lies along the vertical midline. Conventions as in Figure 20.

S

N

T

I

input from two visual field positions disposed symmetrically about the vertical midline. The polarity of the map is now, however, similar to that of "double temporal" eyes (Gaze et al. 1963, 1965). Most rostral tectum (which receives input from the nasal field of the normal map) receives a dual input from nasal and temporal poles of the field; the most caudal tectum (which normally receives from the temporal pole) now receives an input from the vertical midline. The dorsoventral ordering of the map is normal. The characteristic temporal field expansion of tectal representation and ventral curvature of field position rows now occurs in the vertical midline of the map. Again, assuming no translocation of retinal cells between operation and mapping, the polarity of the original fragment (temporal retina or nasal field) remains as it was in the intact eye rudiment, while the map in the complementary regenerate (nasal retina or temporal field) has formed as a mirror image of the original fragment. An example of a normal map obtained from a temporal fragment is shown in Figure 26 . This map is indistinguishable from the normal maps generated by other groups in the series (compare Figures 20 and 21) or maps from unoperated animals.

Figure 27 illustrates a normal map obtained from a ventral fragment. Again it is in all respects indistinguishable from other normal maps. Figures 28 and 29 illustrate reduplication patterns from ventral fragments. They are similar in appearance to those obtained from "double ventral" eyes, resulting from the combination of two ventral fragments with mirror image polarity (Straznicky et al 1974). Each tectal locus receives input from two field positions disposed symmetrically about the horizontal

FIGURE 26. Normal visuotectal map from left field to right tectum obtained from a temporal peripheral fragment created at stage 32 and mapped in tadpole life. Conventions as in Figure 20.

S

N   T

I

FIGURE 27. Normal visuotectal map from left field to right tectum obtained from a ventral peripheral fragment created at stage 32 and mapped in tadpole life. Conventions as in Figure 20.

Top figure: grid of numbered points (1–21) arranged in columns within an irregular oval outline, with an arrow pointing toward the lower right.

Bottom figure: circle labeled S (top), I (bottom), N (left), T (right), containing numbered points 1–21 connected by lines.

FIGURE 28. Reduplicated visuotectal map from left field to right tectum obtained from a ventral peripheral fragment created at stage 35/36 and mapped in tadpole life. The map is poorly ordered but the axis of symmetry would appear to lie along the horizontal midline. Conventions as in Figure 20.
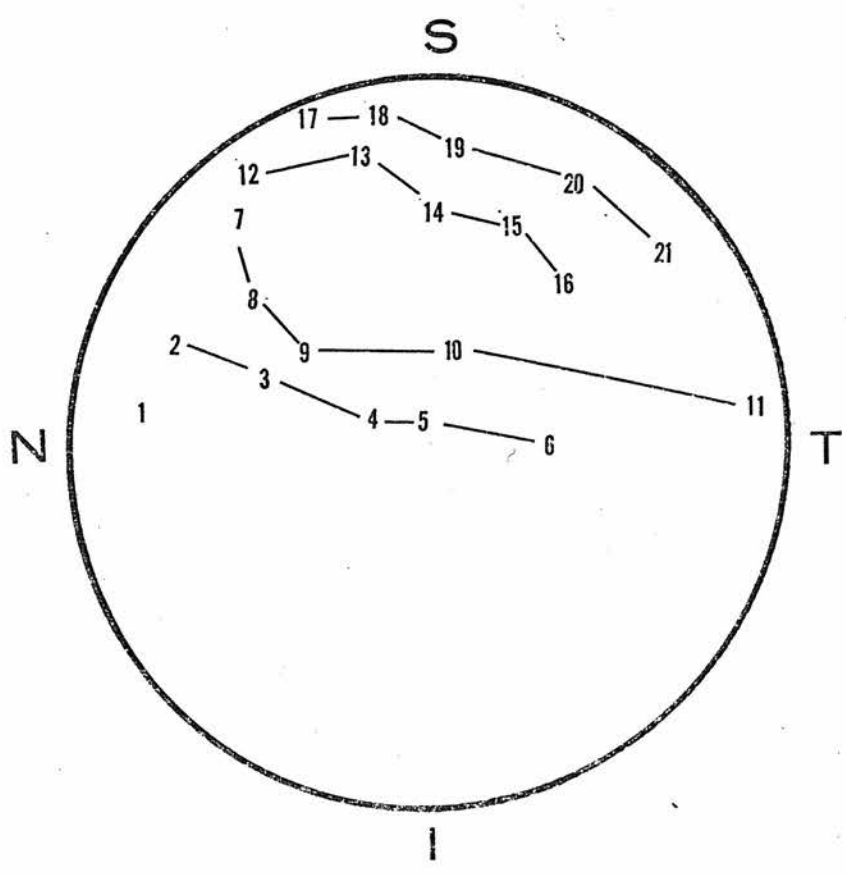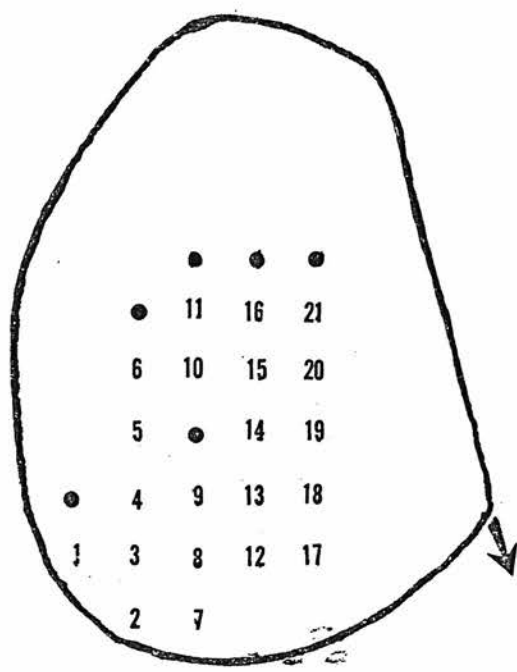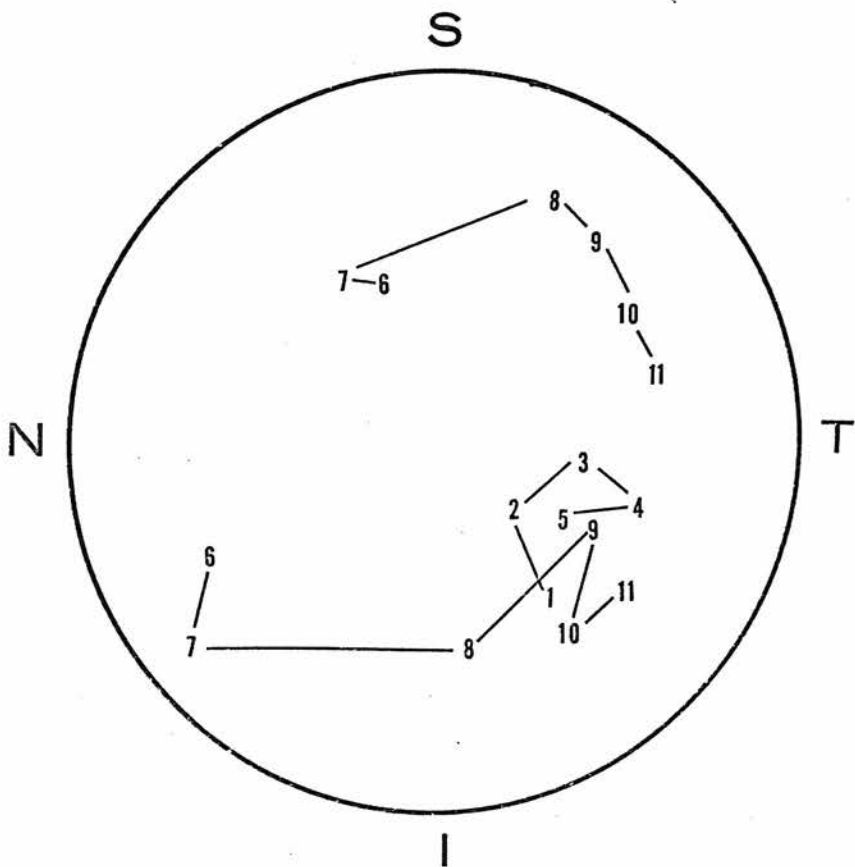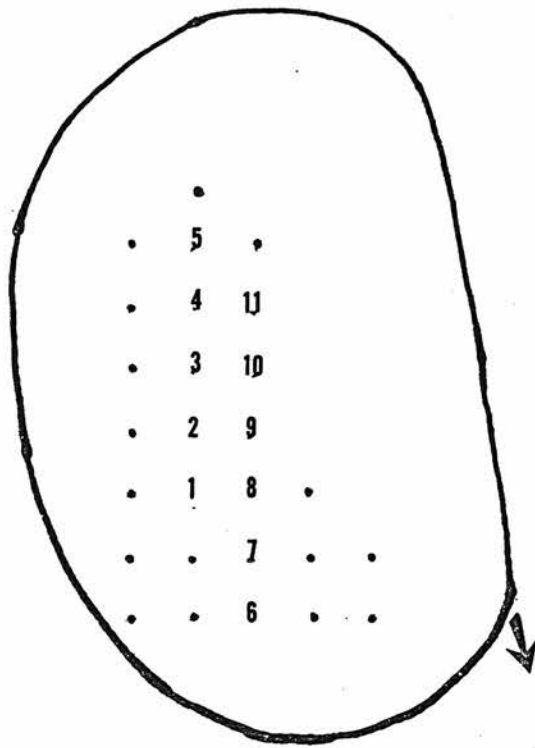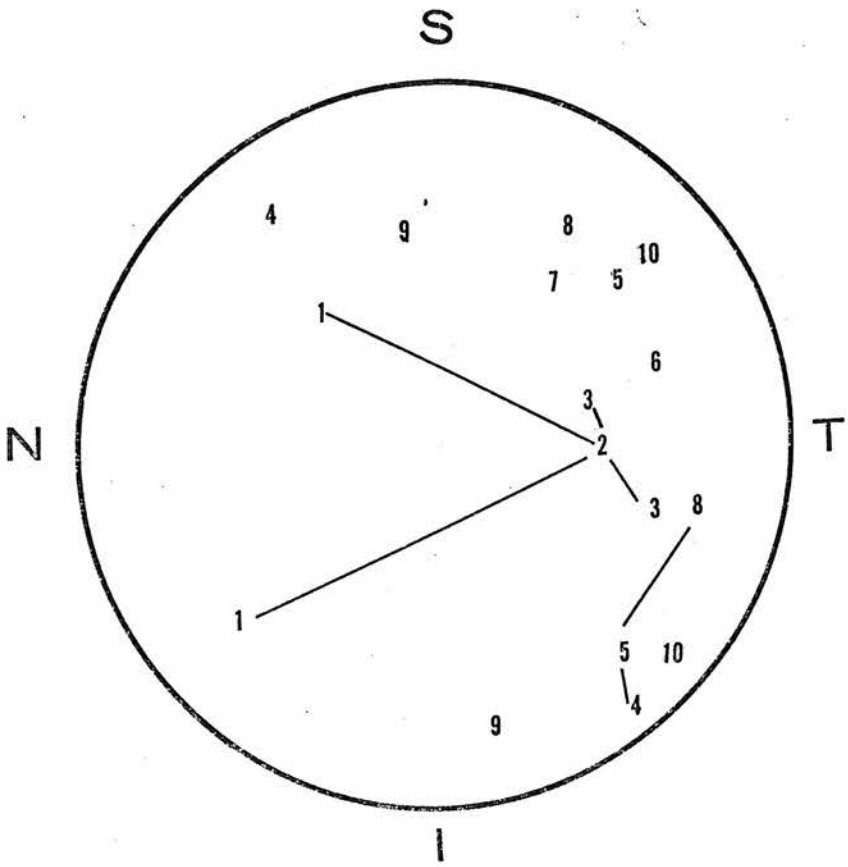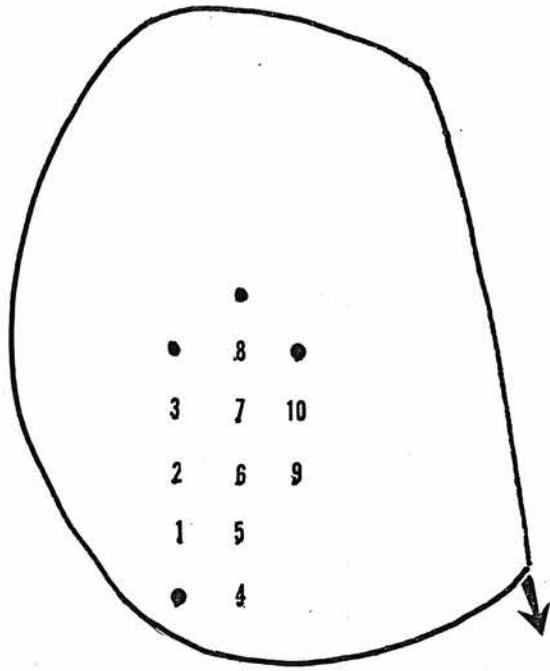
FIGURE 29. Reduplicated visuotectal map from left field to right tectum obtained from a ventral peripheral fragment created at stage 35/36 and mapped in tadpole life. The map is poorly ordered but the axis of symmetry lies approximately along the horizontal midline. Conventions as in Figure 20.

S

N   T

I

midline. The ordering obtained in these maps was too poor to
allow detailed comparison with "double ventral" maps. However
it will be seen that ordering in the nasotemporal field axis
(and corresponding rostrocaudal tectal axis) is normal; the
lateral edge of the tectum receives a dual input from both dorsal
and ventral poles of the field; the medial edge of the tectum
(which would normally receive from the dorsal field) receives
input from the horizontal midline of the field. It was not
possible to examine the maps in sufficient detail for the
characteristic cartwheeling of field rows found in "double ventral"
maps (Straznicky et al 1974), nor for the expanded representation
of the central field.

Only a few dorsal fragments were prepared, in view of the
finding from "compound eye" experiments that double dorsal eyes
failed to connect with the tectum (presumably due to absence
of the ventral fissure). Of these only two of the fragments
yielded assayable maps. Figure 30 shows the production of a
normal map from the first such fragment (although 2 field positions
are duplicated). Figure 31 shows the map from the other. In this
case the map on the tectum is present only on the extreme rostro-
lateral aspect of the tectum and comes from two circumscribed
regions at the dorsal and ventral poles of the field. There is
no apparent internal order within the map. In this respect the
map is similar to pattern 1 maps found in optic nerve regeneration
studies (Gaze & Jacobson 1963; Gaze & Keating 1970). However,
two differences are apparent. The localized regions projecting to
the tectum in conventional pattern 1 maps are at the nasal and/or
temporal poles not dorsal and ventral; secondly, the field positions

FIGURE 30. Visuotectal map from left field to right tectum
obtained from a dorsal peripheral fragment created at stage
32 and mapped in tadpole life. The map is normal with the
exception of positions 15 and 16 which are reduplicated.
Conventions as in Figure 20.

18

5 11 17

4 10 16

3 9 15

2 8 14

1 7 13

6 12

S

N

T

I

12a 12 13

6

6a

14 15

16

7 8

9

1 2

3 10 17
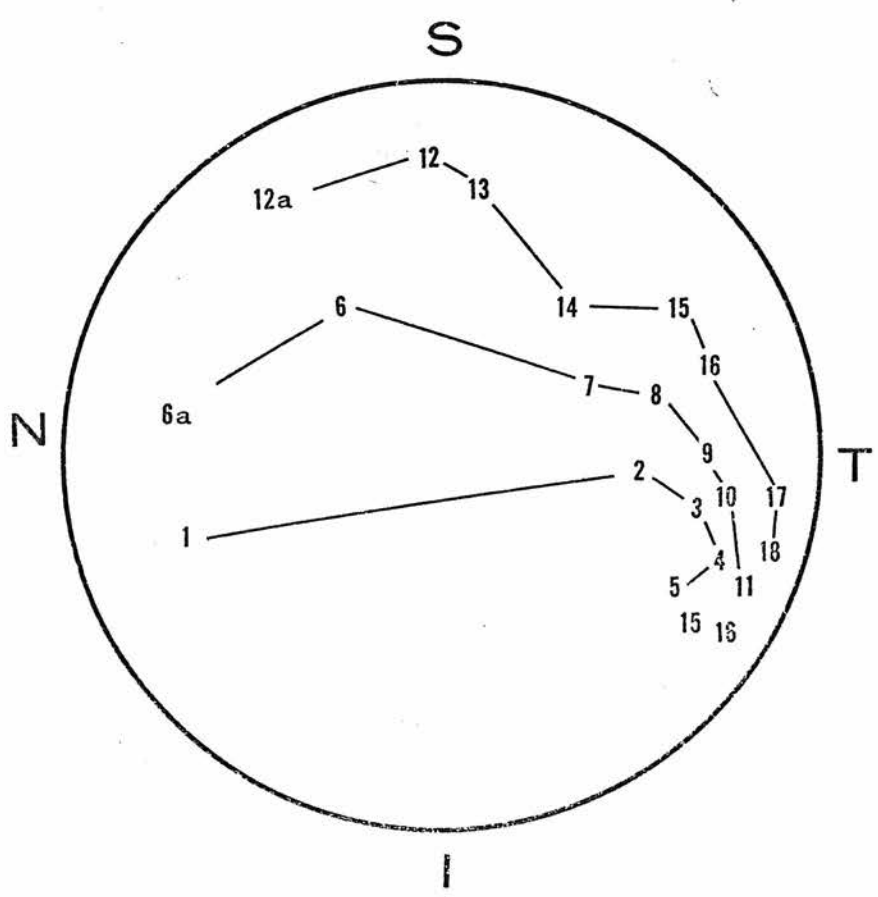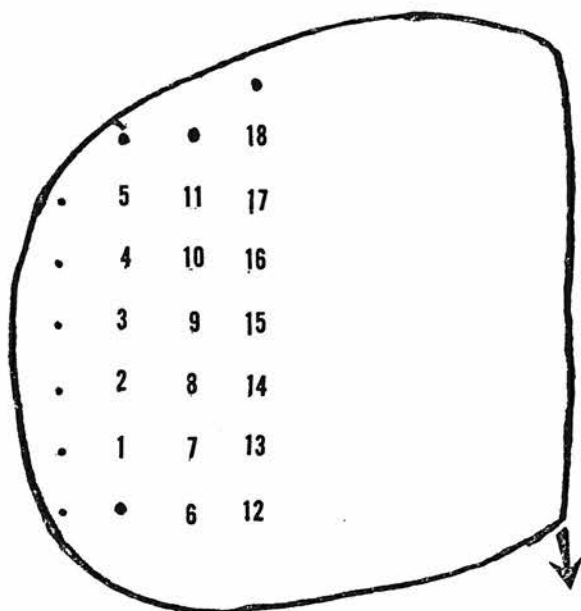
4 18

5 11

15 16

FIGURE 31. Visuotectal map from left field to right tectum
obtained from a dorsal peripheral fragment created at stage
32 and mapped in tadpole life. Only rostrolateral tectum
received localizable input from the visual field. This
input was disordered but came from two field regions at dorsal
and ventral poles in reduplicated fashion. Conventions as
in Figure 20.

S

N

T

I
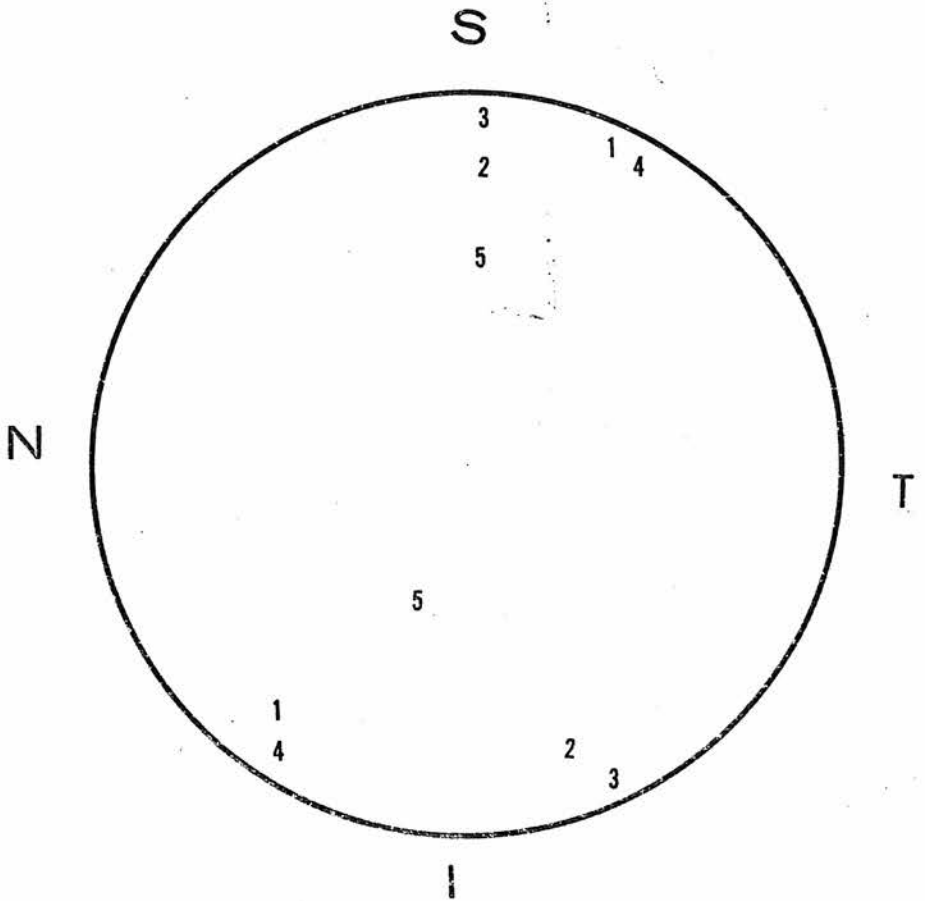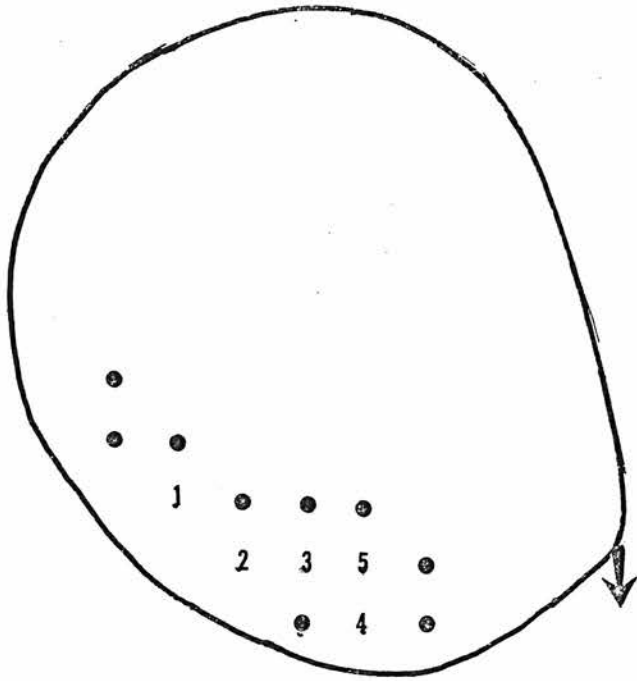
within the map in Figure 31 are reduplicated between the two regions.
For this reason it is possible that the map represents a "double
dorsal" map in an incomplete stage of assembly. The regeneration
maps found in cases $\frac{1}{2}V(p)$ 1 (Table 3c) and $\frac{1}{2}D(c)$ 1 (Table 3d) do
not show this reduplication, while the regeneration map obtained
from the ventral peripheral group $(\frac{4}{5}V(p)$ 3, Table 3c) again shows
a reduplication of points between the projecting regions. While
the evidence from this disordered map alone cannot be conclusive
it seems likely that dorsal fragments are similar in their
behaviour to the other fragments studied here (see also Hunt &
Berman 1975).

In aggregate the entire series shows two characteristic
modes of variation between classes of fragments. Firstly, the
incidence of reduplication is a characteristic proportion of the
total number of peripheral fragments for each group. Thus
nasal peripheral fragments undergo reduplication in 72% of cases,
ventral peripheral in 63% and temporal peripheral in 60% of cases.
This difference in incidence parallels that reported by Berman
& Hunt (1975) for "half eye" fragments. Secondly there is a
characteristic difference in the degree of ordering of the maps.
Nasal peripheral fragments yielded the most orderly reduplication
maps (as indicated by non-intersection and regular polarity of
field position rows), temporal peripheral reduplication maps were
somewhat more poorly ordered and reduplication maps from ventral
peripheral fragments were extremely poorly ordered (see Figures
28 and 29).

A.2.   Oblique Fragments

The results of the previous (orthogonal fragment) series
have broadly borne out the hypothesis that fragments containing
the centre will yield normal maps and those lacking the centre,
mirror-reduplicated maps. This suggested the possibility of the
retinal pattern vectors being distributed in a radially symmetrical
fashion about the centre. If this were the case the central
fragments should continue to yield normal and peripheral fragments
mirror-reduplicated maps, irrespective of the angle the plane of
ablation makes with the major (anteroposterior and dorsoventral)
axes of the eye. This hypothesis has been tested by the construction
of "nasoventral" and "temporodorsal" fragments, whose planes of
ablation form an angle with the major axes rather than running
parallel to them. All operations in this series were performed
at stage 35/36. The results in this case show a marked difference
from those encountered above (Table 4)

Morphological Observations

The rounding up process encountered in orthogonal eye
fragments was also found in the present series to present a
similar appearance and to follow a similar time course. Again,
eye morphology was scored in terms of size, fissure position and
pigmentation pattern.

The fissure position was again observed to be usually
normal. In 5 of the 22 cases the fissure was displaced. In 4 of
these (2 NV(p), 2TD(p)) the resulting maps were normal both in

Table 4:   Results from Oblique Eye Fragment Experiments

CODE	STAGE AT OPERATION	MAP	EYE SIZE	PIGMENTATION	FISSURE
$\frac{1}{2}$NV(c) 1	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(c) 2	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(c) 3	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(c) 4	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(c) 5	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(p) 1	35/36	?	normal	normal	DT
$\frac{1}{2}$NV(p) 2	35/36	normal	normal	normal	T
$\frac{1}{2}$NV(p) 3	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(p) 4	35/36	?NN	normal	normal	V, N & D
$\frac{1}{2}$NV(p) 5	35/36	normal	normal	normal	V
$\frac{1}{2}$NV(p) 6	35/36	NN	smaller	silver	V
$\frac{1}{2}$NV(p) 7	35/36	normal	normal	silver	V
$\frac{1}{2}$NV(p) 8	35/36	normal	normal	silver	V
$\frac{1}{2}$NV(p) 9	35/36	NN	small	normal	V
$\frac{1}{2}$NV(p) 10	35/36	?	normal	silver	V
$\frac{1}{2}$NV(p) 11	35/36	NN	small	partially silver	V
$\frac{1}{2}$TD(p) 1	35/36	?	small	normal	V
$\frac{1}{2}$TD(p) 2	35/36	normal	small	normal	TV
$\frac{1}{2}$TD(p) 3	35/36	normal	small	normal	NV
$\frac{1}{2}$TD(p) 4	35/36	normal	small	normal	V
$\frac{1}{2}$TD(p) 5	35/36	normal	normal	normal	V
$\frac{1}{2}$TD(p) 6	35/36	normal	normal	normal	V

? = unanalysable

terms of lack of reduplication and in terms of lack of rotation comensurate with the rotation of the fissure position. The fifth case was the NV(p) fragment showing marked optical abnormality and reduplicated projections. Here 3 fissure s were present in roughly ventral, nasal and dorsal positions. Thus in general fissure abnormality was not an indicator of map abnormality. Eye size showed a somewhat greater correlation with map abnormality in this series than in the previous one. In the temporodorsal group 4 of the 5 eyes were smaller than the control eye. However, all maps here were normal. In contrast in the NV(p) group, the 3 interpretable reduplicated maps were obtained from eyes smaller than control. The other 5 interpretable normal maps were obtained from eyes of normal size. The NV(c) eyes were all normal sized.

