NEURAL DEVELOPMENT AND REGENERATION IN THE VISUAL SYSTEM OF TELEOSTS

by

SANSAR C. SHARMA, B.Sc., M.Sc., (Panjab)

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neural function. Our present knowledge of neurogenesis stems
from experimental modifications of the developing nervous system
i.e. ablation and explantation of peripheral organs or brain parts,
before or after the formation of peripheral connections, and the
study of the effects of such manoeuvres on the developing organs.

INTRODUCTION

After stimulation of both the eyes in larvae of Rana fusca,
marked reduction in size of the optic lobes after metamorphosis,
particularly in the outer optious layer, was shown by Steinman
(1906). However, the reduction in size was confined to the
contralateral side of the optic lobe when Burton (1911 and 1920)
removed one eye in larvae of Rana fusca.

Quantitative analysis of the degree of hypoplasia in the
larvae of Hyla religiosa, after unilateral elimination of the
eye, was performed by Carnell (1919 and 1921). There was a
marked reduction in the size of the outermost optious layer as
compared with the other layers of the cortex, which showed a
slight thinning. Moreover, there was an increase in the cell
population on the outermost layer in the ipsilateral cortex.
This analysis was done between the time of operation and
metamorphosis. In this work the enlargement of the optic lobes
after metamorphosis was not taken into account.

Embryologists and Neurophysiologists have long studied the nervous system, each from their own point of view, but there has been very little co-ordinated investigation of the genesis of neural function. Our present knowledge of neurogenesis stems from experimental modifications of the developing nervous system i.e. excision and enucleation of peripheral organs or brain parts, before or after the formation of peripheral connections, and the study of the effects of such manoeuvres on the developing organs.

After enucleation of both the eyes in larvae of Rana fusca, marked reduction in size of the optic lobes after metamorphosis, particularly in the outer opticus layer, was shown by Steinitz (1906). However, the reduction in size was confined to the contralateral side of the optic lobe when Durken (1913 and 1930) removed one eye in larvae of Rana fusca.

Quantitative analysis of the degree of hypoplasia in the larvae of Hyla religiosa, after unilateral enucleation of the eye, was performed by Larsell (1929 and 1931). There was a marked reduction in the size of the outermost opticus layer as compared with the other layers of the tectum, which showed a slight thinning. Moreover, there was an increase in the cell population on the outermost layer in the ipsilateral tectum. This analysis was done between the time of operation and metamorphosis. In this work the enlargement of the optic lobes after metamorphosis was not taken into account.

Kollros (1948 and 1953) has shown in detail the effect of unilateral enucleation of the eye in embryonic stages on the development of the optic lobes in frogs. He pointed out that during the normal development of the tectum, differentiating cells first form the three outermost layers in the late embryonic stages as optic nerve fibres begin to cover the tectal surface. There was an increase in the number of cells in the outermost layers during embryonic stages in the normal tectum; however the tectum contralateral to the enucleated eye showed few cells when compared with the other side. After metamorphosis, the difference in the number of cells between the normal and affected lobes was 50%. Moreover, in the deeper layers, there was a marked reduction in the number of cells in the affected lobe; the reduction was confined mainly to the lateral side of the tectum. A reduction in the mitotic rate was also noted.

McMurray (1954) reported experiments on the developmental relationship between the eye and optic tectum of Xenopus laevis, studied by means of cell and mitotic counts; after repeated crushing of the optic nerve before metamorphosis, 45% loss of cells in the contralateral side was observed. The degree of cellular loss was reduced if the optic nerve was allowed to form connections with the tectum. She pointed out that if the operations were performed at a time when the rate of proliferation of tectal cells had diminished, the system would subsequently be incapable of making up the deficit.