The pigmentation pattern of the experimental eyes was normal in the TD(p) and NV(c) groups. Four animals in the NV(p) group, however, showed totally silver eyes. One of these yielded a double nasal map, two normal maps and one an uninterpretable map. In addition one further eye in this group showed a partial silvering of the dorsal aspect. This eye also yielded a double nasal map. Again, however, abnormal pigmentation of the eye was not an indicator of map abnormality.

Electrophysiological Mapping

The results are tabulated in Table 4, classification of map configurations being as for Table 3. Twenty-two results are shown, comprising central and peripheral nasoventral fragments

and peripheral temporodorsal fragments. As in the previous
series, all 5 nasoventral central fragments were normal. Figure 32
illustrates such a map. However, the results from the peripheral
fragments did not yield a majority of mirror- reduplicated maps.
All 5 interpretable temporodorsal peripheral maps were normal
(see Figure 33), although responses were not localizable in the
caudomedial aspect of the tectum with a corresponding deficit in
temporodorsal field. In addition, 5 of 8 interpretable nasoventral
peripheral maps were normal. The remaining 3 were double nasal.
Figure 34 shows a normal map obtained from this group and Figure 35
a double nasal map. Figure 35 is an entirely typical double-
nasal-type map. The axis of symmetry is, as for maps obtained
from nasal peripheral fragments in the previous series, situated
in the vertical midline. It should be noted that this is not
the type of map that would be expected if the fragment had mirror-
reduplicated about the plane of ablation. Had that occurred the
axis of symmetry would have been expected to form an angle of about
45° with the vertical midline. Figure 36 shows another example
of the mirror-reduplication obtained in this series. It will be
seen that this map is not a typical double nasal configuration.
While the majority of the map is organized similarly to a double
nasal map with the axis of symmetry on the vertical midline, there
is a region of further reduplication in the temporoventral aspect
of the field (positions 7, 8 and 9). This additional region
appears to be a mirror image of points in the original nasoventral
quadrant of the retina (temporodorsal field) projecting to the
centre of the tectum. It is the only case found in the present
studies of the partial reduplication found by Feldman & Gaze (1975a)
and Berman & Hunt (1975). However, in this case the partial

FIGURE 32. Normal visuotectal map from left field to right tectum obtained from a nasoventral central fragment created at stage 35/36 and mapped in tadpole life. Conventions as in Figure 20.

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FIGURE 33. Visuotectal map from left field to right tectum
obtained from a temporodorsal peripheral fragment created
at stage 35/36 and mapped in tadpole life. The polarity of
the map is normal. Note the unresponsive regions of caudomedial
tectum and corresponding field deficit in temporodorsal
field. Conventions as in Figure 20.

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FIGURE 34. Normal visuotectal map from left field to right
tectum obtained from a nasoventral peripheral fragment
created at stage 35/36 and mapped in tadpole life. Conventions
as in Figure 20.

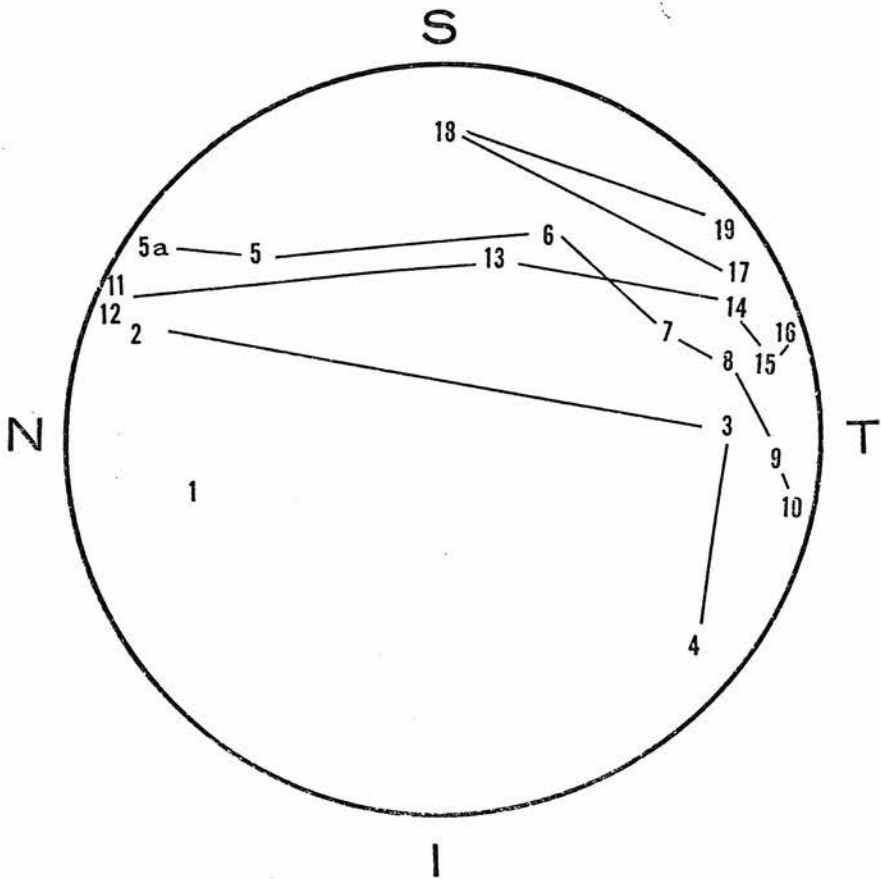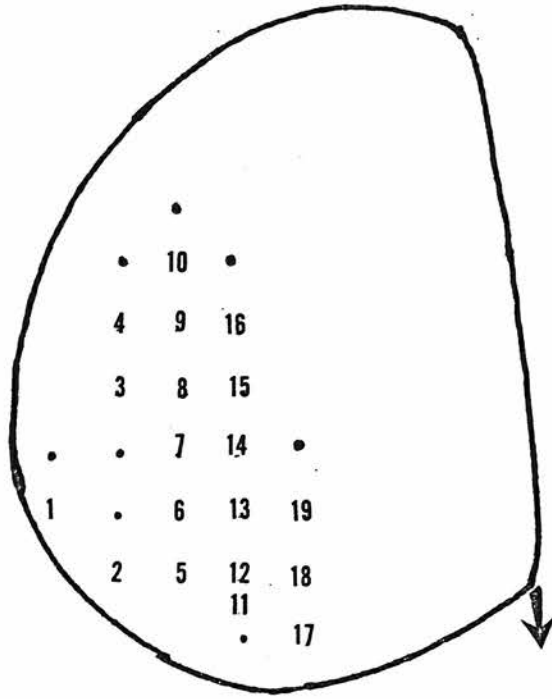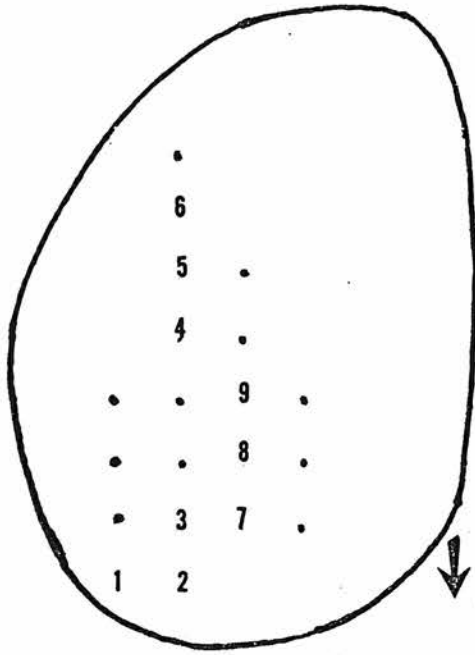FIGURE 35. Reduplicated visuotectal map from left field to right tectum obtained from a nasoventral peripheral fragment created at stage 35/36 and mapped in tadpole life. The axis of symmetry lies along the vertical midline. Conventions as in Figure 20.

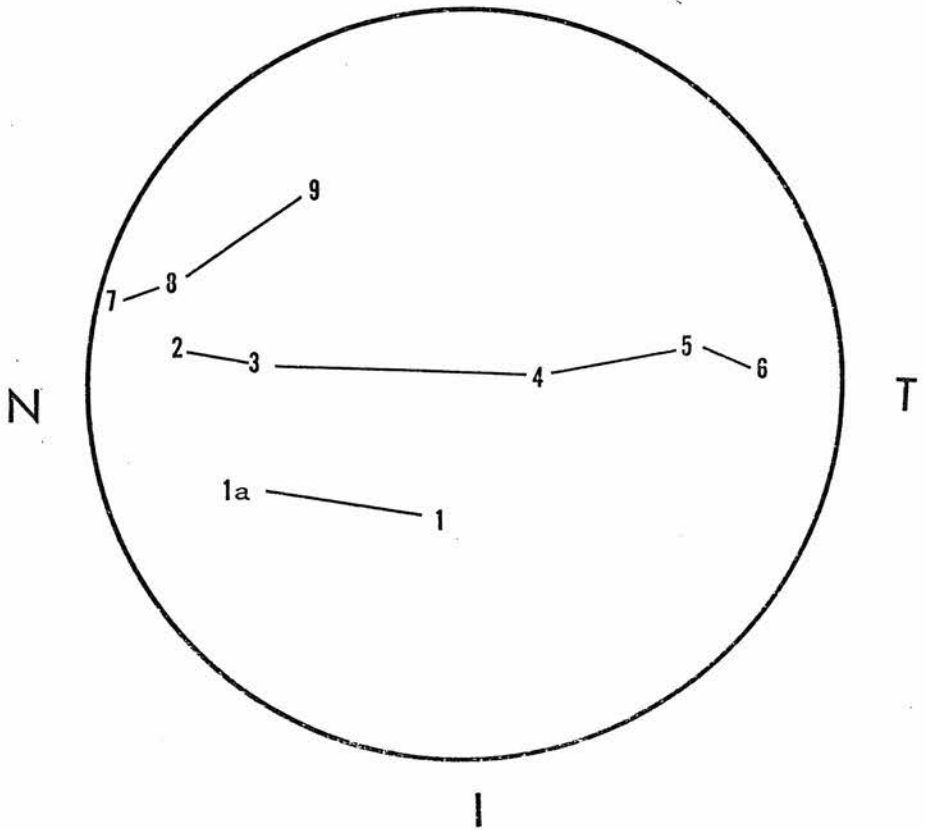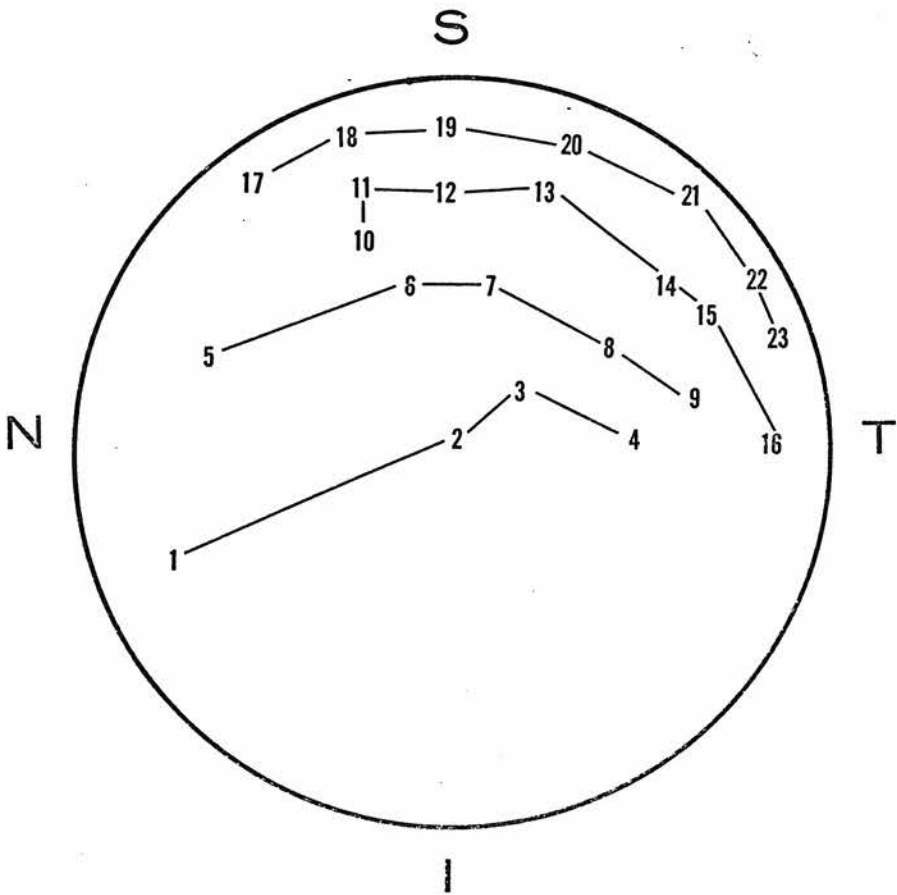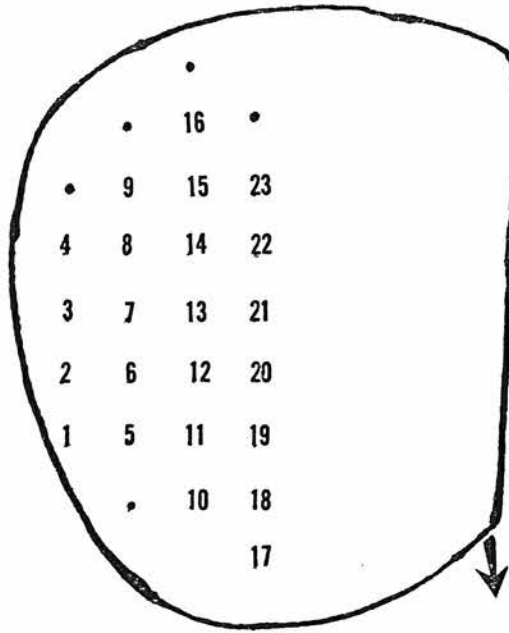FIGURE 36. Reduplicated visuotectal map from left field to right tectum obtained from a nasoventral peripheral fragment created at stage 35/36 and mapped in tadpole life. Note that the map shows a duplication about the vertical midline and also a small region of duplication (positions 7,8,9 and 10) at the temporal pole with axis of symmetry about the horizontal midline. Conventions as in Figure 20.

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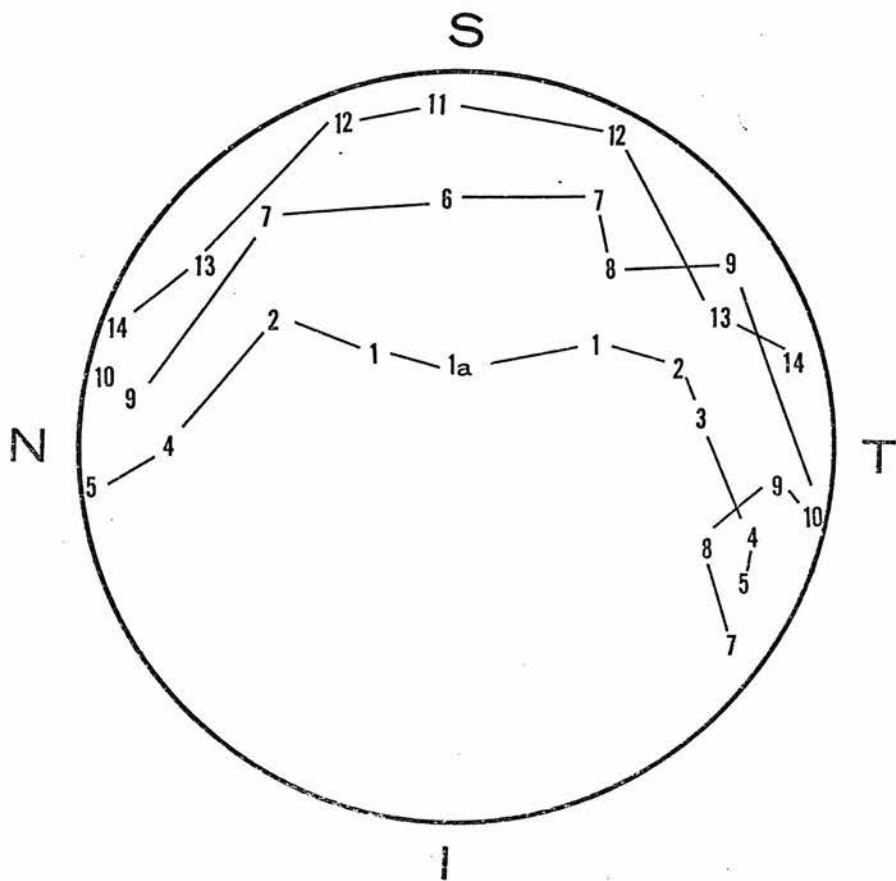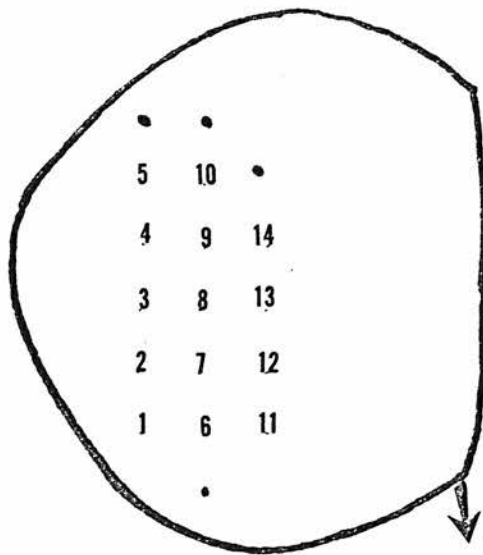
duplication is superimposed on an already duplicated map, resulting in a triplication of points. In addition to these reduplicated maps, reduplication was also found in a fourth case classified as uninterpretable due to severe optical abnormalities of the lens and cornea.

B.   PATTERN RESTORATION AFTER MIDLINE TRANSECTION

B.1.   Series 1 ; (Vertical Midline Transections)

This series comprises a total of 22 animals in which the left eye rudiment was transected along the vertical midline at a variety of embryonic stages from 29/30 to 37/38. The results from all stages in this series were identical. In all cases normal maps resulted when the retinotectal map was assayed electrophysiologically in late tadpole life (Table 5). Figure 37 shows a comparison map from a normal tadpole at stage 57. Figure 38 shows the map from a Series 1 operation performed at stage 32, while Figure 39 shows a stage 37/38 Series 1 operation. It will be noted that the normal tadpole map shows features not found in the adult map. Tectal coverage is not complete until stage 61. Prior to this stage caudomedial tectum where cell division and differentiation are not yet complete is without optic input.

Temporal field positions show a characteristic clustering. That is to say, temporal field occupies more tectum than does nasal field. The final difference is the curvature found at the rostral edge of the tectum. This results in a further apparent distortion of the linearity of the map. In fact, the tectal

FIGURE 37. Normal visuotectal map from left field to right
tectum obtained from an unoperated control eye mapped in
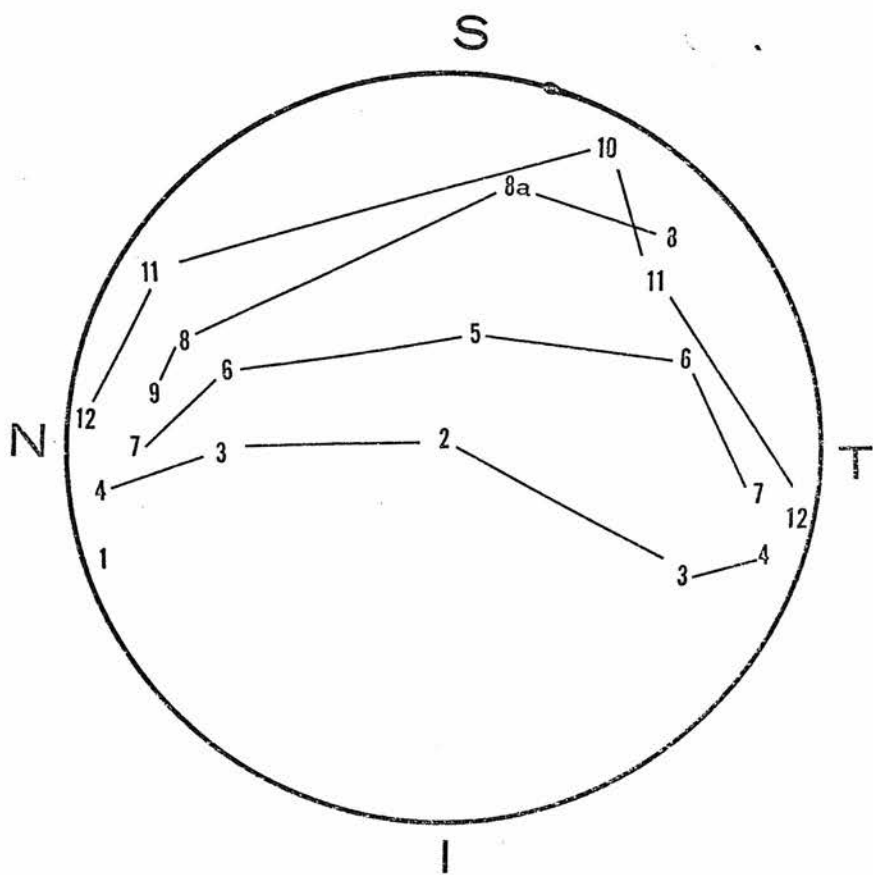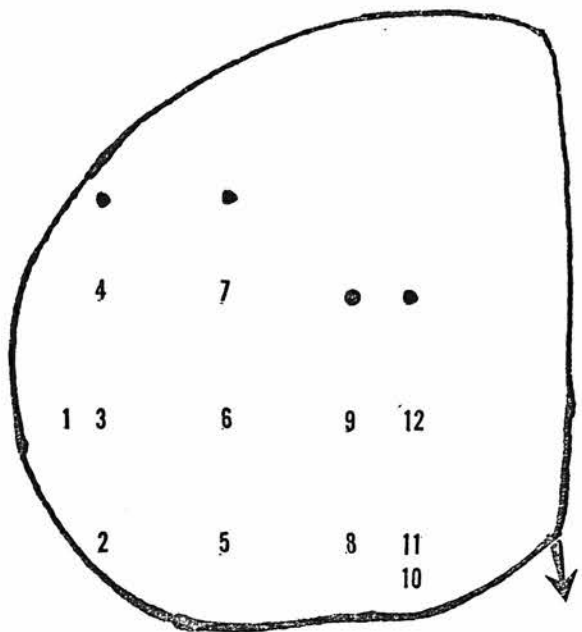tadpole life. Conventions as in Figure 20.

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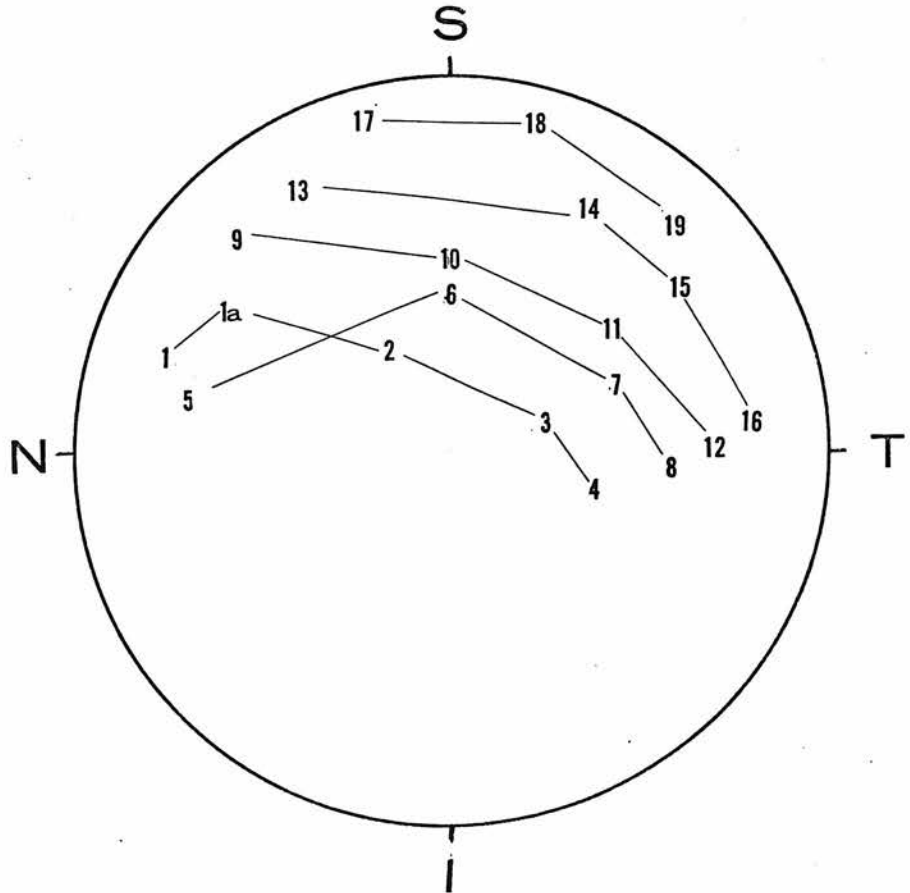
FIGURE 38. Normal visuotectal map from left field to right tectum obtained from a left eye transected along the vertical midline at stage 32 (Series I) and mapped in tadpole life. Conventions as in Figure 20.

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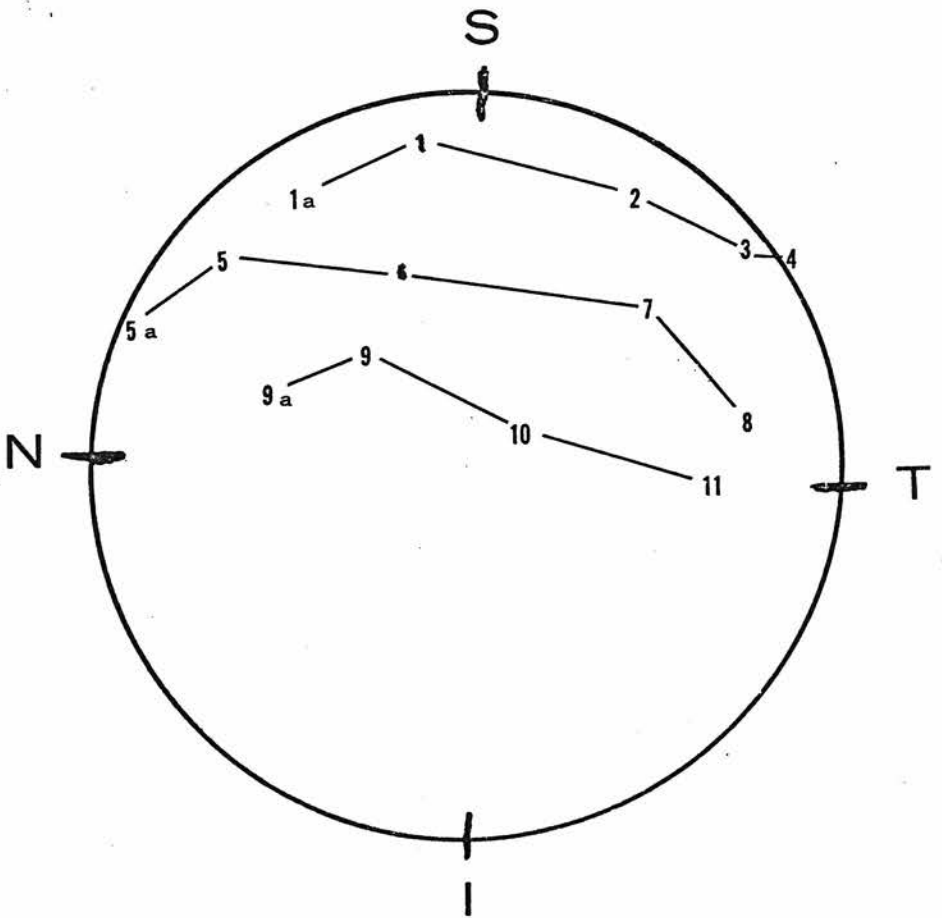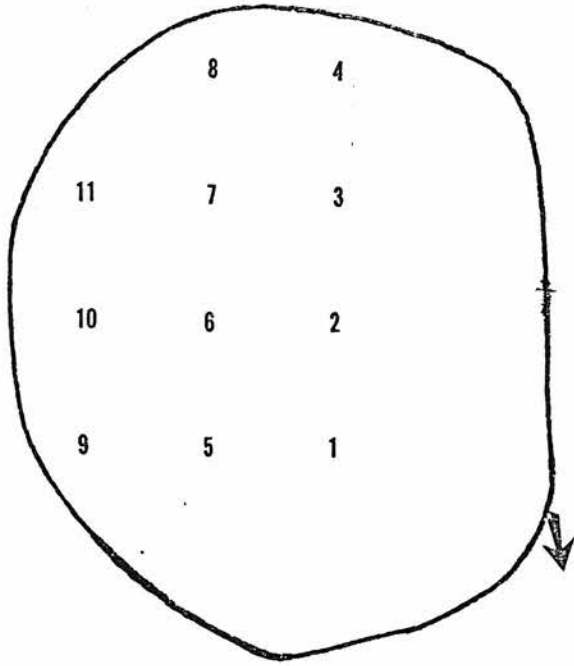
clustering of nasal field positions is due to the fact that
the most rostral positions are situated differently in depth
due to rostral curvature. Thus the most nasal field positions
may be found at electrode penetrations situated deeper than those
for somewhat more temporal field positions. According to the
convention employed here the more superficial tectal positions
(and more temporal field positions) are designated by a number
alone; deeper tectal positions (and more nasal field positions)
are designated by the number of the superficial positions and a
subscript. Thus 1, 1a, 1b and 1c would represent a set of positions
located progressively deeper in the tectum and more nasal in the
field. As with the adult map, the most ventral field is inaccessible
to electrode mapping as they project to tectal loci situated
around the lateral edge of the tectum. There was some tendency
evident in experimental maps towards loss of the projection from
the most nasal field. This was most marked in the latest stages
operated (37/38) as can be seen in Figure 39. Here the most
nasal part of the projection was compressed into the most rostral
100-150 u of the tectum. The reason for this is not clear. It
does, however, reflect simply an exaggeration of the non-linearity
of the maps from unoperated normal eyes. In no case was the
characteristic "double nasal" mirror-reduplication map pattern
found in 40% of Hunt & Jacobson's (1974b) experiments, present
in this series. Nor did the two halves of the field project in
register, each to the entire tectum. Rather the two halves mapped
to distinct non-overlapping regions. Histological examination of
of the retinae after mapping revealed a normal distribution and
size of the cellular layers. No persistent effect of transection
was found. The neural retina was coherent and continuous without

FIGURE 39. "Normal" visuotectal map from left field to right
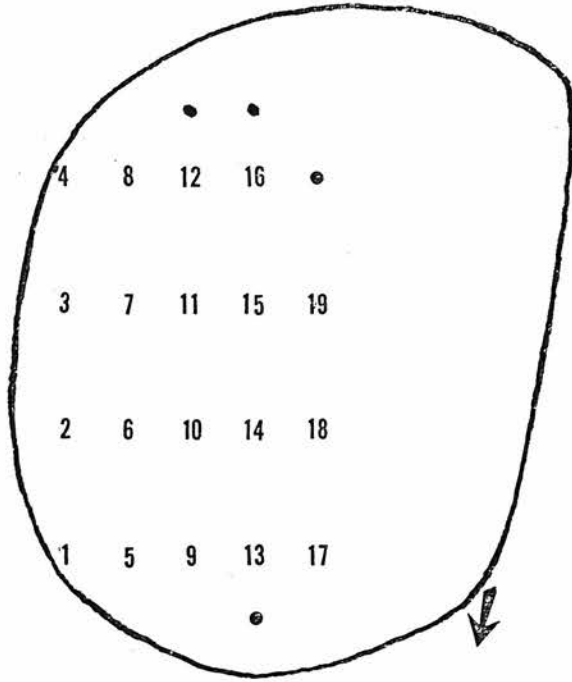tectum obtained from a left eye transected along the vertical
midline at stage 37/.38 (Series I) and mapped in tadpole life.
Note the extreme compression of nasal field onto the most
rostral tectum. The reduplicated positions at the rostral
edge (field positions 2, 2a and 3) are an occasional feature
of the normal tadpole map. Conventions as in Figure 20.

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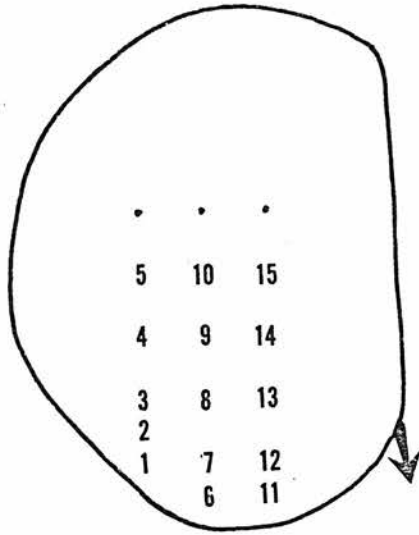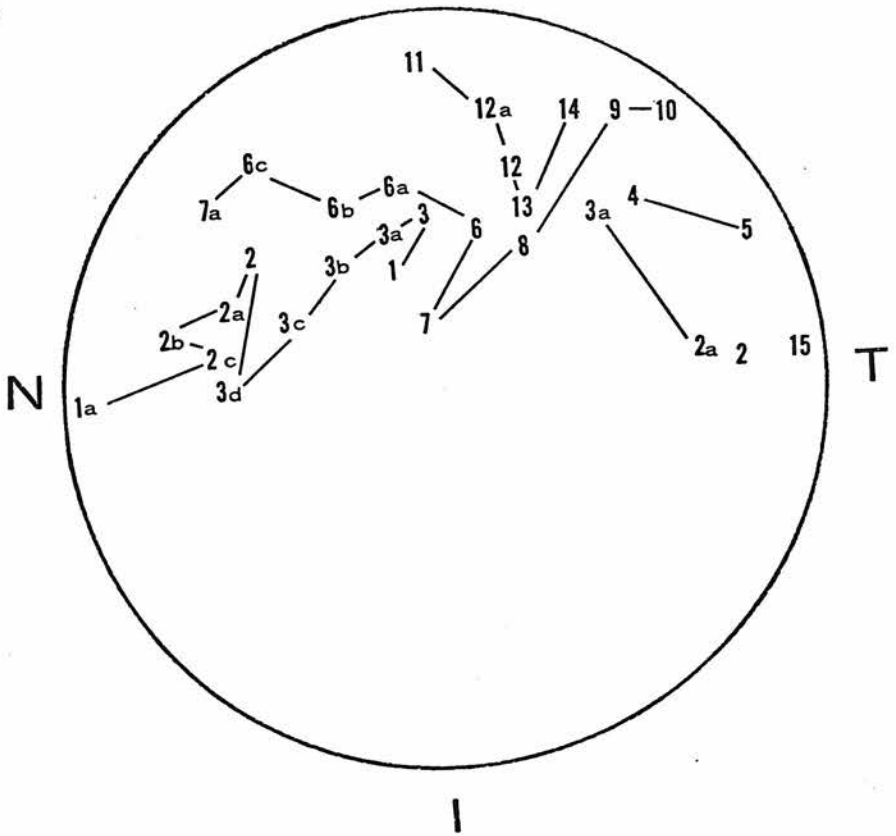
any obvious scar along the line of transection.

B.2.   Series 11 :(Horizontal Midline Transections)

This series comprises a total of 17 animals in which the
left eye rudiment was transected along the horizontal midline
(dividing dorsal and ventral halves of the rudiment) at a
variety of stages from 29/30 to 37/38. Again all cases in this
series consistently gave normal maps (Table 5). Figure 40 shows
the map from a stage 32 transection and Figure 41 that from a
stage 37/38 transection. No case of "double ventral" mirror-
reduplication, as reported in 40% of cases by : Hunt & Jacobson
(1974b), occurred in this series. In no case did the dorsal and
ventral halves of the retina project in register to the whole tectum.
Instead each half projected to distinct and non-overlapping tectal
regions. Histological examination again revealed no persistent
effects of transection either on cellular layering patterns or on
the continuity of those layers.

Thus the result of a total of 39 midline transections from
stages 29/30 to 37/38 was 100% of cases producing maps with
normally disposed axes.

B.3.   Series 111 : (Ionic Strength Controls)

Series 1 and 11 operations were performed in 100% Niu-Twitty
solution and allowed to recover for 24 hours post-operatively in
in 50% Niu-Twitty solution. The results obtained from those
series were inconsistent wth those reported by Hunt & Jacobson

FIGURE 40. Normal visuotectal map from left field to right tectum obtained from a left eye transected along the horizontal midline at stage 32 (Series II) and mapped in tadpole life. Conventions as in Figure 20.

18 12

17 11

16 10

20 15 9 6

19 14 8 5

13 7 4 2

3 1

S

1
2
6
4
3 5
3 a

8 10 11 —12
7a 7 9
N 8
15 16
9 17 18 T
13 14
13a
19 20

I

FIGURE 41. Normal visuotectal map from left field to right
tectum obtained from a left eye transected along the horizontal
midline at stage 37/38 (Series II) and mapped in tadpole life.
Conventions as in Figure 20.

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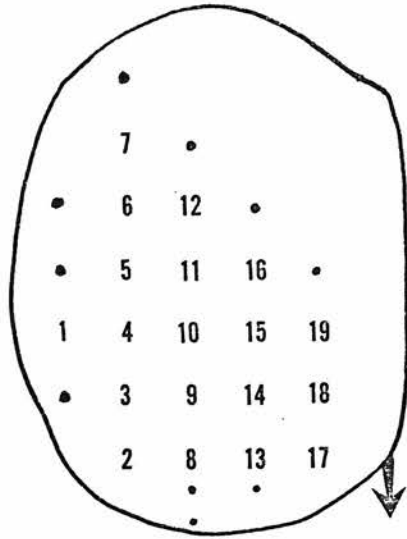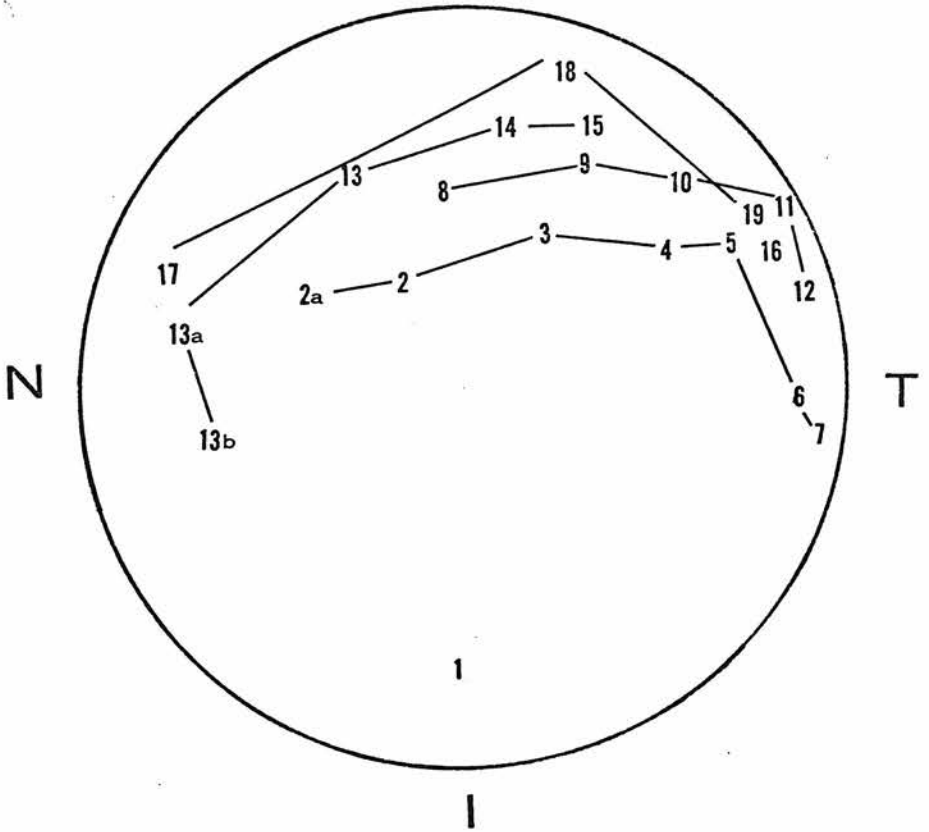
(1974b). Since the operations performed by those authors used
a 25% operating solution and a 20% rearing solution thereafter
(Hunt: personal communication) it is possible that differences
in post-operative ionic conditions account for these differences.
Series 111 therefore comprises a sample of 17 animals subjected
to vertical midline transection at stage 32 in a 25% solution
(20% Holtfreter/ 5% Steinberg). Operated animals were kept in
this solution for 24 hours post-operatively. Fifteen of the 17
animals again gave normal maps, as for series 1 and 11. The
remaining 2 animals, however, yielded mirror-reduplicated "double
nasal" maps, similar to those reported by Hunt & Jacobson (1974b).
These results are displayed in Table 6. A sample map is shown
in Figure 42 . It will be seen that in contrast to the normal
map, each tectal locus receives input from two visual field positions,
disposed in a mirror-symmetrical fashion about the vertical midline.

The nasal and temporal extremities of the field project to rostral
tectum and the vertical midline projects caudally. Thus both halves
of the eye project across the tectum with the normal polarity being
reversed in the temporal half field.

B.4. Observations on Healing Rate

The gross healing rate of Series I (stage 32: operated in
100% Niu-Twitty) and Series III (stage 32: operated in 20%
Holtfreter's / 5% Steinberg's) transections were compared by serial
observation during the first 24 hours after operation. These

TABLE 5:   STAGE OF OPERATION OF SERIES I AND II MIDLINE
             TRANSECTIONS

STAGE	29/30	31	32	35/36	37/38
SERIES I	4	3	5	4	6
SERIES II	3	6	1	2	5

Series I comprises animals whose left eye rudiments were transected
along the horizontal midline; Series II, animals whose left eye
rudiment was transected along the horizontal midline. All operations
were performed in 100% Niu-Twitty solution. The table shows the
number of animals in each group.

TABLE 6:   MAP CONFIGURATIONS PRODUCED AFTER MIDLINE
             TRANSECTIONS

	NORMAL	REDUPLICATED
SERIES I	22	-
SERIES II	17	-
SERIES III	15	2

Series I and II are described in Table 5 (above). Series III comprises
transections at stage 32 of the left eye rudiment performed in 25%
solution with 24 hours healing in that solution. The table shows the
number of animals in each group.

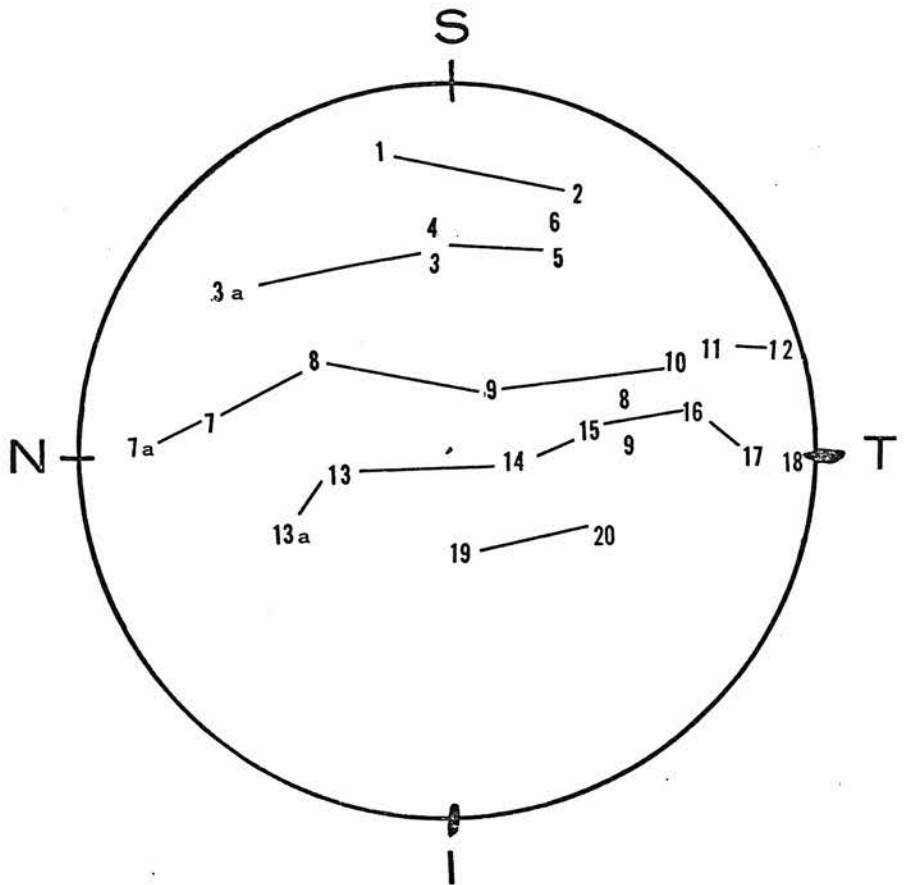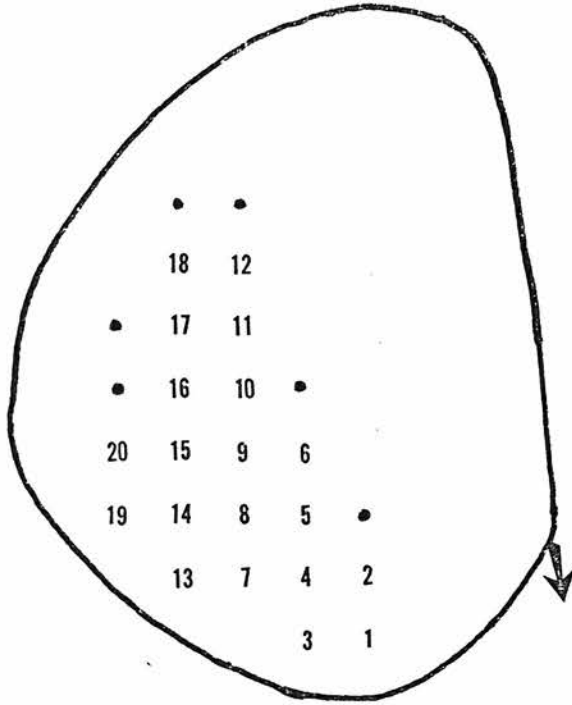FIGURE 42. Reduplicated visuotectal map from left field to right tectum obtained from a left eye transected along the vertical midline at stage 32 (Series III). The axis of symmetry lies along the vertical midline. Conventions as in Figure 20.

S

6

1 — 2 3 5 4

5 3 2 1

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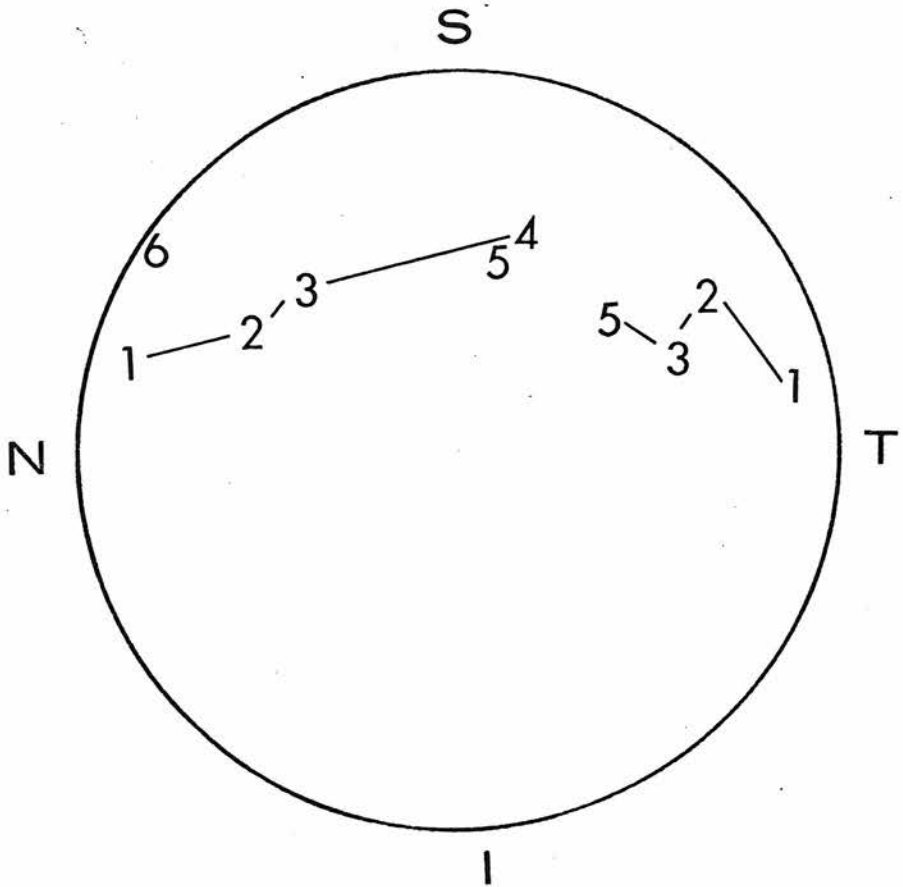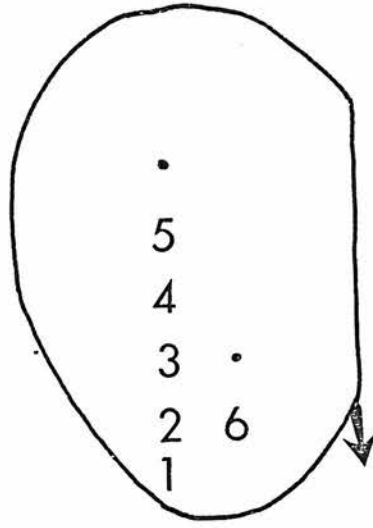
observations were made on two separate occasions, comprising
three operated animals from each series on both occasions.
The healing rates within a series were in accord with each other
to within one hour. The following results, then, pertain to
a sample of 12 animals.

Figures 43a and 43e show camera lucida drawings of a
Series I and a Series III eye respectively, immediately after
operation. To facilitate comparison healing was divided into
four stages: apposition, healing, morphological normality and
differentiation. The eyes were considered to have reached the
apposition stage (Figures 43b and 43f) when the two fragments
of the eye were still clearly separate but apposed to each other.
The healing stage (Figures 43c and 43g) was defined by the
elimination of a clear separation between the fragments and
the restoration of surface continuity. As can be seen from
Figures 43c and 43g the eyes still showed evidence of the
operation in the irregularity (and often the "dumb-bell" shape)
of their morphology. The morphological normality stage was def-
ined by the restoration of a more normal rounded profile and
the clear delineation of the ventral fissure (Figures 43d and
43h). The differentiation stage was defined by the appearance
of lens formation and served as an indication of the re-entry
of the operated eyes into the normal developmental sequence.
This stage was not clearly separable from the preceding one but
represented its culmination and there was considerable variation
between animals in the time at which differentiation was reinitiated.
This stage was not diagrammed as it was signalled only by a

FIGURE 43. Stages in the healing of Series I and Series III
midline transections (a) cameral lucida drawing of a Series
I transection immediately after operation. (b) the same eye
at the "apposition" stage. The fragments have become apposed
but still show a boundary (dotted line). (c) the same eye at
the"healing" stage. The fragments have now fused at the
boundary and are no longer distinguishable as separate. (d)
the same eye at the "morphological normality" stage. The
eye now has a normally rounded profile and a clearly delineated
ventral fissure. (e) a Series III transection immediately after
operation. (f) the same eye at the "apposition" stage. (g) the
same eye at the "healing" stage. (h) the same eye at the
"morphological normality" stage. The pigmented portion of the
eye is shown shaded. All drawings are oriented with the nasal
pole facing left and the ventral pole facing to the bottom of
page. (i) graphical summary of healing rates. The healing
stages are shown as a function of time since the operation.
A - apposition stage; H - healing stage; N - morphological
normality stage; D - differentiation stage. The definition
of the stages may be found in the text (p. 118). Filled
circles Series I, open circles Series III.
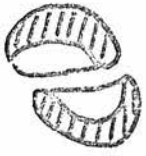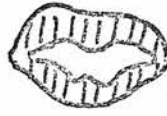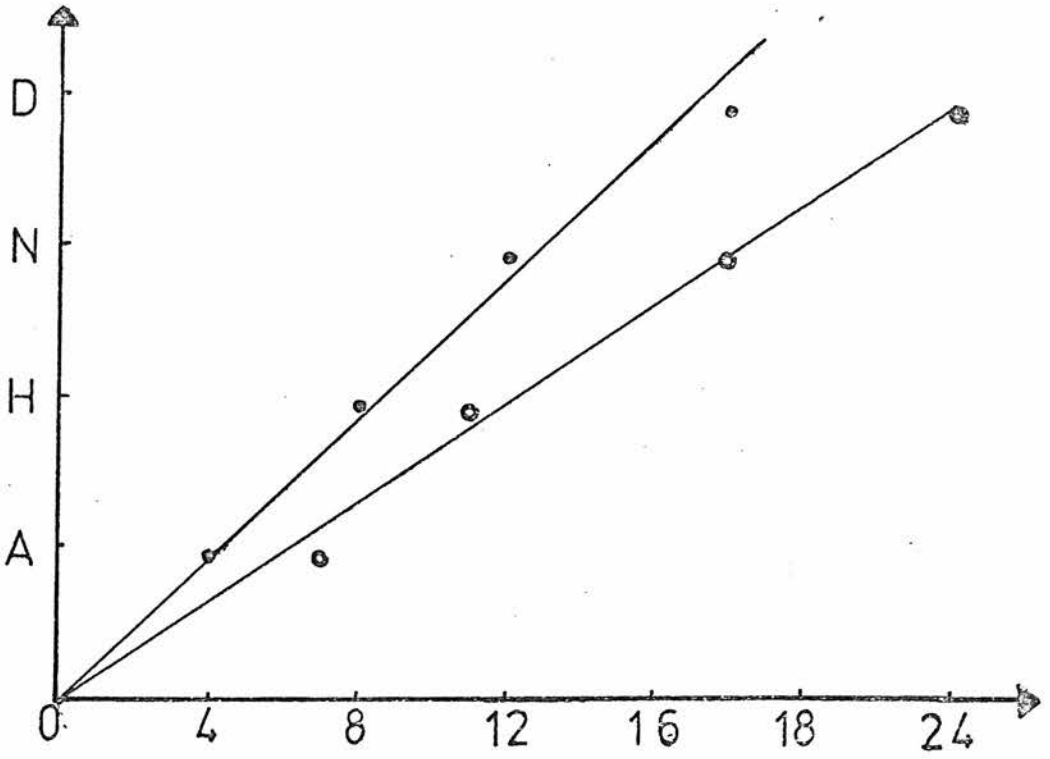
change in the optical properties of the lens cells.

Series I eyes were found to heal significantly faster than did Series III eyes. Furthermore, although not evident from the diagrams, it was found that cell adhesion in general in the 25% solution was poorer and over the course of the observation cell clumps were seen to detach from the embryos. Apposition was achieved by 4 ± 1 hour of operation in Series I and only by 7 ± 1 hour in Series III. Healing occurred at 8 ± 1 hour in Series I and at 11 ± 1 hour in Series III. Morphological normality was restored in Series I at 12 ± 1 hour and in Series III by 18 ± 1 hour. Differentiation was reinitiated by 18 hours in all Series I eyes and by 24 hours in all Series III eyes. Figure 43i summarises these findings in a semi-graphical form. It should be noted that since the stages were arbitrarily defined the slope of the healing curve is not biological meaningful however the enhanced healing rate in the 100% solution is clearly evident. The Series III fragments spend an additional three hours in isolation prior to healing.

C.   THE RETINOTECTAL MAP FROM EYE PRIMORDIA EXPLANTED TO CULTURE AT EARLY NEURULA STAGES

A pilot series of 16 transplants was successfully made as described in Chapter 5. Of these 4 died during larval life and in 5 others the eye was completely resorbed. Of the remaining 7 animals mapped at stage 57/58 three were found to have failed to achieve connection with the optic tectum. Thus maps are available for only 4 animals from this series.

Two of the maps showed disordered projections arising only from a restricted area of the visual field, somewhat analagous to the "pattern 1" regeneration maps described by Gaze & Jacobson (1963) and Gaze & Keating (1970) in studies of regeneration after optic nerve section. The remaining two maps both showed clearly ordered projections. Figure 44 shows the map obtained from case Ex.4. This was a stage 13 left eye explant tranplanted to the enucleated right orbit of a stage 36 host. Despite the fact that responses were obtained only from the most dorsal aspect of the visual field, it will be seen that the response points are ordered approximately as for a normal right eye. Thus either the explant did not acquire an anteroposterior axis in culture, being polarized in the host orbit; or such axes as were acquired were not irreversibly determined. In either situation the axes of the transplant projection have been acquired from the host.

The other successful case, Ex. 1, was a stage $12\frac{1}{2}$ explant rendered semisynophthalmic by removal of the prechordal plate and transplanted into the left orbit of a stage 37/38 host. Again a complete map was not obtained, responses this time coming from the nasal field only (Figure 45). The middle of the field projects to rostral tectum and the nasal pole to caudal tectum. Dorsoventral ordering, from the two rows of field positions available, would seem to be normal. Thus the anteroposterior axis of the map is the inverse of that expected for a normal left eye. The axis was therefore not acquired after transplantation to the host orbit. The interpretation of the finding that only nasal field projects to the tectum in this case is unclear. It

FIGURE 44. Visuotectal map of right field to left tectum
obtained from an eye rudiment explanted to culture at
stage 13, transplanted to the enucleated right orbit of
a stage 36 host and mapped in tadpole life. Although
responses were obtained only from dorsal field the map
is normal in its polarity. Conventions as in Figure 20.
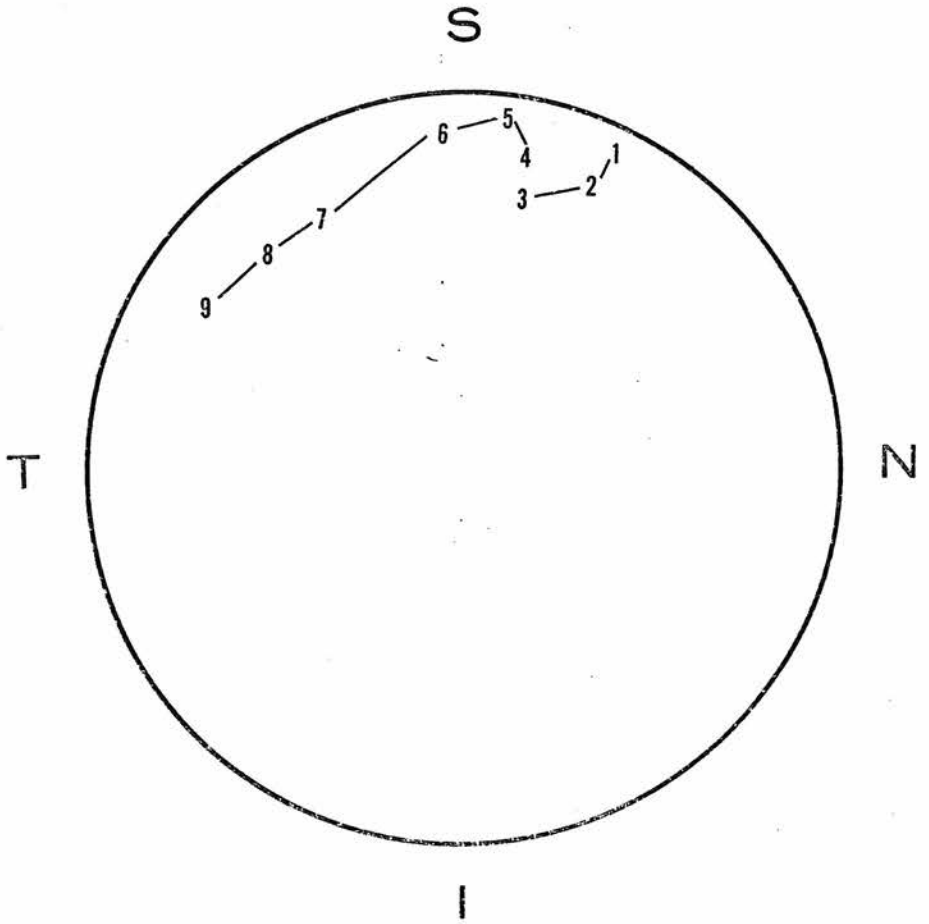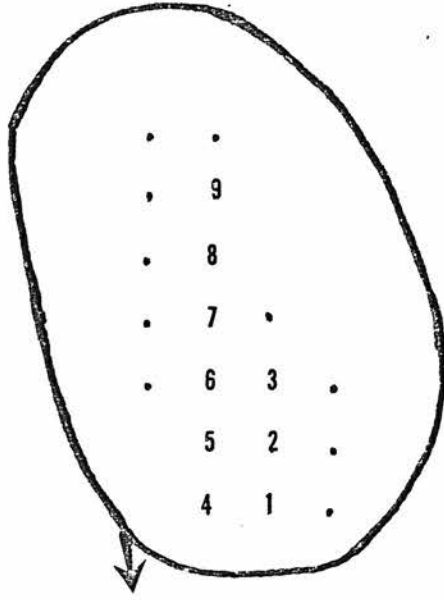
FIGURE 45. Visuotectal map from left field to right
tectum obtained from an eye rudiment rendered
semisynopthalmic by removal of the prechordal plate
at stage $12\frac{1}{2}$ explanted to culture until transplanted
to the enucleated left orbit of a stage 37/38 host
and mapped in tadpole life. Responses were obtained
only for nasal field. The map is normal in its
dorsoventral but inverted in its nasotemporal organisation.
Conventions as in Figure 20.
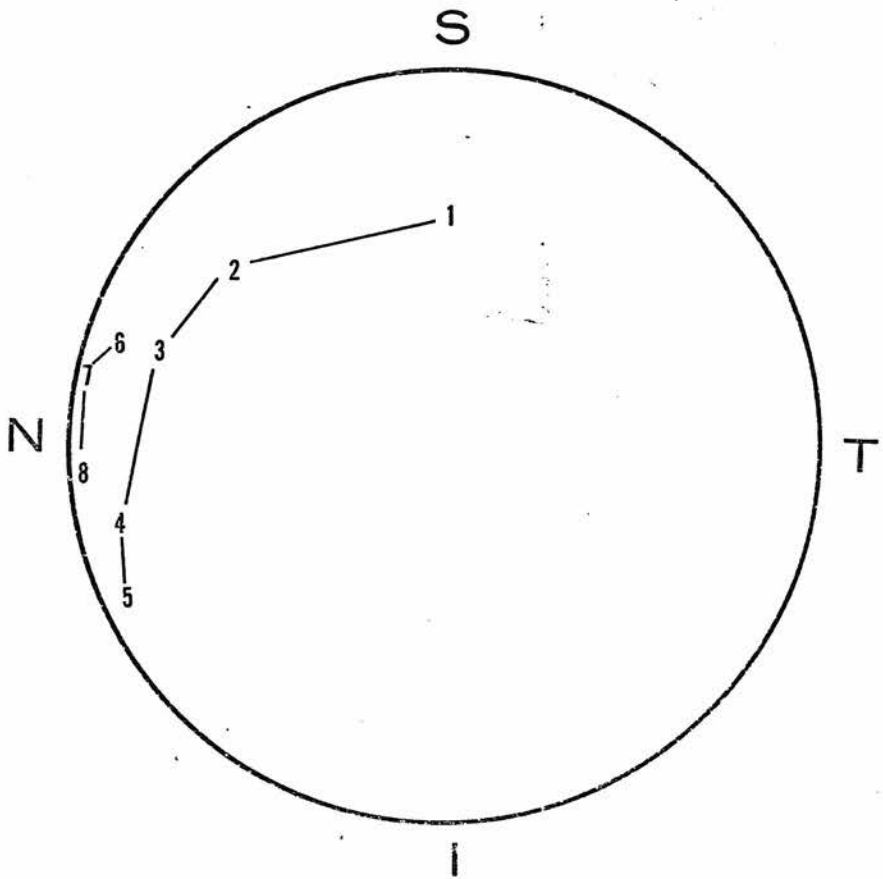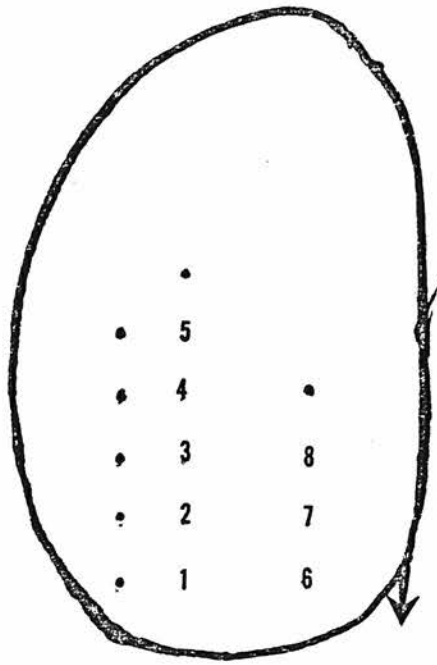
resembles a half field projection from a double nasal eye
(Gaze et al 1963, 1965) rather than the expected finding of
double temporal maps from semisynophthalmic eyes (see Chapter 4).
It may simply represent the mapping from a normal eye implanted
in the host orbit with anteroposterior inversion in which the
nasal fibres have failed to develop and the remaining temporal
fibres have spread across the available tectum. Whatever the
interpretation the clear finding from this case is that the
explant paradigm used here is inadequate to prevent the genesis
of endogenous retinal axes. In case Ex. 4 discussed above,
while stable axes do not seem to have been acquired in vitro
transplantation at stage 36 to a normal host was not adequate
to prevent the specification of the host axes.

Since the orbit of stage 39 hosts has been shown to
be capable of polarizing transplanted pre-determination stage
eyes (Hunt 1975) and since eye primordia explanted to culture
as early as stage 22 possess presumptive axes (Hunt & Jacobson
1972b), the results obtained in this pilot series were perhaps
not altogether unexpected. For this reason the paradigm was
not explored further. It should be noted that the finding of a
graft specific axis in case Ex. 1 above does not necessarily
imply the existence of such an axis from the stage of explantation
($12\frac{1}{2}$). The axis may have developed in the fragment, which
contained the anterior portion of the embryo, at any time during
the culture period. It does however seem likely that the axis
does exist from such an early stage as part of the entire embryo
primary axis.

D.   THE ACQUISITION OF DEVELOPMENTAL AXES: EXPERIMENTS
      WITH CYCLOPEAN EYES

Of an initial sample of 18 animals with successful
transplants of cyclopean eyes, 6 were available at late tadpole
stage 50's in which the eye had not been resorbed. In none of
the six was a map obtainable on the optic tectum. In no case
was the tranplanted eye completely normal to external examination.
In two the eye was small and defective, with lens and cornea
formation absent or incomplete. In one case the eye was simply
a pigmented knot of tissue totally enclosed by the choroid.
Of the remaining three only one showed ventral fissure formation.
In this eye two fissures were clearly present situated in the
nasodorsal and temporoventral quadrants, at approximately 180°
from each other.

Histological examination revealed absence of optic nerve
formation in all six cases. Figure 46a shows a transverse section
through the fundus of the transplant, at the site where an optic
nerve head would be present in a normal eye. The layers of the
retina can be seen to be fairly normal, but the ganglion cell
axons are piled up in a disorderly fashion running in bundles
across the ganglion cell layer. At no point are they seen to
leave the eye. As a result the optic fibre layer is thicker
than that observed in the normal eye. Figures 46b shows a transverse
section through the dundus of the normal host eye in the same
animal for comparison. The optic nerve head is clearly defined.

Agenesis of the optic nerve head with appropriate structures
for the guidance of the optic nerve out of the eye has been

FIGURE 46a. Tranverse section through the fundus of a stage 57 cyclopean eye. The eye was produced by treatment of embryos with Lithium ions at late blastula stages and was transplanted into a stage 35/36 enucleated left orbit. The normal layering of the retina is approximately preserved with photoreceptors (bounded by the pigment layer) at the top of the photograph and the ganglion cell layer facing towards the bottom. The absence of the optic nerve head is conspicuous and as a result the fibrous portion of the ganglion cell layer (the optic axons) is swollen and bundles of axons may be seen transversing the centre of the retina. (Holmes silver stain x 60).

FIGURE 46b. Transverse section through the fundus of the normal right eye in the same animal as that shown in Figure 46a. A clearly defined optic nerve head conducts the optic axons out of the eye. Structures at the bottom of the photograph are fragments of the lens (Holmes silver stain x 60)
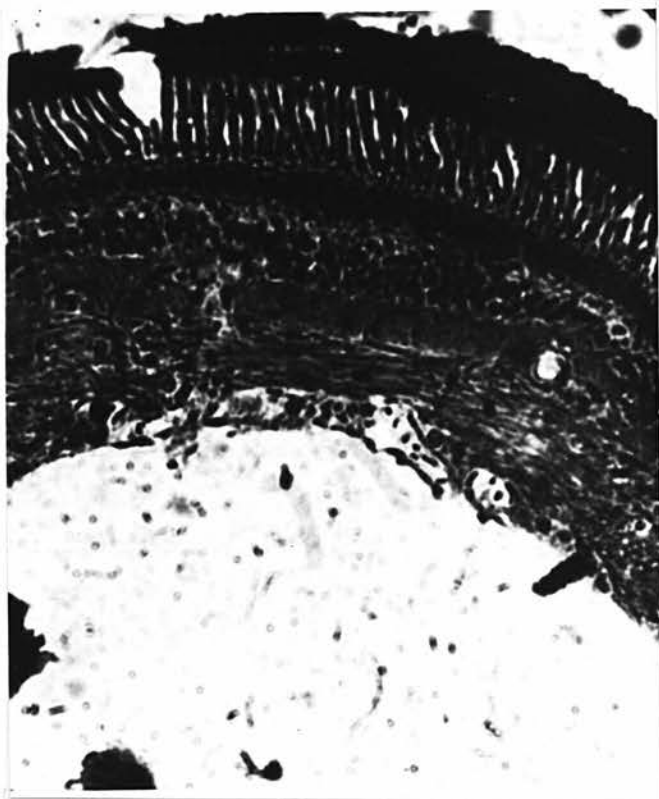
46 a

46 b

reported previously for cyclopean eyes (Szentagothai & Szekely 1955). Those authors suggest that such agenesis is a function of the degree of optic primordia fusion induced by the Li^+ treatment. In early development of the eye the paired primordia are present centrally in the forebrain rudiment. With separation the primordia migrate laterally and dorsally. It may be then that the primordia are initially fused along their presumptive ventral poles. Excessive fusion of the primordia in cyclopean eyes may then well result in agenesis of the ventral apparatus.

Because of the finding of agenesis this project was not carried further.

CHAPTER 8 :   DISCUSSION OF THE RESULTS CONCERNING RETINAL
PATTERN FORMATION

A.   PATTERN RESTORATION IN EYE FRAGMENTS

Orthogonal eye fragments (constructed with the plane of ablation parallel to one of the major axes of the eye) made at early larval stages can give rise to two types of map pattern: either normal or mirror reduplicated (Feldman & Gaze 1975a ; Berman & Hunt 1975). As with comparable cases in other systems (Butler 1955; Bryant 1971; Schubiger 1971) it has been possible to find here two types of fragment corresponding to the two types of map configuration. Eye fragments containing the central retina yield only maps with normal polarity, while those lacking the central retina yield a majority of mirror reduplicated maps. (Chapter 7, A.1.)   A further analogy with such phenomenology in other systems was suggested in Chapter 4 by consideration of gradient models of "distal transformation". It was suggested that the retinal pattern was formed in radially symmetrical fashion about the centre ("proximal" retina). However, when this hypothesis was tested by the construction of eye fragments made at an angle to the major axes (oblique fragments: Chapter 7, A.2.) a different result was obtained. As before fragments containing the centre yielded only normal maps. In this series peripheral fragments lacking the centre also yielded, as a majority result, normal maps. Thus the behaviour of the pattern restoration mechanism was different according to whether the plane of fragmentation followed or made an angle with the major embryo axes.

Before advancing to a possible interpretation of these results, the major conclusions from the present study must be pointed up.

A.1.   Eye Morphology

Eye fragments can "regulate" morphologically. The term
"regulate" is placed in quotes because it covers here two observations
which may involve different mechanisms. In the first place the
eye fragments are observed to round up and assume miniature but
normal morphology. This would appear to be a composite of normal
healing and cell multiplication processes. A more convincing
usage of the term "regulation" would be in reference to the
formation of the ventral fissure. While central fragments contain
the ventral fissure, this structure is absent in nasal, temporal
and dorsal peripheral fragments. Although its position is
determined in the intact eye rudiment at earlier embryonic stages
(Sato 1933; Stone 1966), it can nevertheless be reformed in
peripheral eye fragments. Even in fragments which totally lack
ventral material (dorsal peripheral fragments) such regulative
restoration of the ventral fissure is possible. Straznicky et al.
(1974) reported the absence of a ventral fissure in double dorsal
compound eyes produced by the recombination of two dorsal half
fragments at stage 32. The reason for this difference in fissure
restoration is not clear. It is possible that it is due to the
apposition of two fragments in the case of compound eyes which
may result in the inhibition of mitosis at the cut edge (Feldman
& Gaze 1975a).

In general the "regulated" fissure appears ventrally
(82% of cases) as in a normal eye. In 7% of cases the fissure
appears nasally (once in the nasal peripheral series and 4 times
in the temporal peripheral series); in 3% of cases it appears

126.

temporally (once in the dorsal peripheral series and once in the temporal peripheral series); and in 7% of cases two fissures are formed, one in the nasal half, one in the temporal half of the eye. All misplacements of the fissure, with one exception, occur in the peripheral series (which lack the fissure and thus have to regenerate or regulate it). The finding of the fissure ventrally in the majority of fragments is consistent with either a "field regulation" whereby the fissure is located in the appropriate position, or with a regional determination in which the fissure is produced by the material most proximately situated to the original fissure (i.e. ventral retina). This second possibility is compatible with the nasal position of the fissure in 4 of the temporal peripheral fragments given a marked rounding up of the fragment apposing dorsal and ventral regions of the cut edge in the position of the nasal pole but fails to explain the other cases of fissure misplacement. At present, no definitive conclusions can be drawn as to the mechanism of fissure restoration.

The fissure, as the earliest determined asymmetry of the eye, is an attractive candidate for an "organizer" or "boundary region" of the retinal pattern. Such a suggestion has indeed been made by Sharma & Hollyfield (1974a,b) who found after eye rotation in Rana and rotation of "pre-specification" stage eyes in Xenopus that the degree of rotation of the map corresponded with that of the fissure. However the present results do not substantiate this view. A corresponding rotation of the map was not found in cases where the fissure was misplaced. The maps, whether normal or reduplicated, showed no rotation. In two of the temporal peripheral cases (see Table 3b) maps with a clockwise rotation ($60°$ and $15°$)

were found. The reason for this in unknown. However, the fissure in the 60° rotate was somewhat temporal to its normal position (approximately 15° anticlockwise) and the fissure in the 15° rotate was normally placed. Thus the orientation of the map is disengageable from the orientation of the fissure.

The morphology of the eye does not undergo total regulation. In 60% of the ventral peripheral fragments and 50% of nasoventral peripheral fragments the pigmentation of the dorsal aspect of the eye was totally silver. This is the pigmentation characteristic of the ventral aspect. It suggests that the eye was almost entirely reconstituted from ventral cells which retained this feature of their original determination (although this may alternatively reflect deficits in pigmentation). Again, however, the map configuration is not closely linked to the morphology of the eye. Two of the 5 ventral reduplicated maps had normal dorsal pigmentation as did one of the three naso-ventral reduplications; conversely of the 6 ventral fragments with silver dorsal pigmentation, three yielded normal maps (i.e. maps in which the ventral field coming from the dorsal retina was not a mirror image of the dorsal field) and of the five nasoventral fragments with silver dorsal pigmentation two yielded normal maps, one yielded an uninterpretable map and two double nasal type reduplications.

A.2.   "Duplication" and "Regeneration"

Where reduplicated maps are produced they always correspond to the original nature of the fragment in the intact eye: thus nasal fragments reduplicate as double nasal patterns, temporal

fragments as double temporal patterns and so on. Therefore in the eyes formed by the stage of mapping it is not necessary to invoke any alteration of polarity in the original fragment. The maps may be most simply interpreted as the retention of polarity in the original fragment and the formation of a mirror image pattern in the reconsituted portion of the eye arising at the cut edge. For example, in a nasal peripheral fragment, the map produced retains normal polarity in the temporal field (nasal retina) and a mirror image polarity in the nasal field (temporal retina). Of course such an interpretation is not the only one possible. It does not require the postulation of morphallactic regulation. If regulative reorganization is invoked the mirror reduplication of the maps would have to be produced by some of the cells of the original fragment reversing their polarity by an unknown mechanism. This interpretation is one offered by Berman & Hunt (1975) who regard the finding of mirror reduplication from eye fragments as indicative of "a radical redeployment of locus specificities". While it is clear that under some circumstances the retinal polarity appears to be an interactively maintained property of large multicellular regions and that polarity reversals can occur (Hunt & Jacobson 1973b, 1974b) I have opted for the above mentioned interpretation in which none of the cells of the original fragment alter their polarity. There are two main reasons for advancing this interpretation: firstly, on the grounds that this appears to be the most parsimonious interpretation given that the maps can be interpreted without assuming spontaneous polarity reversal; secondly, because this would bring the retinal reduplication phenomenon into line with what is thought to occur in other reduplicating systems.

The reprogramming experiments indicate a convergence of pattern reduplication configuration onto a final common pathway of either double-nasal or double-ventral type maps. The present results, however, along with the earlier findings from double-temporal compound eyes (Gaze et al. 1963,1965) indicate that the temporal region of the retina also possesses an intrinsic axial organisation. The polarity of the temporal retina in temporal fragments is the same as that displayed in the intact eye. Polarity reversal, where it occurs, is always in the nasal retina. Furthermore it is clear that the polarity of the temporal fragments does not reflect a "respecification" by the orbit since rotated temporal fragments show rotated axes (Hunt and Berman 1975). Thus the temporal retina itself possesses intrinsic polarity vectors, rather than exhibiting a state of axial determination dependent on that of the nasal retina. A similar situation would appear to be the case for dorsal with respect to ventral retina. Dorsal peripheral fragments were capable of yielding normal retinotopically organized maps. Although the finding of double dorsal map characteristics is here equivocal, having been found in only one poorly organized map, a well ordered double dorsal map has been found by Hunt & Berman (1975) by the expedient of preparing the dorsal fragment from an eye rotated prior to the stage of axial determination (which thus possesses the ventral fissure). The characteristics of this map are as expected by analogy with the other three classes of reduplication; the ventral field (dorsal retina) maps with normal polarity, while the dorsal field (ventral retina) maps with mirror-image polarity.

The incidence of reduplication in each of the three

analysable classes is different. Nasal peripheral fragments reduplicate most frequently (72%), ventral peripheral less frequently (63%) and temporal peripheral least frequently (60%). The occurrence of some non-reduplicated maps might be expected due to errors in the positioning of the plane of ablation (i.e. the inadvertant creation of central fragments). However the difference in incidence rate of duplication in different classes of fragments, though not statistically significant, may be worth considering briefly in view of similar differences in incidence rates in other studies.

Berman & Hunt (1975) report that nasal (half) fragments reduplicated in 55-60% of cases, ventral fragments in 35-40% of cases and temporal fragments in 25-35% of cases. The lower absolute value of these statistics is presumably due to a mixture of central and peripheral fragments in each group. However, the relative ordering of incidences is the same as in the present study. Two explanations of this difference suggest themselves: either the "centre" of the pattern does not correspond with the geometrical centre of the eye, or the difference reflects regional differentials in regulative ability. In the first case, it might be that the pattern "centre" is located somewhat temporal and ventral to the geometrical centre of the eye. If this were true fragments excluding the geometrical centre would be expected to include the pattern "centre" more often in temporal and ventral than in nasal fragments. In the second case the differential would be attributed to two processes being set in train in newly constructed fragments: one a tendency to restore morphallactically the entire pattern in the fragment and the other, the processes leading to mirror-reduplication. In fragments where reduplication was underway prior to the completion of regulation, reduplicated maps would result;

conversely in fragments where regulation was completed before the reduplication processes could operate, normal maps might result. It might be that nasal fragments require an increased time for regulation compared to temporal and ventral fragments.

In either case, it appears from the results of Hunt & Berman (1975) using fragments prepared from rotated pre-specification eyes that the increased frequency of nasal reduplicates compared with temporal is a feature of the polarization of the retina and not of the pre-existing morphology of the eye. These authors found that nasal fragments from rotated eyes (i.e. from embryonically temporal fragments) yielded 39% double nasal maps (3 out of 8) while temporal fragments (prepared from previously nasal material) yielded 0% (none out of 5) double temporal maps. Thus the asymmetry in reduplication incidence is a feature of the retinal pattern, not retinal morphology.

Finally, before proceeding to discussion of possible interpretations, the results from oblique fragments deserve further comment. Here the low proportion of reduplication in peripheral fragments and a lack of reduplication of the expected kind throw into question the generality of the hypothesis concerning the role of the centre sustained by the orthogonal fragments. Two cautions should be introduced here: firstly, is the lowered incidence of reduplication due to the later stage of construction of the fragments (35/36) and secondly, is the criterion for reduplication of the "expected" kind a valid one? Berman & Hunt (1975) reported that the incidence of reduplication in post-stage 32 fragments decreased with increasing age. However, this may have been due simply to the fact that with increasing age (and hence increasing size of the eye rudiment)

proportionate cell damage at the cut edge was smaller, hence predisposing towards the creation of an increased proportion of central fragments with increasing age. A small number of orthogonal fragments were constructed in the present studies at stages 35/36 or 37/38. Of 11 interpretable temporal fragment maps in the peripheral group 2 were constructed at ages later than 32. Both of these were reduplicated. The remaining 9 fragments, constructed at stage 32 showed 5 reduplications. Again in the ventral peripheral series 2 of the 8 interpretable maps were constructed at stage 35/36 and both were reduplicated; while the remaining 6 made at stage 32 yielded 3 reduplications. Thus the tentative conclusion may be drawn (albeit from a small sample) that peripheral fragments made at stage 35/36 do not show a lowered incidence of reduplication as compared with those made at stage 32. Thus, it would seem that age is unlikely to be an explanation of the lowered incidence of reduplication in the oblique fragment series. The nature of the reduplications themselves remains to be discussed. The three interpretable cases of reduplication were of the double nasal type, one of the three possessing a small region of additional duplication. The radial model, outlined in Chapter 4, leads to the expectation of reduplication of the polarity of the existing fragment, about the plane of ablation. Figure 47 shows the expected pattern of reduplication from a nasoventral peripheral fragment and a nasal peripheral fragment plotted on a polar coordinate system. In constructing these patterns, it is assumed that regeneration occurs at the cut edge, circumferential values (0 - 360) being heritable in radially elongating clones, and radial values elaborating stepwise with cell division. It will be apparent that two features distinguish the double nasal projection from the expected one: firstly, the

FIGURE 47. Expected patterns of reduplication in the maps
from nasal peripheral (a) and nasoventral peripheral (b)
fragments. The partial pattern values contained in the
fragments have been shown on a radial coordinate system.
Thus the nasal peripheral fragment (a – top diagram)
contains circumferential values 15°– 165° and the
nasoventral peripheral fragment (b – top diagram)
circumferential values 75° – 195°. The radial coordinate
values are not shown for the sake of simplicity. It is
assumed that growth occurs along the cut edge elaborating
the radial values stepwise with cell division and with
circumferential values heritable in clonal lines. This
will result in the formation of a duplicated regenerate
(a & b – middle diagrams) showing mirror-image symmetry
about the plane of the cut edge. The bottom diagram shows
the orthogonal plots for these maps. It will be seen that
the expected duplication for nasoventral peripheral maps
(b – bottom diagram) shows an axis of symmetry at 45° to
the vertical midline and the axes of the duplicated map at
90° to those of the original fragment. The AP axes are shown
as solid arrows and the DV axes as dotted arrows. The regen-
erated portion of the retina is shown shaded.
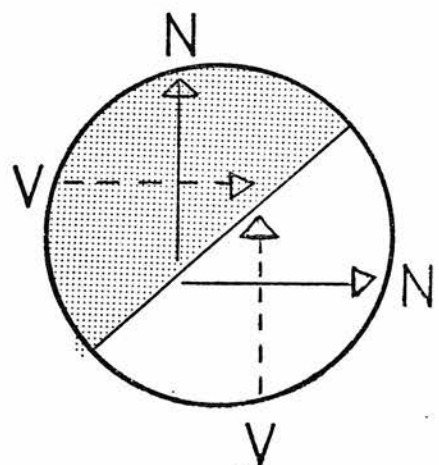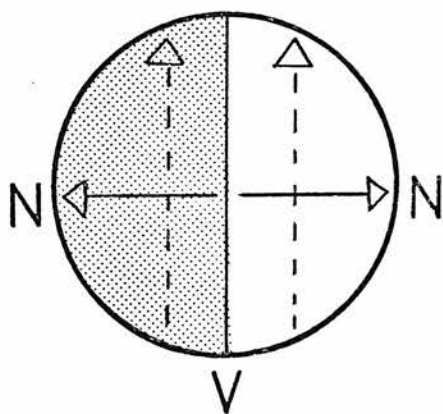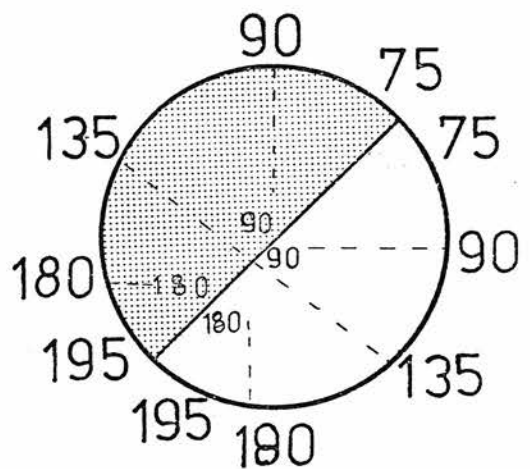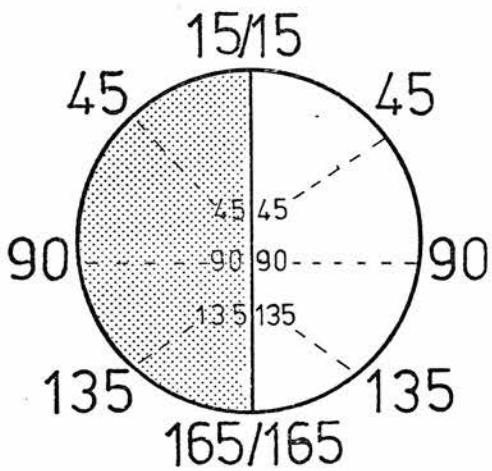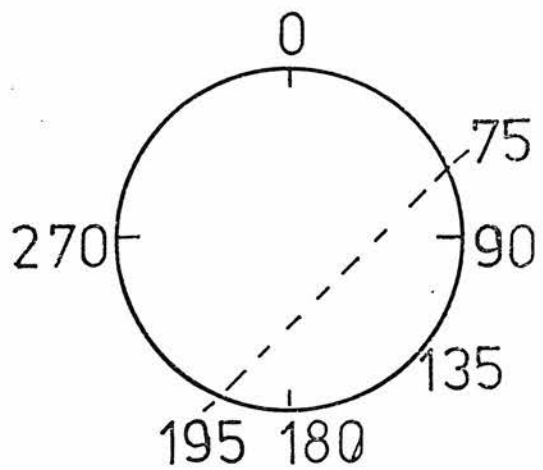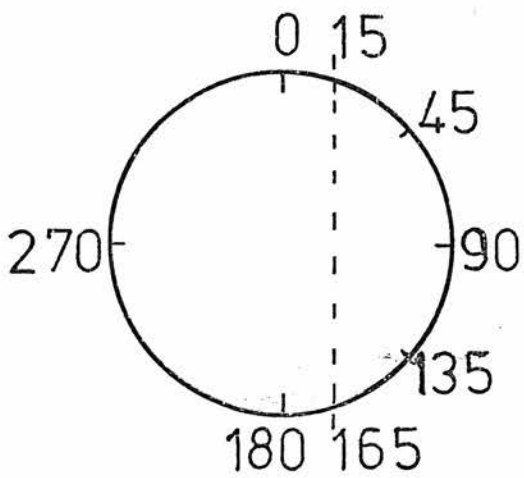
47(a)   47(b)

plane of symmetry is at an angle of about 45° to the vertical

meridian of the field: secondly, the mirror reduplicate is approximately

90° rotated with respect to the map on the original fragment.

Clearly the double nasal reduplicants (see Figures 35 and 36) do

not correspond to this expectation. How then is one to interpret

the finding of double nasal maps from this series? The simplest

interpretation would seem to be that this is due to inadvertent loss

of material from the temporoventral quadrant (only a fraction of

which is included in the nasoventral peripheral fragment) thus

resulting in a fragment akin to a nasal peripheral fragment. The

finding of double nasal maps as the only class of abberant reduplicants

in the present series would be consistent with the suggestion of

a temporodorsal location of the patern "centre". Thus in a

nasoventral peripheral fragment loss of temporal tissue might be

expected in a significant number of cases. That this occasional

loss occurs of the small amount of material from the fractional

quadrants included in the nasoventral peripheral fragments (temporo-

ventral and nasodorsal) is strongly suggested by the map shown in

Figure 36 . Here a limited triplication of field positions has

occurred such that the temporodorsal pole of the field was mirrored

at the nasodorsal and temporoventral poles. Such a map might be

expected where the majority of the retina is being reconstituted

from the nasoventral quadrant of the retina (temporodorsal quadrant

of the field). If mirroring takes place along both cut edges a

triplicated representation of the nasoventral retina, mirrored about

the horizontal and vertical meridians of the field, should result.

This is indeed the type of map found in Figure 36 . The reduplication

about the horizontal meridian is however, only partial, the majority

of the map being a standard double nasal map. This is analogous to

the partial reduplications reported for orthogonal half eyes
(Feldman & Gaze 1975a; Berman & Hunt 1975) where a small sector
of reduplication is superimposed or sandwiched into an otherwise
normal map. It may be that such small aberrant sectors reflect a
"nick" in the original fragment extending up to the midline. Figure
48 shows the conditions of operation producing such maps. The
cases of abberant reduplication illustrated by Berman & Hunt (1975)
show clearly the continuity of the abberant region with the centre
of the map, as would be expected if it were the sector of cells
produced by cell division from a "nick" in the fragment at operation.
It should be noted that such an interpretation assumes that the
pattern reconstituted from fragments results from cell autonomy
of the maintenance and transmission (within clonal compartments)
of their determination states. That is to say the small group of
cells around the edge of the "nick" giving rise to the abberant
sector are presumed to be uninfluenced by the surrounding (majority)
of cells restoring the normal pattern. Such cell autonomy has been
demonstrated in imaginal disc fragments of Drosophila (Tobler 1966;
Garcia-Bellido 1966). Thus it would appear most likely that the
difference of results from orthogonal and oblique peripheral
fragments is a genuine one; that oblique peripheral fragments yield
normal maps; and that cases of reduplication from the latter group
can be attributed to errors in constructing the fragments (inadvert-
ently producing fragments restricted to the nasal portions of the
retina) rather than to reduplication properties of these fragments
(when correctly constructed).

FIGURE 48. Proposed conditions of operation leading to
abberent sectors of duplication in eye fragments. (a)
sector of duplication from a nasal central fragment. The
fragment is shown to contain a "nick" which crosses the
midline. Cell division proceeding autonomously along the
cut edge will restore the remaining temporal portions of
the fragment everywhere except at the nick. Here nasal
cells are present resulting in the formation of a small
sector of duplication. The AP axes of the maps are
represented by solid arrows and the DV axes by dotted
arrows. The edges of the duplicated sector are bounded
by solid lines. The regenerated portion is shaded.(b)
triplication arising from a nasoventral peripheral fragment.
The fragment is shown to contain no temporal tissue and only
a small segment of dorsal tissue. Duplication occurs
along the vertical midline (as for a nasal peripheral
fragment). At the nasal pole where the boundary of the
fragment crosses the horizontal midline duplication also
occurs in a manner similar to that described in (a) above.
Again the extent of the regenerated tissue is shown by
shading and the extent of the abberent sector of duplication
is bounded by shaded lines. The axes of the map are
represented as in (a) above.
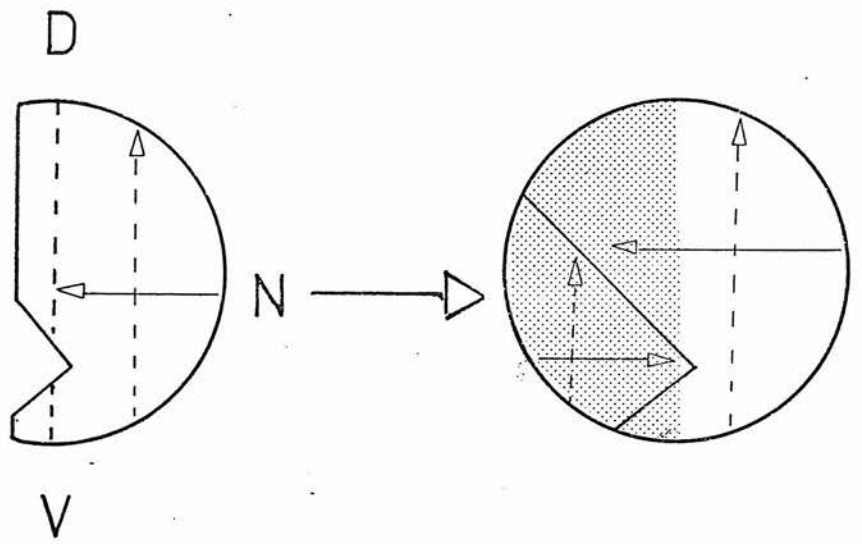
(a)
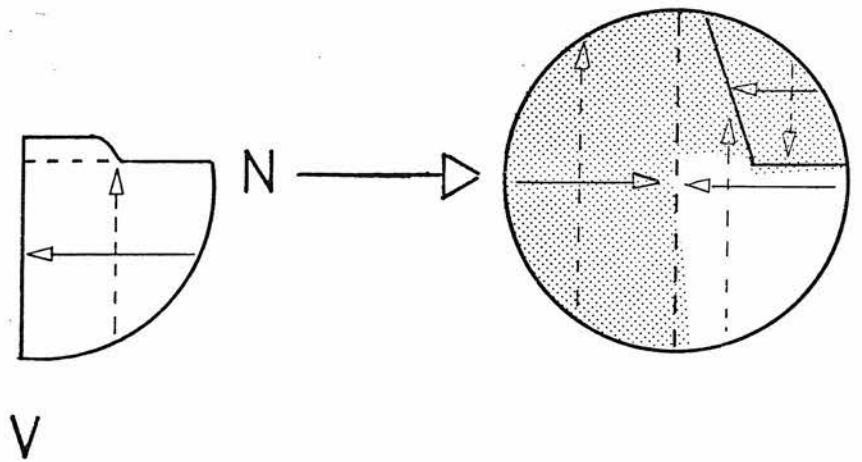
(b)

A.3.   Cellular Activity and Pattern Restoration

To what extent are the present results homologous with
those of other reduplicating systems and to what extent can
homologous interpretations be offered? A caution is pointed out
by Hunt & Berman (1975). In the analogous systems fragments of
one type reconstruct the entire pattern, while the complementary
fragment mirror-reduplicates only those parts of the pattern
contained in the fragment (Butler 1955; Gehring 1966; Bryant 1971;
Schubiger 1971) as shown in Chapter 4, Figure 2. Since pattern
cannot be directly assayed in the retinotectal map, it is not clear
if normal maps are complete while reduplicated maps are partial
pattern mirror-images. Hunt & Berman (1975) have attempted to
answer this difficulty by use of the three eyed competitive
innervation assay (see Chapter 2). They find that both normal and
reduplicating fragments yield a map covering the entire tectum
when mapped in concert with a normal eye. They conclude that the
pattern in the reduplicants is also normal and complete. However,
the difficulties of the three eye assays have already been discussed
in Chapter 2 and the reasoning used by Hunt & Berman cannot be
taken as conclusive. In any case, the possibility of pattern
regulation occuring in both halves of the eye after reduplication
would not substantially inveigh against the suggested homology.

There exists some controversy as to the cellular processes
underlying pattern restoration in analogous fragmentation experiments.
The literature on Drosophila imaginal disc pattern formation
illustrates this. It is clear that proliferation is necessary for
both normal and mirror-reduplicated pattern restoration (Schubiger

1971; Ursprung 1959; Nothiger & Schubiger 1966). Controversy arises as to the locus of cell proliferation in the fragment and the relationship between duplication and total pattern restoration. Nothiger & Schubiger (1966) distinguish reduplication from regeneration (total pattern restoration) on the grounds that duplication simply involves the conservative replication, on a cell by cell basis, of the existing states of determination in the duplicated fragment whereas regeneration involves the mobilization of developmental information not contained in the regenerating fragment. Postlethwait et al. (1971) have challenged this view of conservative replication of states of determination. Bryant (1971) proposed a model of developmental gradients which render duplication and regeneration identical phenomena, a view essentially akin to the "rule of distal transformation" (Wolpert 1971) outlined in Chapter 4. Recent studies have tended to argue against the view of cell by cell replication throughout reduplicating fragments. Ulrich (1971) found in a study of somatic mosaics in halved female genital discs that the number of mosaic spots was smaller in the "new " than in the "old" half but that the size of each spot was larger. This, he argues, is indicative of a "proliferation front" hypothesis, whereby the duplicate is generated mainly by cell division at the cut edge. Schubiger & Alpert (1975) made elegant use of the homeotic mutant, aristapedia which transforms the third segment of the arista into the corresponding leg segment. They found, using a temperature sensitive allele, that if eye-antennal discs were isolated after the temperature sensitive period and the antennal disc was allowed to undergo reduplication then the reduplicating aristas went through a second temperature sensitive period, indicating that states of determination were not being conservatively replicated. They also

found that proliferation was limited in the distal segment (third segment of the arista) of the original fragment and suggested that the distal segments of the duplicate must be produced by more proximally lying cells which grew better. Both the results of Ulrich and of Schubiger & Alpert are clearly consistent with the rule of distal transformation and with the model proposed by Summerbell et al. (1973) for distal elaboration of pattern with cell division (see Chapter 4). Thus it appears likely that pattern formation in the insect imaginal disc, (Gehring 1966; Ulrich 1971; Bryant 1971; Schubiger 1971; Schubiger & Alpert 1975), the regenerating amphibian limb (Butler 1955; Smith et al. 1974) and the developing chick limb bud (Summerbell et al 1973; Summerbell 1974) all represent deployment of homologous mechanisms. Is, then, pattern formation in the amphibian retina underlain by mechanisms of the same kind?

It is not possible to assess the role of cell division in the retina directly, due to the necessity for allowing several weeks to elapse between operation and assay of the result by electro-physiological mapping. That is to say, the map cannot be assayed without allowing cell division. However, as argued in Chapter 4, if the retina were dependent for a component of its pattern on distal transformation through cell division, one ought to be able to construct a map with only radial order by blocking the acquisition of the other pattern component. While attempts to do this by early explantation were not successful (see Chapter 6), such a map has been found serendipitously.

This case is found among those published by Hunt & Jacobson (1974b) resulting from midline transection of the eye rudiment at

stage 32. The map is shown in Figure 49 . The map is reduplicated

and appears to be an abortive attempt to form a double nasal map.

The temporal field sends an appropriately ordered map to the

whole tectum, while the map from the nasal field is scrambled.

The map then allows examination of the radial hypothesis, since

the corresponding positions in ordered and disordered maps can

be readily compared. The map, in effect has a built in standard.

According to the radial hypothesis the disordered map from the

nasal field should retain a degree of ordering relating to distance

from the centre (i.e. the number of mitotic divisions the cells

at that position have undergone) and no ordering related to angular

or circumferential position. To test this prediction, an arbitrary

centre (C) was inscribed in the map as was an arbitrary boundary

(CD). Corresponding map positions in the ordered (O) and scrambled

(D) maps could now be compared with respect to radial position

(distance from C, measured in centimetres) and with respect to

angular position (distance from CD, measured by the angle DCP,

where P is the location of the field position under consideration).

Figure 50a shows this comparison for radial-position and Figure 50b

for angular position. It will be seen that a straight line passing

through the origin and making an angle of about 45° with both axes

can easily be drawn through the points. That is to say, corresponding

points in both maps show a tendency to be located at equal

distances from the centre of the retina. No such best fit straight

line can however, be drawn through the points of Figure 50b. Thus,

there would seem to be no correlation in the angular positions

occupied by corresponding points in the two maps. It is clear,

therefore, that the apparently scrambled map from the nasal field

is not in fact without order and that this ordering reflects a

FIGURE 49. Visuotectal map obtained by vertical midline transection of a stage 32 eye (from Hunt & Jacobson 1974b) analysed in Figures 50 (a) and 50 (b) for radial ordering. An arbitrary centre (C) and boundary (D) have been inscribed. Points in the two maps projecting to the same tectal locus have been compared with respect to distances from C (radial coordinates) and from CD (angular coordinates) as shown in Figure 50.

LEFT OPTIC TECTUM

Caudal

27

26 25 24

23 22 21 20 19

Medial 18 17 16 15 14 Lateral

13 12 11 10 9

$\updownarrow$ 100 μm

8 7 6 5 4

3 2 1

Rostral

|— 150 μm —|

Superior

D

8 5

23

20 15

27 14 19

13

7

2

24

2

26

12

5

1

21

6 7 8

18

17

11 6

1

22

22

16

10 5

4

11

3

23

21

4

10

13 12

Temporal

25 24

20

C

Nasal

26 27

15

9

4

10

9

19

14

9

25

Inferior
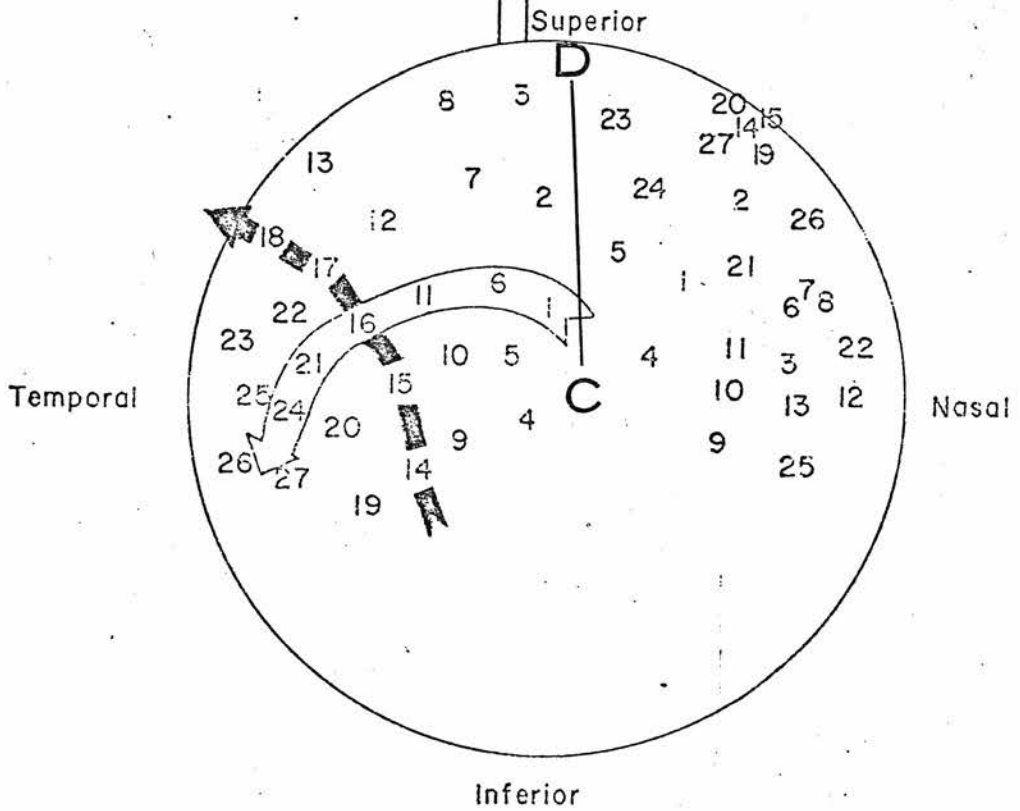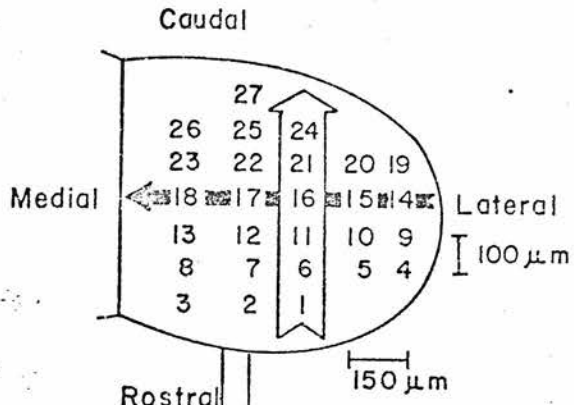
RIGHT EYE'S VISUAL FIELD

FIGURE 50a. Analysis of Figure 49 for radial ordering.
The distances of corresponding points in the ordered
(O) and disordered (D) maps are plotted against each
other. The numbers refer to the field position numbers
in Figure 49. The line represents the theoretical
function (straight line at 45°) passing through the
origin) assuming radial order i.e. that corresponding
points are equidistant from the centre. It will be
seen that the theoretical line fits the points fairly
well.
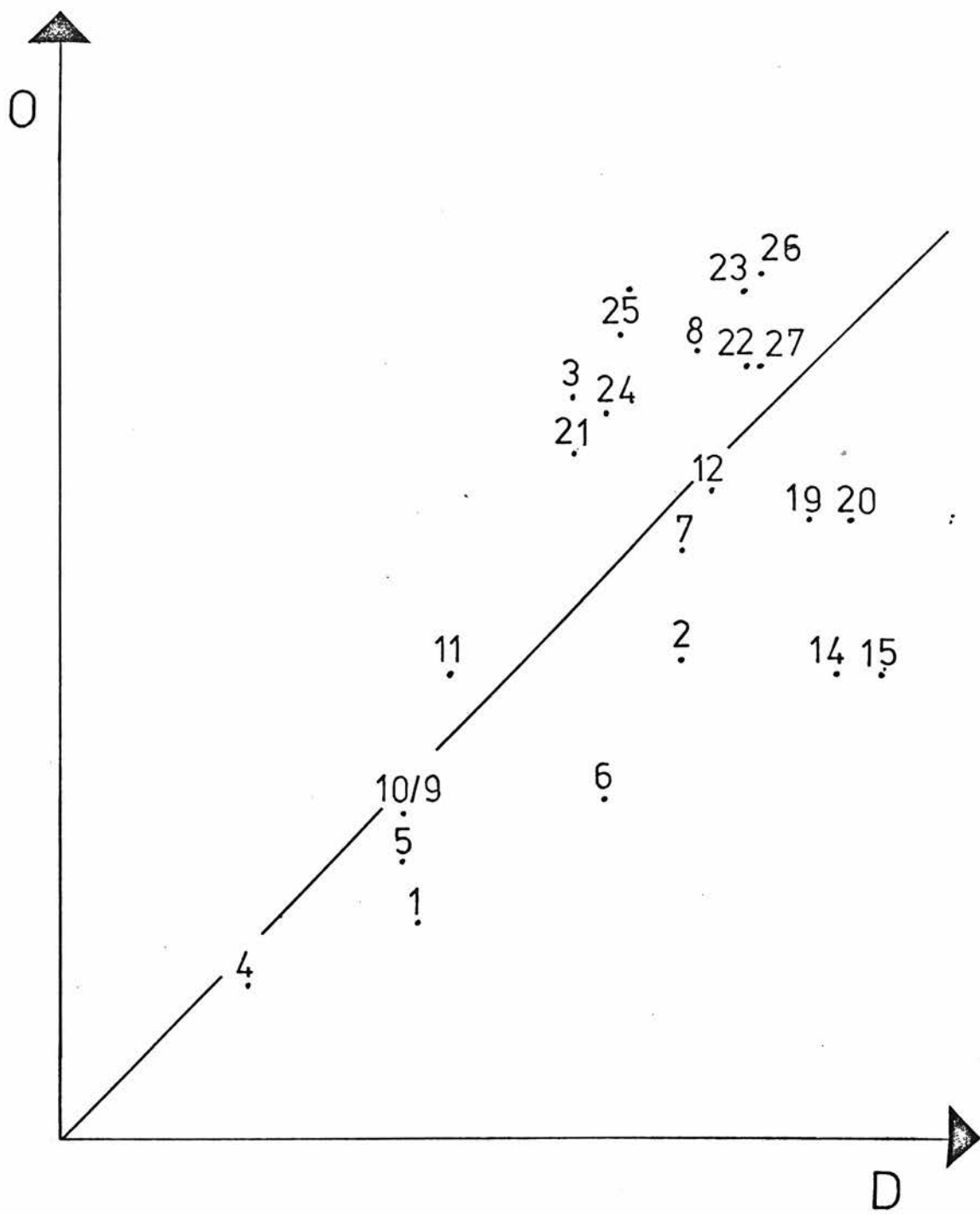
FIGURE 50b.   Analysis of Figure 49 for angular order.
The angular distances of corresponding points in the
ordered (O) and disordered (D) maps from (CD) are
plotted against each other.   There is no evidence
of order.
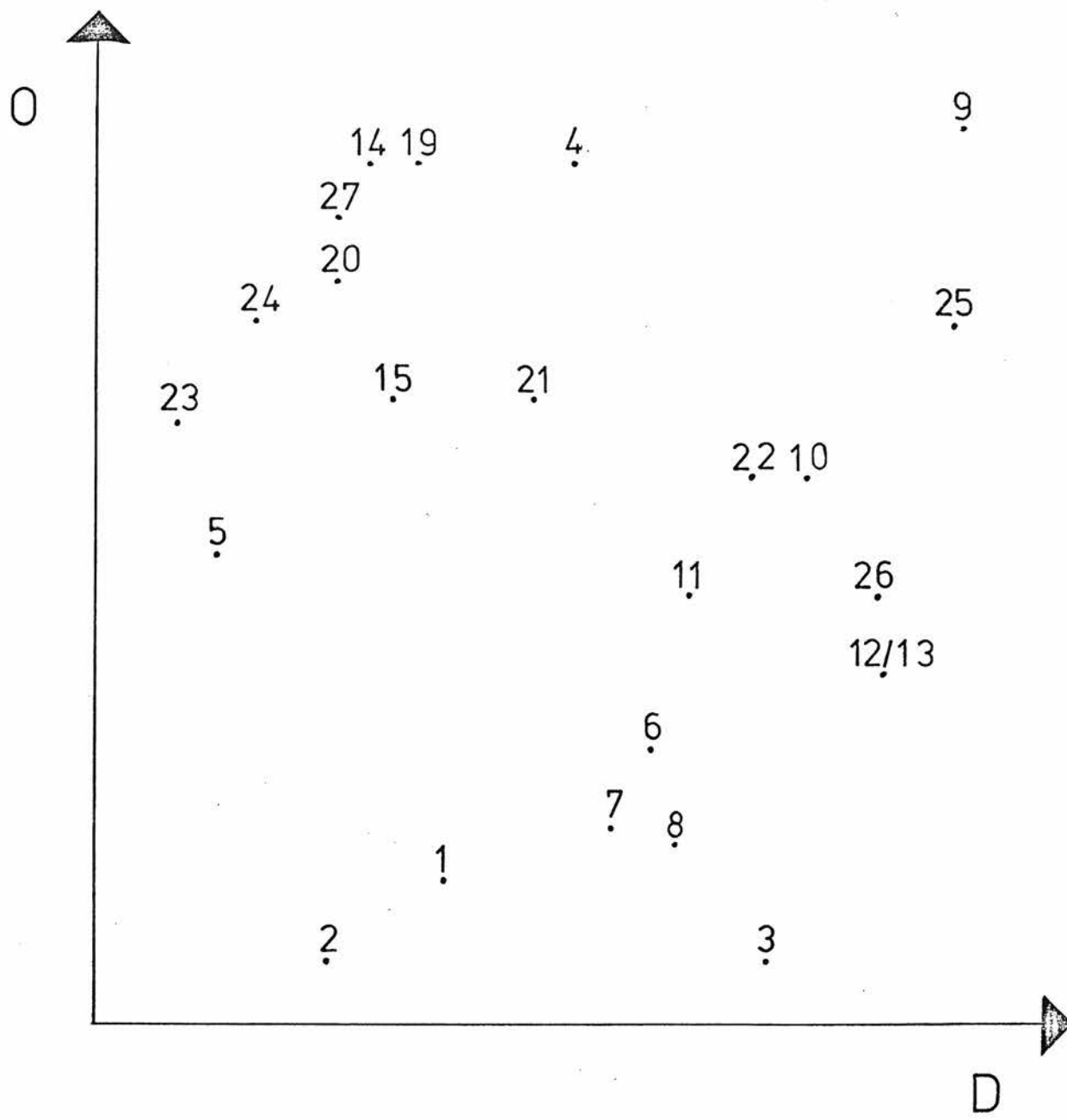
mapping in terms of distance from the centre, as predicted by the radial hypothesis. It is not, of course necessary that this radial ordering be achieved by cells differentiating as a function of the number of divisions they or their progenitors have undergone. Some other component of cell behaviour might conceivably underlie this ordering. Nevertheless, mitotic "counting" suggests itself as an attractive possibility. It should also be noted that the finding of a radial component in the map need not imply that the remaining pattern information is distributed angularly. While a single case cannot be conclusive, it suggests a role for cell division in the elaboration of retinal pattern akin to that proposed for the chick limb (Summerbell et al 1973).

If then centroperipheral cell division in the retina (Straznicky & Gaze 1971) imposes a radial organisation on the map, how is the remaining order maintained? Mathematically, as argued in Chapter 4, the simplest way of doing this would be to establish an angular or circumferential ordering, thus specifying positional information to the retina on a polar coordinate system. However, the predictions of such a model, while consistent with the orthogonal fragment results is contraindicated by those of the oblique fragments (see above). This discrepancy of results can be interpreted in rather a simple way, by assuming a stepwise determination of the retinal pattern.

The results may be explained if the retina at stage 32 is thought of as divided into 4 compartments, corresponding to the 4 quadrants: nasodorsal, nasoventral, temporodorsal and temporoventral. Each compartment is restricted in its developmental potential such

that it can only give rise to cells forming the appropriate quadrant of the pattern. Within each quadrant cells would be identical in potency. Figure 51 shows the predictions from such a model for a nasal peripheral and a nasoventral peripheral fragment. The eye fragment is shown, in both cases, as being reconstituted by growth at the cut edge, the cells arising there transmitting their quadrantic determination to their progeny. The nasal fragment, comprising material from only two quadrants, forms a mirror duplicate while the nasoventral fragment, comprising material from three quadrants reforms a normal map, with the exception that the temporodorsal quadrant of the retina is replaced by a mirrored nasoventral quadrant. Such a mirroring would be clearly detected in the mapping, however, and maps of this type were never found. It may be, however, that in the temporodorsal peripheral fragments (Figure 33   ) the absence of responses from caudo-medial tectum and the temporodorsal field deficit represent just such a mirroring of the temporodorsal quadrant in nasoventral retina. This quadrant projects beyong the lateral edge and therefore would show up as a field deficit. An additional assumption is therefore necessary to explain the results. Some form of interaction between the quadrants, dependent on the number present, might accomplish this. Possible modes of interaction will be discussed in the following chapter.

One difficulty with this view is worth mentioning here. It would appear to contradict the earlier suggestion of a cell autonomous replication of determined state, invoked to explain cases of partial reduplication. Be that as it may, the model may be able to harmonize the apparent discrepancy of results between orthogonal

FIGURE 51. Expected duplications from nasal peripheral
and nasoventral peripheral fragments assuming quadrantic
determination of developmental fate. In the top diagram
the intact retina with a primary pattern of four
quadrants is shown (a) the duplication of the remaining
two quadrants of a nasal peripheral fragment. It is
assumed that growth occurs along the cut edge and that
progeny of cells from a given quadrant are capable of
forming only tissue of that quadrant (b) restoration of
the remaining three quadrants of a nasoventral peripheral
fragment and duplication of the nasoventral quadrant in
place of the missing temporodorsal quadrant. Assumptions
as are in (a) above.
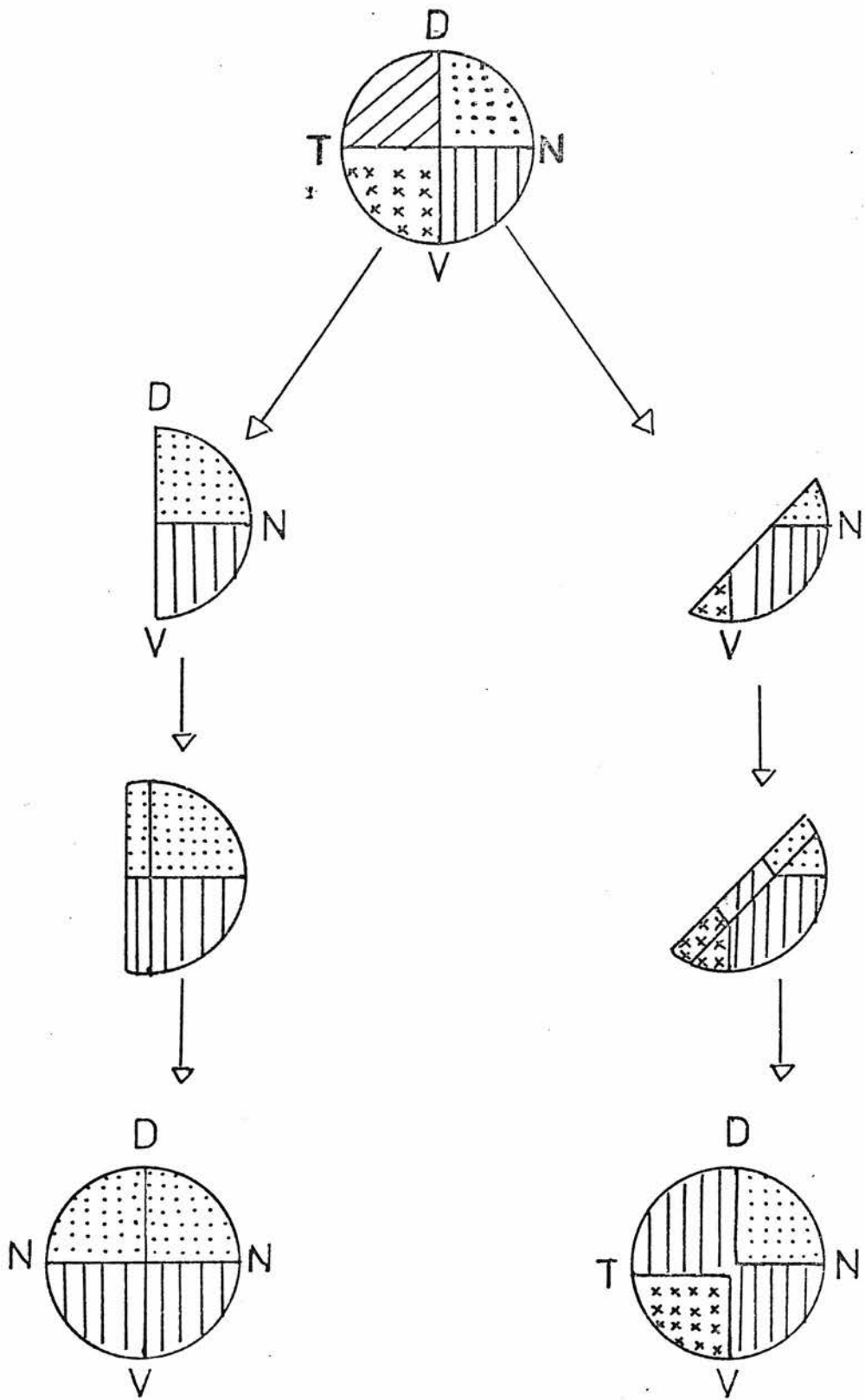
51(a)   51(b)

and oblique fragments. Furthermore, such a stepwise compartment-
ation of a pattern field is characteristic of <u>Drosophila</u> imaginal
discs (Becker 1957; Bryant & Schneiderman 1969; Bryant 1970;
Garcia-Bellido et al. 1973) and developmental potency within such
compartments appears to be restricted (Ulrich 1971).

B.   DISCUSSION OF THE MIDLINE TRANSECTION RESULTS

The results presented here indicate that transection of
larval eye rudiments may result in the reversal of polarity in one
of the two halves of the transected rudiment. Thus, as argued by
Hunt & Jacobson (1974b), polarity, at the stages studied, would
appear to be a systemic rather than a cell autonomous property
of the retina. The occurrence of polarity reversal of "reprogramming"
(Hunt & Jacobson 1974b) is rather strictly dependent on the
conditions of operation. In none of the 39 cases comprising series
1 and 11 was a "reprogrammed" map obtained. In series 111 where
such maps did occur this configuration represented a minority case
(2 out of 17 animals). As in the results reported by Hunt &
Jacobson (1974b) only "double-nasal" reprogrammed maps were
obtained after vertical midline transection.

B.1.   The Acquisition of Axial Commitment

The present results extend those of Hunt & Jacobson (1974b)
by examining the effect of transection on pre-specification stage
rudiments. Four eye rudiments at stage 29/30 were transected
along the vertical midline and 3 along the horizontal midline. In
none of these 7 cases did anything other than a normal map result.

Thus the introduction of tissue discontinuity at the stage of determination of the anteroposterior axis did not prevent the creation of the normally ordered map configuration in this, or the subsequently determined dorsoventral axis. Similarly normal maps were produced by 3 rudiments transected along the vertical midline and 6 along the horizontal midline at stage 31. Thus tissue discontinuity during determination of the dorsoventral axis did not prevent normal dorsoventral patterning of cell differentiation. It may be that integrity of the tissue is not necessary for the expression of pre-existing presumptive polarity (Hunt & Jacobson 1973a), or alternatively, that the discontinuity would have to be maintained for a greater period of time to modify this expression. The present results cannot decide between these alternatives.

B.2.   Maintenance of the Determined State

The finding of normal maps as a majority case following midline transection post-stage 32 suggests, but does not neccessitate the conclusion that in the majority of instances polarity vectors are relatively stable in eye fragments. It may be that after transection the polarity vectors do undergo change in one or both of the fragments but restabilize in normal configuration in the majority of cases. The former alternative is strongly suggested by failure to find, in a single case, reversed axial commitments in the progenitor population of cells comprising isolated eye fragments (Chapter 7, Feldman & Gaze 1975a; Berman & Hunt 1975). That is to say, temporal fragments never give rise to double nasal maps or nasal fragments to double temporal maps. Is then the reprogramming phenomenon a genuine one? Given that eye fragments

are known to be capable of mirror reduplication, is it not possible that the results are due to fragment loss after transection and mirror-reduplication by the remaining fragment? Two arguments militate against this possibility. Firstly, the transected rudiments were kept under close observation during the post-operative period for precisely this reason. The small proportion of transections which failed to heal normally or in which a fragment was lost were discarded during the first 24-36 hours of operation. Secondly, Hunt (1975) has conclusively demonstrated the reality of the reprogramming phenomenon by "fragment salvage" experiments (Chapter 3). Finally it is worth noting that Hunt & Jacobson (1974b) obtained mirror-reduplicated projections in 40% of cases. Since their isolated fragments (Berman & Hunt 1975) yielded reduplicated maps in, on average, 40% of cases one would have to conclude that something approaching 100% of Hunt & Jacobson's transections were subject to fragment loss if one wished to use this as an explanation of the map configuration.

Since, as mentioned above, isolated fragments do not alter their polarity the "reprogramming" phenomenon must involve a genuine interaction between the two fragments involved. This is also suggested by the finding of stable intermediate configurations in fragment salvage experiments, in which one axis has been reprogrammed but not the other (Hunt 1975).

B.3. The Mechanism of Reprogramming

Hunt & Jacobson (1974b) suggest that the reprogramming phenomenon is due, not so much to transection of the eye, as its

144.

refusion. This suggestion is made on the basis of the fusion
of whole eyes with resulting reduplicated maps. However since
the eyes were fused with mirror symmetrical relation (temporal
to temporal or dorsal to dorsal) no inference of reprogramming
is necessary here. However, as argued above, the fragment
salvage experiments and the eye fragment experiments would seem
to suggest that reprogramming requires a genuine interaction
between the two fragments.

The observation that ionic conditions affect the occurrence
of reprogramming, is entirely consistent with this line of
argument. Ionic conditions might canalize the process in two
ways: either by affecting the healing rate, or by affecting
electrotonic cell coupling. In the first case manipulation of
ionic conditions would alter the time at which endogenous
processes within each fragment were able to interact, exogenously,
with each other; in the second case anisotonic conditions may
simply uncouple cell communication (Loewenstein et al 1967)
resulting in the self-differentiation of the fragments. Thus
the first mechanism would reflect a temporal balance between
endogenous and exogenous factors while the second a total abolition
of exogenous factors. The finding that the healing rate is
slower in the Series III conditions (when reprogramming occurred
in the present study) is consistent with the first alternative.

The most intriguing feature of the reprogramming results
is the finding of mirror-reduplication (an axial inversion)
rather than tandem-reduplication (reduplication with the same

polarity). Hicklin et al (1973) found the formation of anterior parts at the boundary of grafted head ends to decapitated <u>Hydra</u> when the graft integrity was interrupted by a cut. Swett (1926) also found tandemly reduplicated limbs after transection of <u>Amblystoma</u> limb buds provided healing of the fragments was prevented by interposing neutral tissue. Mirror reduplication can be brought about in <u>Tubularia</u> when the dominant head end (hydranth) fails to inhibit hydranth formation throughout the length of the stem. This can occur in ageing individuals (Rose 1967) or in animals artificially lengthened by grafting (J. Cooke: personal communication). However this situation is not an adequate model for the present results, inasmuch as they would seem to represent the result of, rather than the failure of interaction.

There are also suggestions in the work of Harrison (1921) on limb bud fragment recombinants of a similar phenomenon. AP harmonious DV disharmonious recombinants were made by combining the anterior half of one bud with the posterior of the other (the analogue of the work in Hunt & Jacobson 1973b). Reduplications resulted when the posterior half was the one left <u>in situ</u> and not when the anterior half was left <u>in situ</u>. Thus the present results would not appear to be without precedent but they do not appreciably enhance our understanding of the mechanisms involved. It may be significant for the asymmetry of results in Harrison's recombinants that the "boundary" region of the AP polarity in the limb bud is located posteriorly (Saunders 1972).

Recent results indicate that this region behaves formally
in graft combinations as if it were the source of a gradient
(Tickle et al. 1975) In view of the extensive analogy
between limb bud and neural retina results touched on already,
these results might suggest an avenue for proceeding to a
mechanistic understanding.

It would at present be an overinterpretation of the
data to carry further the discussion of mechanisms. The
results allow us to infer that reprogramming requires an
interaction between the fragments and therefore that polarity
at the stages studied is a systemic property of the retina.
It seems likely that reprogramming represents a balance
between endogenous and exogenous polarity variables in the
fragments. The importance of these results is in the constraints
they place on theorizing about the nature of the interactions
occurring across the retina at stage 32. In Chapter 9, a
model for retinal pattern formation is developed which
successfully accounts for much of the data currently available
on retinal pattern. However the present findings on midline
transection are not yet explicable.

CHAPTER 9 :   A MODEL FOR RETINAL PATTERN FORMATION

A.   THE NATURE OF THE PROBLEM

A number of disjointed interpretations and hypotheses have
been presented in the preceding chapters in relation to the
various studies on retinal pattern formation. It is now apposite
to attempt to link these into some sort of general and testable
explanatory schema. Some final points of terminology and
interpretation must, however, first be sketched in.

A.1.   The Determination Process

Although no experiments have been presented in these
studies concerning the events of axial determination, a few general
points are worth noting.

The term "determination", which has been used throughout the
discussion, can have only an operational definition. It refers
to the observation that, after stage 32, the axes are not modifiable
in the intact eye rudiment by the experimental procedures so far
used (rotation, ectopic grafting, explantation to culture and delay
in achieving tectal innervation). It should not be taken to
imply (although such may indeed be the case) any necessary change
in cell behaviour at stage 32 , corresponding to a triggering
event or the activation of novel gene readout. For example the
events of stage 28–32 may simply represent the continuing evolution
of some cell state variable(s) beyond a threshold of irreversibility
(see for example the model proposed by Meinhardt & Geirer 1974).

Finally the determination events should not be confused with

the specification of positional information, as they have been in the interpretation offered by Hunt & Jacobson (1972a,b ; 1973a). In Chapter 1 it was argued that positional information and polarity may be distinct elements of pattern formation. There is no evidence available concerning the specification of positional information in the retinal ganglion cells or its timing. Such information would be difficult to obtain in the Xenopus system since (Chapter 2) distinct pattern elements cannot be assayed here. There are indications of such data in the chick system (Kahn 1973; Crossland et al 1974) where eye rudiment lesions on day 2 of incubation result in "holes" in the subsequent map. The interpretation here, however, is confounded by the possibility of retrograde degeneration in the tectum following retrograde degeneration in the isthmo-optic nucleus which projects to the retina.

A.2.   The Determined State and Reprogramming

It is worth pointing up the nature of the problems raised by the reprogramming data. Does "reprogramming" of the temporal fragment in midline transections or nasal right/temporal left (AP harmonious, DV disharmonious) involve an "instructional" effect on the part of the nasal fragment? If so the reprogramming of the AP axis of the temporal fragment involves not an entrainment to that of the nasal fragment but to an axis at 180° to it. Secondly, in the case of nasal right/temporal left combinations, the DV axis is also reversed in the temporal fragment. Yet presumably the extent of the dorsoventral axis including any "gradient sources" or "boundary" regions, is the same in both

fragments. Why then, if the DV and AP axes are independent (see however discussion of this assumption by Goodwin 1972; Straznicky et al 1974), should the nasal fragment possess the ability to reverse the DV axis of the temporal fragment? One crucial control has been missed out from the experimental series of Hunt & Jacobson (1973b). The reprogramming of the DV axis might be due either to its being brought into alignment with that of the nasal fragment, or with that of the orbit. The missing control to test this possibility would involve the construction of combinations in which the nasal fragment was AP-normal, DV-inverted and the temporal fragment AP-normal, DV-normal. This could be accomplished by transplanting a left nasal fragment, 180° rotated in place of a right nasal fragment in a right eye. If the DV reprogramming were due to the surround, the DV axis of the temporal fragment would be expected to remain normal. If on the other hand it were due to the nasal fragment, the DV axis of the temporal fragment should invert. Circumstantial evidence against such a surround effect is available. It can reasonably be asserted that the nasal fragment is necessary at least to trigger the process since reversal of neither AP nor DV axis is found in isolated temporal fragments, rotated through varying angles (Hunt & Berman 1975). It would also seem that the continued activity of the nasal fragment is necessary. Hunt (1975) in the fragment salvage experiments described in Chapter 3, found that the reprogramming phenomenon had a distinct time course and that removal of the nasal fragment at intermediate times resulted in AP-inverted, DV-inverted maps as stable intermediates. Thus the completion of the first stage of the process (AP reversal) is not sufficient to initiate the second stage (DV reversal).

However, such indirect evidence will not substitute for the
critical experiments.

A final point deserving comment is the nature of the
terminology adopted by Hunt & Jacobson to describe the phenomenon
and the view of the process implied in the terminology. The
postulation of a "reprogramming" event, implies, quite correctly,
that an axial commitment is changed. It also implies, however,
at least in the usage of Hunt & Jacobson, a "reprogramming"
fragment and a "reprogrammed" fragment. The nasal fragment is
presumed to remain unchanged in its polarity characteristics,
while the temporal fragment is presumed to change. However, the
axes in the nasal fragment also appear to undergo change in the
maps presented (Hunt & Jacobson 1973, 1974). The "characteristic
bowing" of the axes, described by Hunt (1974) is evident in
both fragments. It might then be more reasonable to conceive the
process as involving a change in both fragments, which might,
but does not necessarily, involve an interaction between both
fragments.

It has been suggested that mirror-reduplicated pattern
formation in the neural retina fragment paradigm presents an
appearance similar to that found in other fragment systems:
namely a stepwise creation of pattern discontinuities or
compartments, analagous to that observed in insect imaginal discs
and a growth related mode of distal transformation suggested by
the necessity for proliferation in imaginal discs and analysed
formally in the chick limb. In addition, however, a simplistic
formulation in terms of cell autonomous developmental restriction

in clonal compartments is contraindicated by the finding of
systemic interaction at stage 32 (in reprogramming experiments)
and at stage 35/36 (in normal pattern formation in oblique fragments).
The results summarized in Chapter 3 (section A) are clearly
consistent with the first two elements of the situation above:
viz, the elaboration of pattern with growth (microphthalmic eyes:
Hunt 1975) and the absence of the entire pattern, transmitted in
clonal lines, at stage 32 (FUdR ciliary margin extirpation:
Hunt 1975).

The eye fragment results, suggesting compartmentation,
bear on the nature of the "interpretation" mechanisms (see Chapter
1) of the pattern forming system; while the reprogramming experiments
bear on the positional specification mechanisms. In general in
contemporary developmental biology, we possess greater information
about the former than the latter. Insofar as is possible, the
discussion below will discuss these two aspects of the problem
separately.

B.   THE INTERPRETATION OF POSITIONAL INFORMATION IN THE
      RETINA

At a minimum the retina, conceived of as a two dimensional
cell sheet, must possess two components of positional information
(Sperry 1945; Szekely 1954; Jacobson 1968a). It has been argued
in Chapter 8 that one component of information is radial (specifying
position from the centre) which may be built directly into the
division programme at the ciliary margin. This component represents
the analogue of the proximo-distal "clock" postulated in the
vertebrate limb (Summerbell et al 1973). Here the specification

and the interpretation of positional information bear a direct relation to each other. A mechanism for such a "clock" is discussed by. Holliday & Pugh (1975) involving sequential modification of base sequences in the nuclear DNA, although clearly other mechanisms are possible.

The eye fragment data was interpreted in the last chapter as indicating a quadrantic compartmentation of the retina. On this interpretation by stage 32, two pairs of compartments (nasal/temporal and dorsal/ventral) are established. At this stage, the retina comprises approximately 300 cells with a radius of 10 cell diameters (Jacobson 1968b; Straznicky & Gaze 1971), and thus about 75 cells per quadrant. Each quadrant in its outer annulus would contain 15 cells. Thus the worst resolution (achieved in the widest annulus) is 15 cells. That is to say within each compartment regions are uniquely specified (given also radial specification) to the level of 15 cells. Further growth will, of course decrease this resolution, necessitiating the creation of further compartments. If one assumes that the size of each compartment is 75 cells and that further compartmentations also proceed by binary restriction, every time a compartment achieves a "critical mass" of 150 cells it will be bifurcated. Such a bifurcation would then create a poorest resolution (at the outer annulus) of 15 cells, and a best resolution of 7.5 cells (at the inner annulus). Since the electrophysiological mapping technique resolves to approximately 5 retinal cell diameters (see Chapter 5) this will result in an _effective_ experimental resolution to 3 cells (at the poorest) and 1.5 cells (at the best). Thus we can now represent the earliest stages of pattern formation as in Figure 52 . At stage 28 only a radial organisation will be

FIGURE 52. Proposed elaboration of the retinal pattern
during development (a) stage 28: radial order only (b)
stage 31: creation of four polyclonal compartments
imposing a quadrantic primary pattern on the radial
order (c) further compartmentation at later stages
creating more refined subdivision of the retinal
pattern field. Throughout development the radial order
is assumed to extend with growth.
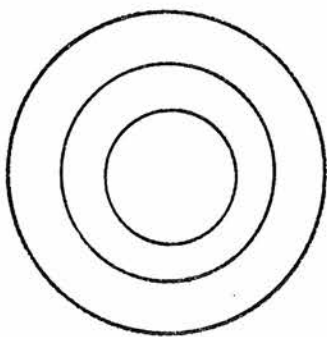
a
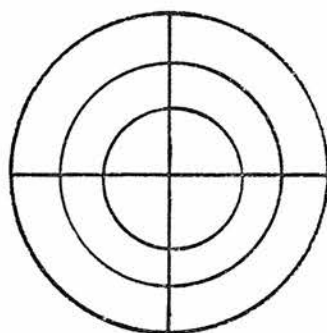
b
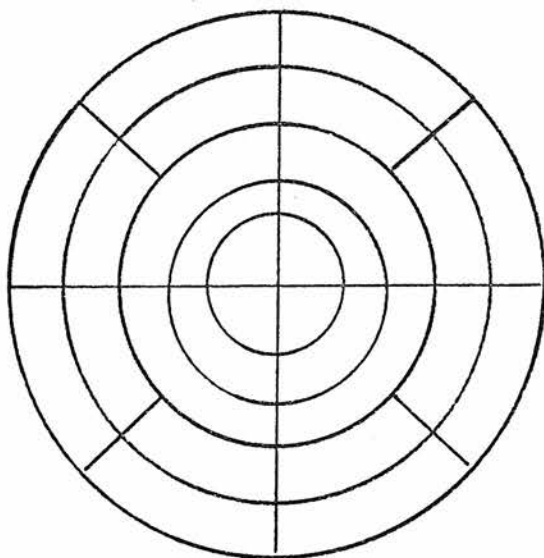
c

fixed (which in itself may not be clear, since mitosis has not yet become restricted to the margin). Between stages 29/30 and 31 quadrantic compartmentation is fixed, forming the ancestral cells of the future nasodorsal, nasoventral temporodorsal and temporoventral quadrants of the adult retina. Prospective potency of cells during normal development may then differ between quadrants. At these stages all cells within a quadrant will exhibit common prospective fate, further circumscribed only by radial value. At a later stage (late 30's to early 40's assuming an 8 hour cell cycle time) a further compartmentation divides the progeny of each compartment. Further such compartmentations, at critical mass, could occur until the retina reached its adult complement of 50,000 cells (in Xenopus). To divide the adult complement into compartments of 75 cells approximately 666 compartments would be required, which in turn would require between 254 and 510 such bifurcations. If the events producing the bifurcations are not independent (i.e. if they occur throughout the retina simultaneously in all compartments as for the stage 29-31 events) only between 6 and 7 such events after stage 31 are required

$$\left(4 \sum_{o}^{n} 2^n = 666 \right).$$ n is therefore between 6 and 7 and indicates the number of events after stage 32 necessary to create 662 compartments. As mentioned in the last chapter, such stepwise creation of differentiation commitments has been found, in somatic recombination experiments in the Drosophila leg disc (Bryant & Schneiderman 1969) eye disc (Becker 1957) and wing disc (Bryant 1970; Garcia-Bellido et al 1973). It is also, of course, characteristic of the determination of organ primordia in the amphibian primary embryonic field, within which more refined differentiation occurs only subsequently. It is possible that the mechanism outlined has not only analogical or topological relations to that in these other situations but

that it is in fact strictly homologous in several of them. If
this were true it might explain why reduplication rather than
pattern reformation is the standard finding for <u>Drosophila</u> discs
(Gehring & Nothiger 1973). Thus if there were a central junction
of clonal compartments and a radial component excessive tissue
destruction of halved discs might lead to the absence of central
fragments. French (1974) has indeed argued for a radial model
identical to that outlined in Chapter 4 for <u>Drosophila</u> leg discs.

The proposal of a stepwise determination of retinal pattern
is thus consistent with findings from other systems and allows
of a parsimonious use of developmental information, while allowing
the simplest number (2) of developmental decisions to be made by
cells at each stage. Thus it contains in its interpretational
simplicity a corresponding precision of pattern formation. Yet
why should such a stepwise mode of determination be so commonly
encountered, as opposed to a simultaneous and continuous
specification of all pattern elements such as that found in <u>Hydra</u>?
The situation in hydroids may not of course be strictly comparable
in that they do not appear to undergo cellular determination, as
commonly understood. Rather, they involve the continuous
specification of positional information and a corresponding lability
in the differentiated state of cells. Such a mechanism may be
an evolutionarily primitive state of mechanisms found in higher
organisms, where the role of cell division and determination may
become more prominent (Summerbell et al 1973). Maynard-Smith
(1960) has argued that a stepwise and multiplicative mode of
determination is a formal necessity. However, this argument
rests on the assumption of a Turing-type mechanism (see Chapter 1)

and the limits of its precision. The only indicator we have of
the limits of precision that might be possible in other mechanisms
is the empirical generalization that developmental fields never
exceed 100 cell diameters in linear extent (Wolpert 1969) and the
size limitations of this magnitude imposed by diffusion mechanisms
(Crick 1970). A further possible reason for the stepwise mode
is encountered in considering the mode of growth of the retina.
Unlike the linear growth of the limb bud, this system grows
radially. Therefore if the entire pattern was specified at an
early stage, it would be subject to unequal distortion at the
centre and at the periphery. Thus the peripheral elements would
be formed with less precision. While it is possible to imagine
a set of interpretational gradient thresholds ordered in such
a way as to offset this growth induced distortion, it would require
a highly precise packing of thresholds at the periphery which
would result in a corresponding lack of reliability or "fail-safe"
potential. A similar argument may be a possibility in relation
to imaginal disc pattern formation.

This aspect of the model thus makes a number of predictions
which are, in principle at least, testable. Unfortunately, the
elegant techniques involving somatic recombination developed in
insect work to demonstrate clonal compartments, are not available in
amphibia. Nor will the extirpation work used in the present studies
allow of such demonstration, for the potential to reconstitute a
compartment may well reside at the level of the primary quadrants.
Ciliary margin FUdR extirpation at later stages might achieve this
aim, as would translocation of marginal material. If such translocat-
ion were technically feasible in mid-40's stages the model clearly

predicts a subquadrantic type of mirroring.

C.   THE SPECIFICATION OF POSITIONAL INFORMATION

One component of "positional information", as discussed
above, is built into the radial growth programme itself. It
is this component which confers polarity on the isolated fragments.
It now remains to discuss the possible nature of the pattern
"landscape" which creates the proposed compartmentation of the
retina. In looking for relevent data for the construction of an
appropriate model one is again hampered by the inability to
assay pattern in the system. There is, indeed, no entirely
convincing demonstration that positional information has been
specified in the retina at the stages discussed. Therefore, it
must be recognized in what follows that this assertion is of the
nature of an assumption and that the attempt made here constitutes
the erection of a working hypothesis rather than a final explanation.
Pre-eminently, the data suggesting reprogramming will be drawn
on, and in particular the intriguing observation (Hunt & Jacobson
1973b; Hunt 1975) that, in nasal right/temporal left combinations,
the dorsoventral axis as well as the anteroposterior axis is
reprogrammed. Reference will also be made to the subtle but
reproducible alterations of pattern axes found after various
fragment recombinations: the cartwheeling found in double ventral
maps (Straznicky et al 1974) and the hooking of axes found in
reprogramming experiments (Hunt 1975).

The simplest model for retinal positional information
might involve a single "gradient" in each axis, with a single

interpretational threshold in each gradient. Thus in the antero-
posterior axis, all cells above the threshold would form nasal
and all those below the threshold temporal tissue. However, it
is difficult to see how such a model alone will account for the
reprogramming results. Five more elaborated models will be
discussed and compared here.

The first four models will be discussed only briefly.
Though they accord with the current picture of retinal pattern
formation in postulating a Cartesian coordinate grid for
positional specification, they are unable to account adequately,
despite their very different underlying mechanisms, for dorsoventral
reprogramming. The fifth model evades this difficulty by
utilising a non-Cartesian coordinate grid.

C.1.   Orthogonal Models

Model 1:   Direct Quadrantic Organisation

Two "gradients" in each axis, their sources located
centrally and propagating peripheral, would pattern the axis into
two compartments, assuming that a boundary preventing overlap
is set up between the spheres of influence of the two sources.
The reprogramming results might be accommodated on such a model
if it is assumed that following post-operative trauma, one
source in each axis is regulatively restored more rapidly than
the other and succeeds in capturing the tissue normally patterned
by the complementary source. The model also directly explains the
eye fragment results if it is assumed that a source can only be

reformed in tissue which it had previously patterned. That is to say, nasal tissue cannot regulate for the removal of a temporal source and vice versa. Thus orthogonal peripheral fragments, possessing only three of the four tissue types, mirror duplicate; while oblique peripheral fragments, possessing all four tissue types retore the normal map. The model predicts (a) an "organizing" role for the centre (b) that reprogramming is dependent on rapid healing time (since contact between the fragments must be restored after the first but before the second source has regulated). The model however has the defect that it requires special pleading for the finding of dorso-ventral reprogramming. In addition it would predict cases of both anteroposterior and dorsoventral mirroring after destruction of the centre (for example in midline transection) and such maps have not been found in such situations.

Model 2: Inhibitory Gradients

This model assumes a single "gradient" in each axis, one originating at the nasal pole and the other at the ventral pole, the gradients being of an inhibitory nature. They might for instance reflect a graded threshold for inhibition (c.f. Webster 1971) across the axis. All cells above a given threshold would produce an inhibitor and would be nasal or ventral cells, while those below the threshold would destroy the inhibitor and would be temporal or dorsal cells. Considering the nasotemporal axis, the introduction of a discontinuity in the cell sheet (by midline transection or fragment recombination) and the consequent relief of inhibition would begin a regulatory process in the temporal fragment. With short healing times the inhibition (from the nasal

tissue) would be restored before the temporal cells had completed their regulation and a normal map would be restored. With longer healing times the most nasal of the temporal cells would have completed their regulation prior to the restoration of inhibition and would have been able to set up an entire field within the temporal tissue. Here tandem reduplication should result (although the maps might be scrambled due to the mismatch between the radial component and the compartmentation). With intermediate healing time, assuming a relatively slow passage of the inhibitory information across the tissue, the most nasal of the temporal cells might be reincorporated into the pattern of the nasal fragment, while the most temporal of the cells continued regulating and achieved nasal values before the inhibitory signal from the nasal fragment reached them. Here the mirror reduplicated maps of the reprogramming experiments would be produced. The model explains the eye fragment results by assuming that formation of the regenerate is underway, with the compartment values at the cut edge, before regulation has altered these values. It may indeed be possible to explain the characteristic differences in duplication frequencies if nasal fragments regulate less rapidly than do temporal fragments. The model thus predicts that reprogramming is a feature of healing rate, as in model 1, but this time it occurs at intermediate rather than rapid healing times. It predicts no organizing role for the centre. However, again it has the disadvantage of not directly explaining dorsoventral reprogramming and in addition is not consistent with the findings from other gradient systems that, in the short term, regulation of pattern value occurs only at the boundary or cut edge (see for example Hicklin et al 1975).

Model 3:   Polarized Active Transport

In each axis a morphogen "gradient" is established by
active transport against a concentration gradient. Failure of
the necessary pumping mechanism in the reprogrammed fragments
would result in the morphogen at the junction establishing a
gradient of reversed slope by passive diffusion. Thus a mirror
reduplicated map would result. The eye fragment results are again
explained on the basis of cell heredity in the regenerate. The
model predicts that interference with energy metabolism would
tend to produce this result. It may be of relevence that Hydra
treated with the mitochondrial poison oligomycin D shows such a
pattern of partial axial reversal (Wolpert et al 1974) and that
bicaudal phenotypes in insect embryos can be produced by inter-
ference which results in mitochondrial damage (Sander 1975). The
model again suffers from the disadvantage of requiring additional
explanation for dorsoventral reprogramming. In addition, it is
not immediately apparent why the transport mechanism should break
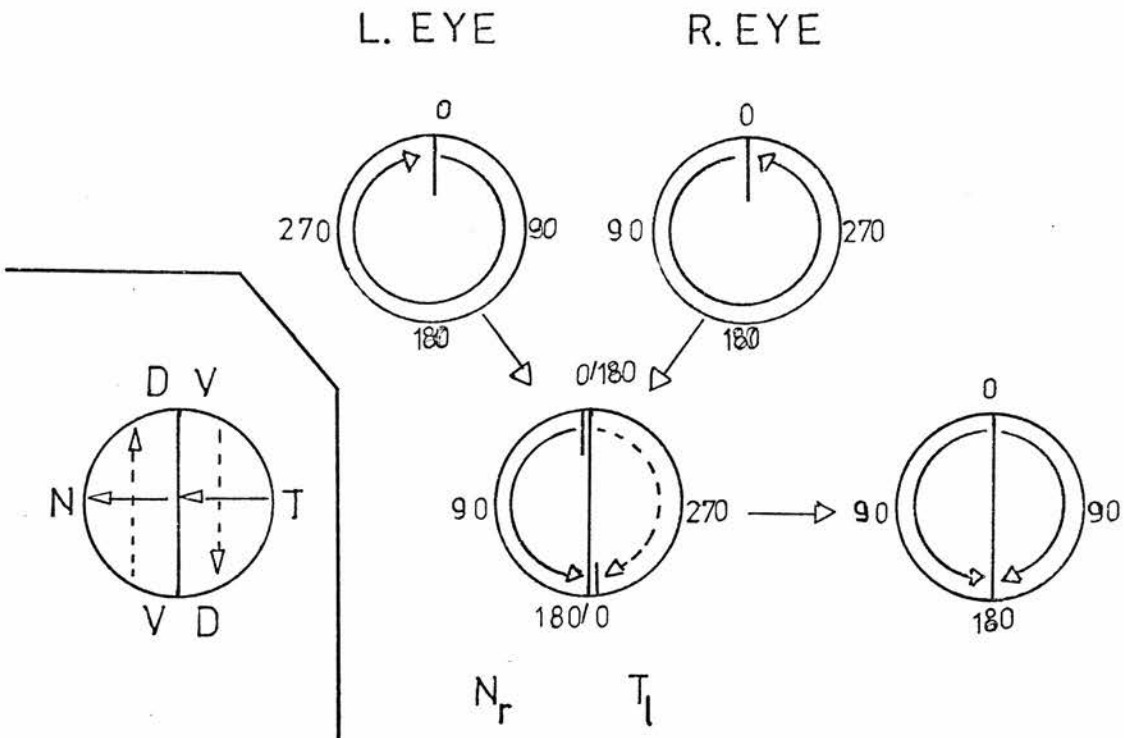down only in the reprogrammed fragment.

Model 4:   Double Gradient

In each axis two gradients of opposite slope are maintained
with sources at either pole (c.f. the sea-urchin double gradient
model: Runnstrom 1933). Failure of the gradient propagation in
the reprogrammed fragment would result in the entire axis being
patterned by a single gradient, a mirror-image polarity being
conferred by the radial component. The eye fragment results would
be explained by a similar lack of one gradient. This model encounters

similar difficulties to the previous one: namely the requirement
of special pleading for dorsoventral reprogramming and the
difficulty of accounting for a reproducible breakdown of only
one gradient.

C.2.   Circumferential Specification

In previous chapters a model based on a radial coordinate
system has been briefly outlined. While the discrepancy of results
of the orthogonal and oblique fragment series militated against
such a model, it may be premature to dismiss it in the light of
a single experiment. Indeed there is much that such a model can
still account for parsimoniously. An objection stressed in all
the previous models was their inability to directly explain the
reversal of the dorsoventral axis which occurs along with antero-
posterior reversal in nasal right/temporal left combinations
(Hunt & Jacobson 1973b; Hunt 1975). It will be apparent that such
a difficulty is obviated in the case of a radial model, with
circumferential propagation of one component of positional
information. Figure 53 illustrates this. The direction of
circumferential propagation in a stage 32 left and right eye is
shown as from dorsal clockwise in the left eye and anticlockwise in
the right eye. Combination of a nasal right with a temporal left
fragment results in a combinant with mirror-imaged propagation
directions in both fragments. This might result in both fragments
being patterned with identical (but mirror reduplicated) circum-
ferential values by the dorsally located boundary in the nasal
fragment, producing a double nasal map. Figure 54 illustrates
the situation for nasal/ventral recombinants. A nasal right/ventral

FIGURE 53. Proposed explanation of reprogramming of
developmental axes in nasal right/ temporal left eye
fragment recombination experiments by means of a
circumferential signal. Intact left and right eyes
are shown at the top of the figure. The circumferential
signal is assumed to be polarized in its propagation
from a dorsal boundary clockwise in the left eye and
anticlockwise in the right eye. Combination of a nasal
right and a temporal left fragment is shown.   The
propagation polarity in each fragment is indicated by
an arrow. This polarity is such that only the boundary
of the nasal fragment is able to propagate its signal.
This signalling occurs in both fragments to create a
mirror-reduplicated map of the "double nasal" type
(bottom right).   The inset at the bottom left shows the
disposition of the presumptive orthogonal axes of the
fragments at the time of combination. The nasotemporal
axis is represented by a solid arrow and the dorsoventral
axis by a dotted arrow.

L. EYE   R. EYE

0   0

270   90   90   270

180   180

0/180

D V

N   T   90   270   90   90

V D   180/ 0   0

180

N_r   T_l

left recombinant would produce exactly the same sort of possibility for reprogramming as did the nasal right/temporal left recombinant. Again reorientation of the axes to produce a double nasal map is unproblematic. However the nasal right/ ventral right recombinant produces the opposite recombination of propagation polarities. Here reprogramming by the dorsal boundary of the nasal fragment would not be possible. However, it has already been noted that the nasal right/ventral recombinant map shown by Hunt & Jacobson (1973b) could in fact be adequately accounted for by both fragments expressing their original polarities, if and only if the ventral fragment was from a right eye (see Chapter 3). Thus, formally, a circumferential polarization possesses the capacity to explain coordinate reversal of both axes, which here would represent conventions of the mapping technique rather than real biological variables in the retinal pattern. Can these formal aspects of a circumferential polarization be assigned a biological reality corresponding to the known features of the system?

We know that the retina undergoes a sequence of polarization events between stage 29/30 and stage 31 (Jacobson 1968a). On the present model these cannot be equated with an anteroposterior and a dorsoventral axis. However, it is necessary that the conceptual replacement of these axes utilize such variables as to produce the apparent determination of these axes from eye rotation experiments. The formal model illustrated in Figures 53 and 54 possessed two variables: a direction of propagation and a source. The two axial polarization events may correspond to the determination of these two variables. If the stage 29/30 event is equated with the

FIGURE 54a. Proposed explanation of reprogramming of
developmental axes in nasal right/ventral left eye
fragment recombination experiments by means of a
circumferential signal. The intact eyes, the
propagation polarities and the formation of the
recombinant are shown as in Figure 53. Again, as in
Figure 53, the polarity of propagation is such that
the dorsal boundary of the nasal fragment can signal
to both fragments creating a mirror-reduplicated
map of the "double nasal" type. The inset on
the bottom left shows the disposition of the
presumptive orthogonal axes of the fragments at
the time of combination.

R. EYE   L. EYE

D N
N   V
V T

0 / 90
90   180 → 90   90
180 / 270   180
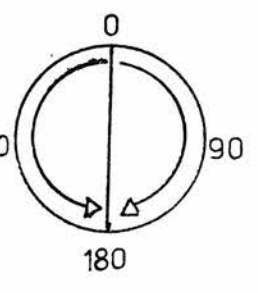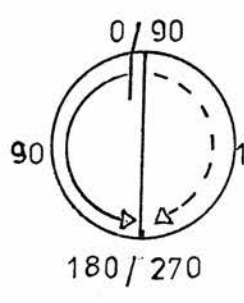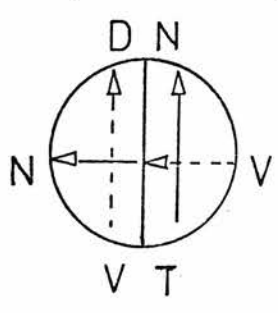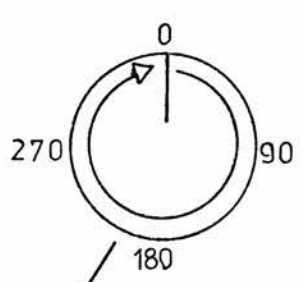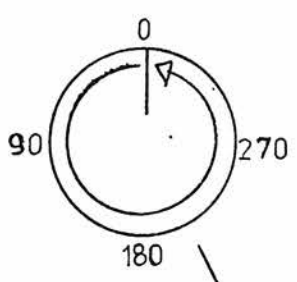
N_r V_l

FIGURE 54b. Proposed explanation of reprogramming
of developmental axes in nasal right/ventral right
eye fragment. Representation of the combination
is as in Figure 53. In this case the propagation
polarity does not allow reprogramming of the ventral
by the nasal fragment. The inset on the bottom
left shows the disposition of the presumptive
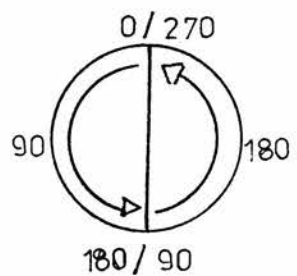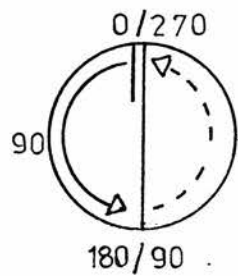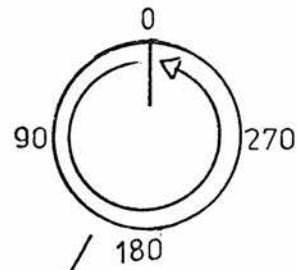orthogonal axes of the fragments at the time of
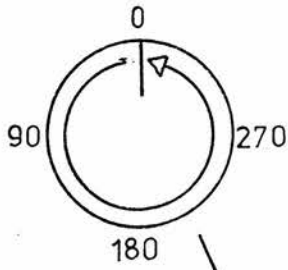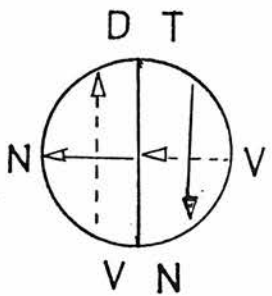combination.

R. EYE　　　　　　　　R. EYE

the establishment of the direction of propagation and the event

of stage 31 with the establishment of the source position the

eye rotation experiments may be re-explained. Rotation prior

to stage 29/30 will of course result in a normal map. Rotation

after this stage but before stage 31 will result in an inverted

direction of propagation with a normally located source (dorsal)

and consequently a map inverted nasotemporally but normal dorso-

ventrally. Rotation after stage 31 will result in a totally

rotated map. This schema is shown in Figure 55 . In the fixation

of propagation polarity a polarization of transmitting junctions

is not sufficient at stage 29/30 (although it would be sufficient

by stage 32) since rotation of the stage 29/30 rudiment will,

then, not suffice to rotate the direction of propagation (anymore

than an inverted clock would sweep out the hours in the opposite

direction). Thus a landmark must be fixed in the eye perimeter

(such as the nasal pole) towards which the junctions transmit.

Fixing the direction of propagation would, then, consitute the

determination of this landmark. By stage 32 the retina in an

intact state is resistent to perturbations of its geometrical

relations with the surround (see Chapter 3.), but reprogramming

is possible between partial retinal fragments. Circumferentially

oriented propagation of positional information, as seen above,

would account for most of these results. It is heartening in

this respect that the ultrastructural study of Dixon & Cronly-

Dillon (1972) shows the maintenance of gap junctions around

the circumference after stage 32. Jacobson's (1973) fluorescein

injection studies indicate that these junctions will allow

passage of small molecules. It is not yet known whether trans-

mission at these junctions is polarized as would be predicted on

FIGURE 55. Results of the early eye rotation experiments
as predicted by the orthogonal hypothesis (Jacobson 1968a)
and by the radial hypothesis. The top line shows the
prediction for 0° and 180° rotation at stage 29/30 on the
basis of the orthogonal hypothesis. This hypothesis
postulates that an AP axis is determined at stage 29/30
and a DV axis at stage 31. 0° rotation leaves the newly
determined AP axis undisturbed. 180° rotation inverts
this axis. The DV axis develops subsequently in the
correct orientation producing an AP inverted/DV normal
map. The second line shows the predictions for the
stage 29/30 experiments on the basis of a circumferential
signal. It is assumed that at stage 29/30 a "landmark" is
determined at the pole of the eye facing anterior and
that at stage 31 a boundary from which the circumferential
signal propagates is determined at the pole of the eye
facing dorsal. 0° rotation will leave the newly determined
nasal "landmark" undisturbed and a normal map will be
formed after determination of the dorsal boundary. 180°
rotation will invert the "landmark" but the boundary
will be determined subsequently in the correct location
resulting in the formation of an AP inverted/DV normal
map. The bottom two lines show the results of 0° and
180° rotations at stage 31, on the basis of the two
hypotheses. Both hypotheses predict completely inverted
maps.

0° ROTn.   180° ROTn.   MAP
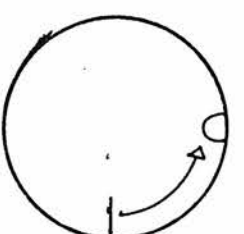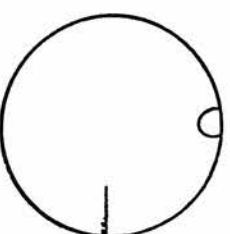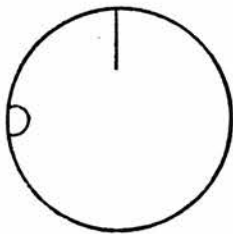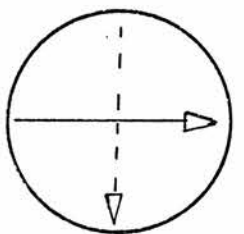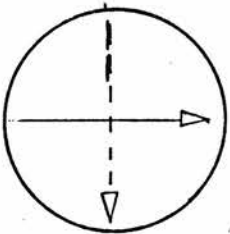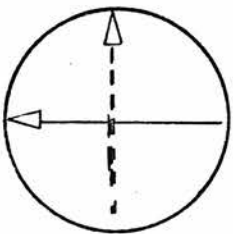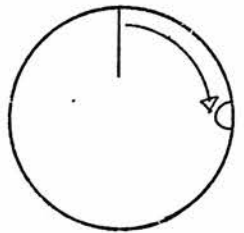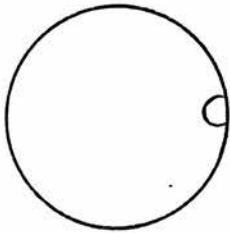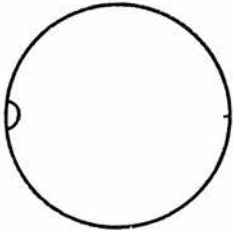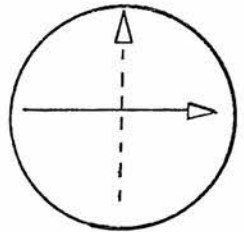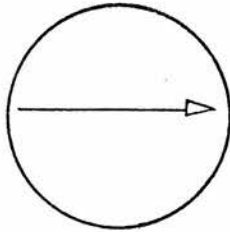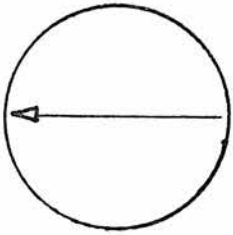
the present model.

The location of the boundary dorsally and the direction
of propagation as towards nasal tissue was not entirely arbitrary.
Figure 56 shows how, with the assumption of such locations, the
characteristic alterations of map patterns in double ventral and
double nasal eyes may be explained on the current model. In
nasal fragments (and indeed in dorsal and temporal fragments)
the circumferential pattern boundary is represented at the
growing zone at the ciliary margin. However, in the case of
ventral fragments, this boundary is internal to the tissue and
is not represented at the ciliary margin. It is assumed in
Figure 56 that respecification of the circumferential signal
takes place only in the presence of the dorsal boundary. Thus
nasal fragments are capable of regulating towards normality and
the restoration of the complete pattern. In the case of ventral
fragments only the central tissue can regulate. The ring of new
cells at the periphery are represented as possessing the subset
of circumferential values present in their progenitor population.
Two map types are shown for such ventral fragments after prolifera-
tion. In type 1 the growth annulus possessing exactly the
inherited values of the original periphery, surrounds a regulated
centre. This may be compared with the "double cartwheel" map
type derived from ventral fragments (Figure 57). In the type
11 situation the growth annulus possesses sufficient lability
for the circumferential gradient to form additional pattern values
$(275^{\circ} - 360^{\circ})$. This type may be compared with the "single
cartwheel" map shown in Figure 58 . It is apparent that if pattern
distortions occur in ventral fragments due to partial regulation

FIGURE 56A. Proposed explanation of the map derived
from "double ventral" compound eyes on the basis of the
radial model. (a) the eye prior to operation showing
the circumferential values of the poles. (b) the
formation of the compound eye by the fusion of two
ventral halves. Note the position of the two dorsal
regions centrally. (c) the partial set of circumfer-
ential values present in the ventral fragments at the
time of operation. Only a single fragment is shown
for the sake of simplicity. (d) the condition of the
circumferential pattern in the fragment after regul-
ation in the centre and growth at the periphery.
The annulus of new cells at the periphery is shaded.
The circumferential values in this annulus are those
present originally in their progenitor cells at the
periphery of the initial fragment. The centre has now
regulatively restored the full set of circumferential
values $(0^{\circ} - 360^{\circ})$ (e) isoinformational contours
joining points in (d) which bear the same circum-
ferential value. (f) the visuotectal map provided by
a compound eye whose isoinformational contours are
shown in (e).
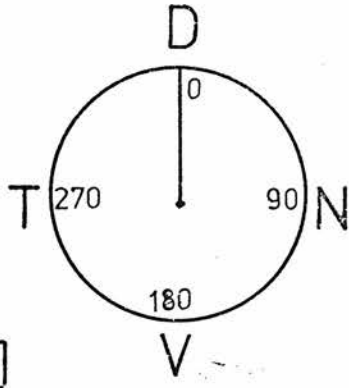
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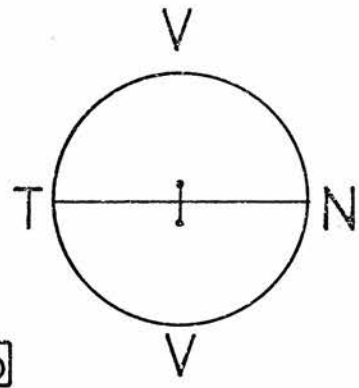
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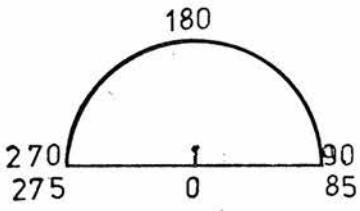
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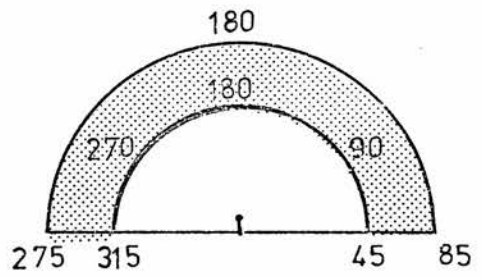
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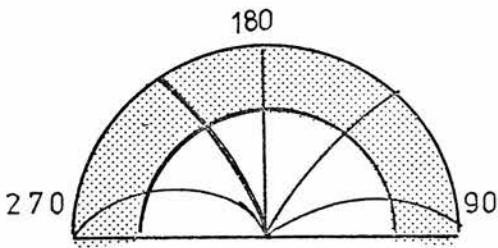
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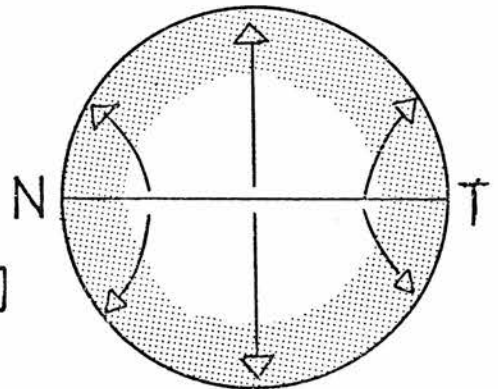
f

FIGURE 56B.   Proposed explanation of the map derived
from "double ventral" compound eyes on the basis of
the radial model. The compound eye is constructed as
shown in Figure 56A (a & b). (a) the partial set of
circumferential values present in a single ventral
fragment (b) the fragment after growth at the peri-
phery and regulation in the centre. The centre has
regulated as described for Figure 56A.   In this
case however the growth annulus at the periphery
possesses sufficient developmental lability to allow
the inherited circumferential values to "run down"
to their lowest level (360°) (c) isoinformational
contours linking points in (b) bearing the same
circumferential value (d) the visuotectal map
produced by a compound eye whose isoinformational
contours are as shown in (c).
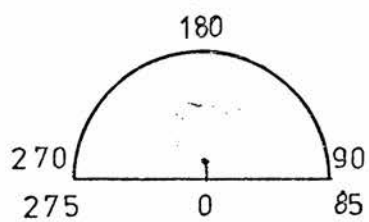
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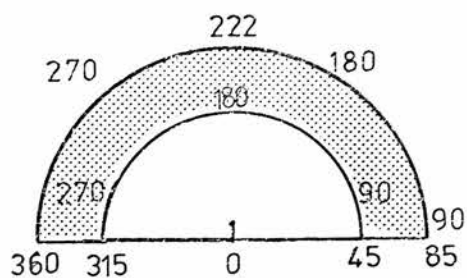
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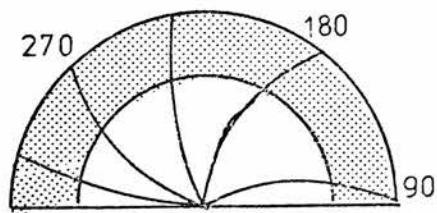
c
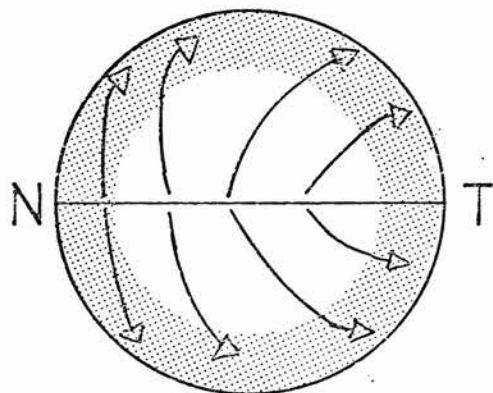
d

FIGURE 57. Visuotectal map obtained from a "double
ventral" compound eye showing "double cartwheeling".
The field rows at the nasal and temporal poles bend
towards these poles instead of running dorsoventrally
(from Straznicky et al 1974).

C

39 38 37

36 35 34 33 32 31

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

R

4   3   2   1

S

15

22
23

14

13

21

12

11

19

9

7

6

30

26

5

29 28 27 33 25

4

1

T

36

35 34 33 32 31 24 17 10

N

39 38 37 25

11 18 5

27

26 19

13

28

21 20 12

14

6   7

22

8

29

9

39 30 23 16
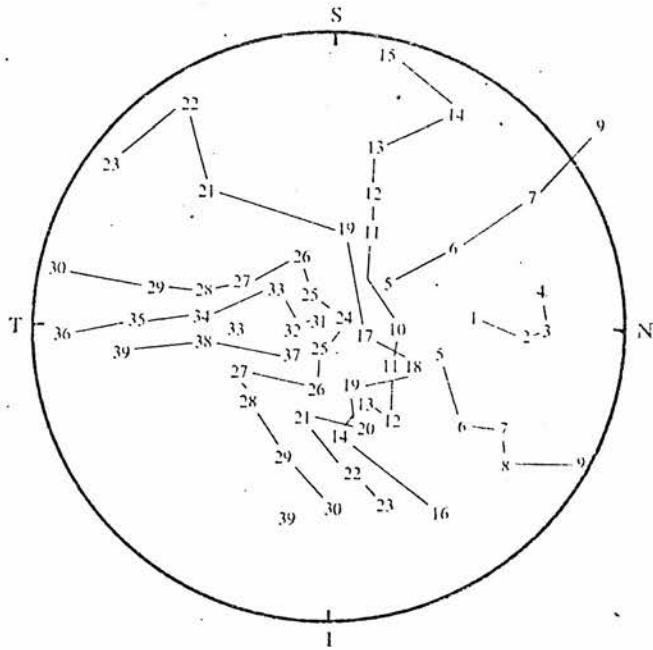
FIGURE 58. Visuotectal map obtained from a "double
ventral" compound eye showing "single cartwheeling".
The field rows bend out of dorsoventral orientation
only towards the temporal pole (from Straznicky et
al 1974).

C

31 30 29 28 27

26 25 24 23 22 21

20 · 19 18 17 16 15

14 13 12 11 10 9

R 8 7 6 5 4

3 2 1

S

· 14

13

20 7 3

6

19 — 18 — 17 16 5 2

15 12 11 1

16 10

T 25 — 24 9 4

26 10

22 27

28 23 21 15 11 4 1

29 16 5 2

17 12

30 — 31 24 18 13 6 3

25 19 14 7

26 20 8

I

N
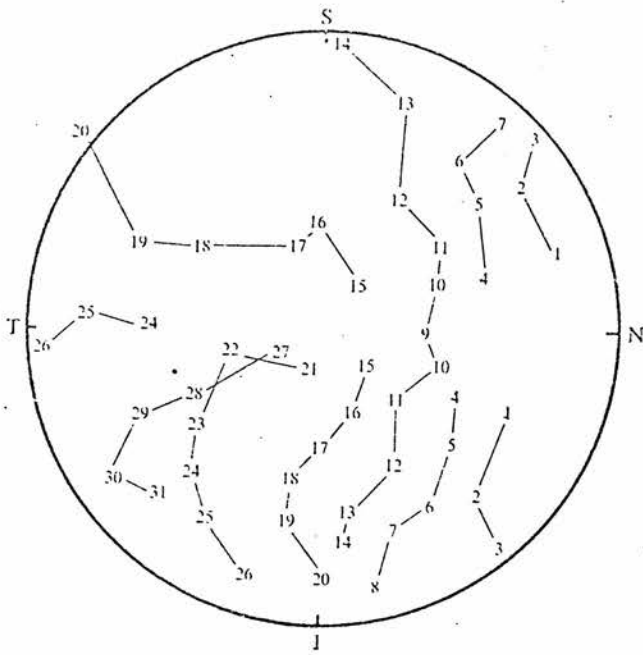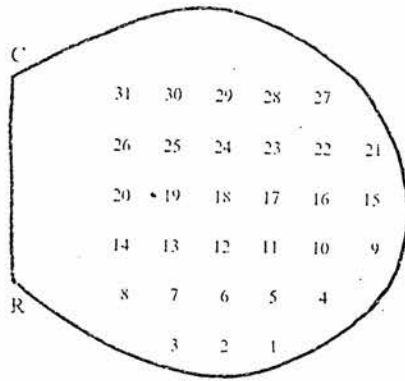
they should also occur in nasal fragments undergoing complete
regulation. The double nasal maps originally published (Gaze et
al. 1963, 1965) show no obvious distortion. However distorted maps
from nasal fragments have been published more recently (Hunt &
Jacobson 1973b, 1974b ; Berman & Hunt 1975; Hunt & Berman 1975).
Such a distorted map is represented in Figure 60 . It will be
evident that the predicted map from a regulated nasal fragment
shown in Figure 59 bears considerable resemblance to this map.
Without additional variables being introduced into the model it
will be apparent that the map configurations shown explain a
further anomaly between the behaviour of double nasal and double
ventral maps: namely that double nasal maps "spread" to occupy
the entire tectum, whilst double ventral maps occupy basically
their normal tectal domains with an overrepresentation of the
centre. On the present model the entire nasal fragment contains
the complete map whilst only the centre in a ventral fragment
contains the complete map. Thus a point-to-point mapping (see
Chapter 2) would explain the differential tectal coverage by the
two classes of eye recombinants. The model raises two further
predictions on these lines. Firstly, the "single cartwheel" map
is regarded here as resulting from residual lability in the
circumferential signalling mechanism. It would therefore be
reasonable to predict that "single cartwheel" maps result from
eyes of a slightly younger stage than do "double cartwheel" maps.
Therefore the proportions of single to double cartwheel configurat-
ions should be manipulable by simple variation in the embryonic
stages used. The second prediction is that double dorsal maps
should behave analogously to nasal and temporal fragments and not
like ventral fragments. That is to say, they should "spread" across

FIGURE 59. Predicted distortions in "double nasal"
compound eye maps on the basis of the radial model.
(a) normal eye (b) "double nasal" compound eye
constructed by fusion of two nasal fragments. Note
that in distinction to "double ventral" eyes (Figure
56A (b)) the dorsal boundaries have representation
at the periphery. (c) the partial set of circumfer-
ential values (355° – 185°) contained in a nasal
fragment (d) regulation from the boundary results
in restoration of the complete set of circumferential
values both at the centre and in the growth annulus
(shaded) (e) isoinformational contours connecting
points in (d) with the same circumferential value
(f) the visuotectal map produced by a compound eye
whose isoinformational contours are as shown in (e).
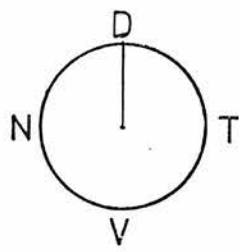
a
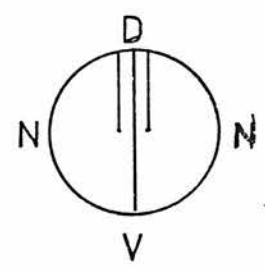
b
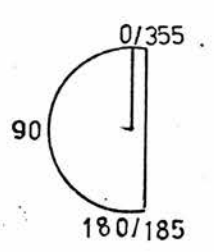
c
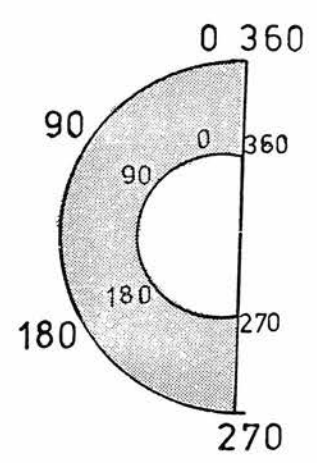
d
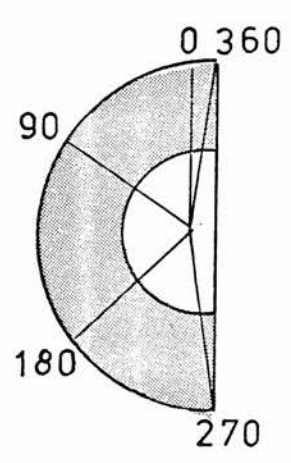
e
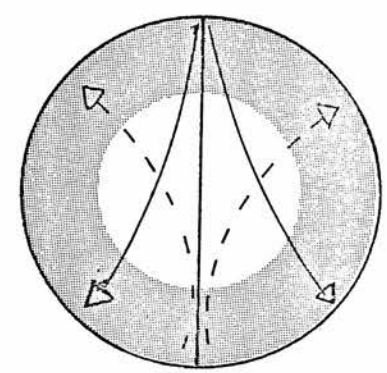
f

FIGURE 60. Visuotectal map obtained from a "double
nasal" compound eye showing "hooking" of the field
rows (from Berman & Hunt 1975).

LEFT OPTIC TECTUM

Caudal

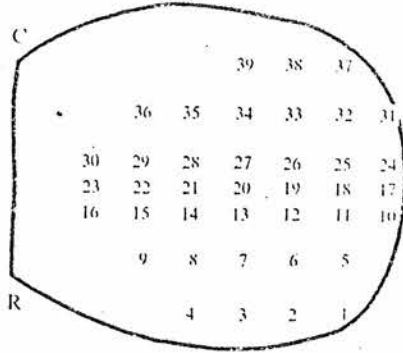| | 30 | 29 | 28 |
27	26	25	24
23	22	21	20	19
Medial	18	17	16	15	14	Lateral
13	12	11	10	9
8	7	6	5	4
3	2	1

⊺ 100μm

Rostral

⊢—⊣
150 μm

S

13 — 8   8   13

18   3 7 12   18

27   18   12 7 3   17   23

17   11 6   6 11   16   22

22   16 11 6 2   10 15   21 26 27

27   26   10   1   4   20   25   30   N

T   21   15   9   14   24   29

25   20   9   19

30   29 24   14   28

28   19

28

I
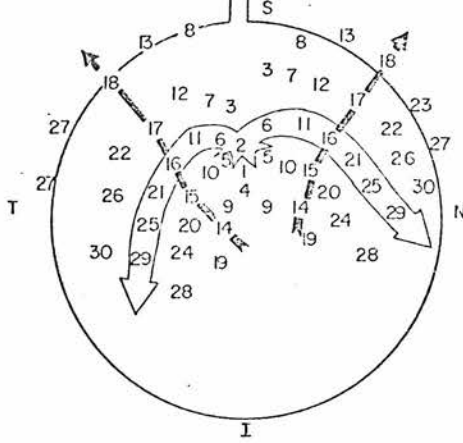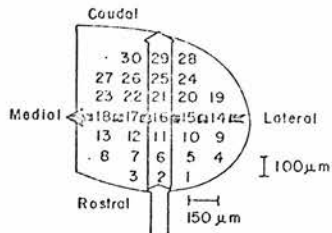
RIGHT EYE'S VISUAL FIELD

the entire extent of tectum and should not show a "cartwheeling" distortion of the map. Hunt & Berman (1975) have presented a double dorsal map prepared by making a dorsal fragment in an eye rotated prior to stage 28. This map does indeed correspond to the predictions. No cartwheeling is evident and gaps exist in the field at the dorsal and ventral extremities, suggesting a spreading of the map around the lateral edge (which is inaccessible to microelectrode recording). There is no overrepresentation of the centre (as is found in double ventral maps).

It remains to determine to what extent the present model is compatible with the eye fragment results. As argued in Chapter 8, a cell heredity model will not explain the results of the oblique peripheral fragments. Some interaction would seem to be required to explain these results. There it was argued that this necessity militated against a circumferential specification of positional information. However a positional signal propagated circumferentially and interpreted with four thresholds (0°, 90°, 180° and 360°) might yield behaviour consistent with results from other systems. French & Bulliere (1975a,b) have shown that grafts around the circumference of leg segments in <u>Blabera craniifer</u> result in the intercalary formation of elements linking the discontinuous values at the graft/host boundary such that the shortest route around the circumference is taken. If an orthogonal peripheral fragment contains only two of the four compartments while an oblique peripheral contains three, oblique peripheral fragments forming intercalary structures by the shortest route around the circumference will reform the missing quadrant while orthogonal fragments will have at least an equal probability of duplicating.

Such a rule does not, of course, provide an explanation.
Nevertheless it may provide an indication that the apparently
inconsistent behaviour of oblique fragments will be assimilable
to a common system rule.

In section B, above, the quadrantic organization of
the retina in early stages was assumed to be the result of two
independent and orthogonal events. Thought of in this way, it
conforms with the Cartesian models of the retinal positional
coordinate grid. In the present section it has been reformulated
in terms of four interpretational "thresholds" for a circumferential
signal. The postulation of quadrantic compartmentation is of
course independent of any particular model for positional
information.

The present model would appear to be inadequate in two
respects. It fails to provide an explanation of reprogramming
after midline transection. The proposed polarization of junctions
shown in Figures 53 and 54 to explain the fragment recombination
results would not allow repolarization of the temporal by the
nasal fragment in the same eye. Furthermore the fragment salvage
experiments (Hunt 1975) indicate an anteroposterior reversed/
dorsoventral inverted map as a stable intermediate (i.e. they
indicate that AP and DV reprogramming may be disconnected). On
the model outlined AP and DV reprogramming are features of the
same event. However, since the model utilizes two variables
(direction and source of propagation) this latter result may be
accommodated. In the nasal right/temporal left recombinant
illustrated in Figure 53 , the dorsal boundary of the temporal

fragment is unable to specify its circumference due to the polarity of the junctions. The fragment is therefore specified from the boundary of the nasal fragment. If the fragments are separated at an intermediate stage the temporal fragment will now possess a nasal pole (the 90° position) and it may be that this allows the boundary of the temporal fragment to reorient the transmission characteristics of the junctions and repattern the fragment (as in the axial determination events illustrated in Figure 55). The inability of the model to explain the midline transection results remains a problem. This fact and the problematic behaviour of peripheral oblique fragments indicate that the behaviour of the pattern formation mechanism in the retina must be more complex than is envisioned here. The model has been elaborated here only as a working hypothesis. Insofar as it makes predictions of a testable nature it will have served a purpose.

D.   CONCLUSIONS

On the basis of the present studies and published data
from other workers a working hypothesis of pattern formation in
the amphibian neural retina has been outlined. The postulates
and predictions of the model are as follows:

1.   Postional information in the retina is specified on a
       radial coordinate system. A radial component, measuring
       distance from the centre, is patterned directly in the
       growth programme itself, in a manner homologous to that
       thought to operate in vertebrate limb morphogenesis
       (Summerbell et al 1973). A circumferential component adds
       the second dimension of information. This is set up
       utilising two processes: firstly, a polarization of
       transmission direction is specified and secondly a boundary
       or source for transmission is determined.

2.   The interpretation of the circumferential signal is, in
       early stages, dependent on four thresholds, patterning the
       retina into four primary quadrants. It is assumed that at
       subsequent stages more refined subcompartmentation occurs
       (c.f. Garcia-Bellido et al. 1973).

3.   The model correctly predicts that of the four orthogonal
       classes of compound eyes, only the double ventral class
       will show partial spreading (by overrepresentation of the
       centre) and "cartwheel" distortions of pattern (Gaze et al
       1963, 1965 ; Straznicky et al 1974; Hunt & Berman 1975).
       Characteristic "hooking" of map axes in other classes of

recombinant eyes is also correctly predicted.

4. As a corollary of the above predictions, the model predicts
    that partial retinae of the double nasal or temporal class
    will "regulate" towards restoration of the complete pattern.
    Such a result is suggested by the competitive innervation
    assay (Hunt & Berman 1975) but this data cannot be considered
    conclusive (see Chapter 2). It should be possible to test
    this prediction by following the regeneration of the map
    from a compound eye after optic nerve section (c.f. Cook
    & Horder 1974).

5. The results of reprogramming experiments are explained
    on the basis of the spatial disposition of the boundary
    and the polarity of transmission. Transmission is equated
    with the gap junctions identified by Dixon & Cronly-Dillon
    (1972). The model predicts that these junctions should
    transmit only in one direction. A further prediction of
    the model is that gap junctions should reappear centrally
    in retinal fragments and there are recent indications that
    this may be the case (Rose & Jacobson 1973; quoted in CIBA
    Symposium 1975, new series 29). The possible role of ionic
    conditions in determining the occurrence of reprogramming
    (see Chapter 7) is also consistent with the proposed role
    for gap junctions. It is important to recognize that the
    postulated role of gap junctions and the associated predictions
    are not "strong" features of a radial model, in that the
    model as a whole cannot be tested by testing these suggestions.
    They represent suggestions as to a particular mechanism for
    radial pattern formation.

6. The model predicts an "organizing" role for the dorsal
boundary which should be testable in early grafting
experiments.

7. There are indications that the model developed here may
not reflect an isolated mechanism confined to the neural
retina pattern (Bryant 1975; French & Bulliere 1975a,b) but
rather a mode of pattern specification adapted to particular
modes of growth. The physical nature of the pattern
components suggested here have not been identified. The
radial component may well depend on events related to cell
division. There is no clear indication as to the physical
nature of the circumferential component, although it is
significant that grafting experiments "round the clock"
(French & Bulliere 1975a,b) have failed to find any region
behaving like a gradient boundary with a polarity "cliff" on
either side. This suggests the possibility of some such
signalling mechanism as phase shifting (Goodwin & Cohen 1969).
On the other hand, the "running down" of the signal, invoked
to explain the "single cartwheel" double ventral maps is
suggestive of substance gradient mechanisms. If evolutionary
homologues of the mechanism proposed here should exist, the
ability to assay subtle axial distortions in the retina
combined with the ability to assay pattern in other systems
should dovetail in the construction of more formal and
precise models.

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