Hypoplastic development of the external geniculate body of mammals was reported after enucleation of the eye of new born animals (Goodman, 1932; Tsang, 1937; Chace, 1943). Similar changes were reported in a series of experiments on new born kittens, where deprivation of patterned vision in one eye for two to three months resulted in severe or moderate atrophy of the geniculate cells (Wiesel and Hubel, 1963, a). However, the number of striate cortical cells which could be binocularly driven from the deprived eye was also greatly reduced (Wiesel and Hubel, 1963, b).

Following early removal of the optic vesicle in chick, Filogamo (1950) reported the production of hypoplasia by degeneration of cells of all layers of the optic lobes after twelve days of incubation.

Relatively little attention has been paid to the marked hypoplasia in fish. Studies on various species of blind fish (Ramsay, 1901; Franz, 1912; Charlton, 1933; Kappers, Huber and Crosby, 1936) have shown reduction in size of the optic lobes with marked reduction in the number of cells in the opticus layer of the tectum. However, White (1948), while working on embryos of Fundulus heteroclitus, has reported that after a complete or partial unilateral removal of the eye, before the appearance of the optic nerve, there followed a reduction in the volume of grey and white matter as well as a reduction in the number of cells in the grey matter of the

contralateral tectum. Furthermore, a marked hypoplasia of
the contralateral optic lobe was reported after removal of
the optic rudiment, as well as in those cases where the eye
was reduced in size during development by exposure of the
embryo to ethyl alcohol, magnesium chloride and magnesium
nitrate. Such embryos took longer to develop and had one
eye smaller than the other.

After removal of one eye in Salmo during embryonic stages,
before the optic nerve formed its connections with the tectum,
Leghissa (1951) reported a marked under development of all
laminae in the contralateral tectum. Few cells were
differentiated, mostly the ones which were not connected with
the optic nerve terminations. His results were mainly based
on the studies of tectal layers, 60-90 days after hatching, by
which time most of the layers were formed.

Following the removal of one eye in newborn Xiphophorus
or Lebistes (Pflugfelder, 1952) most of the cells in the
outermost layer of the contralateral tectum failed to develop.
This was thought to be due to the failure of the tectal cells
to grow in the absence of optic nerve terminals, rather than to
their failure to be produced or to their subsequent degeneration.

Correlations between the structure and function of the
developing nervous system have been sought by some investigators.
Youngstrom (1938) studied the organisation of the nervous system
in Urodele embryos and demonstrated a well defined correlation

between the successive structural patterns and the development

of behaviour. Leghissa (1951), while working on the embryo of

Salmo irridens, described structure and behavioural development

but these studies were not co-ordinated with each other.

Harris and Whiting, (1954); Whiting, (1955); Hamburger,

(1964) and Gideiri, (1966), have correlated the onset of reflexes

i.e. body movements etc., with the formation of reflex arcs.

They studied histologically the pathways followed by nerve fibres

from cutaneous sensory receptors through the spinal cord to the

skeletal muscles. The ontogeny of behavioural and electrical

visual responses in lower vertebrates has been completely

ignored, although a few anatomical studies of the development

of visual centres have been carried out.

Growth and Differentiation in the Nervous System

During the past ten years, with the advancement of anatomical

techniques, especially that of labelling migratory cells with

tritiated thymidine, the origin and migratory movements of

neuroblasts has been fairly well established.

Differentiation of Neurons

As early as 1906, Ziehen (quoted from Cajal, 1926) put

forward an interpretation of the histogenesis of the central

nervous system of vertebrates. He classified the neural tube

cells into three different categories:-

1. Germinal cells of His - These were large cells rounded

and clear, lying directly beneath the internal
limiting membranes.   They were considered to
be the only generator cells in the neural tube
which gave rise to spongioblasts and neuroblasts.

2. Spongioblasts:- These were elongated bipolar cells
which constituted the so called primitive ependymal
cells.

3. Neuroblasts:- These were rounded, unipolar or
bipolar cells which might be distributed amongst
the germinal cells and spongioblasts.

The unipolar neuroblasts were considered to be the primitive
neuroblasts by Held (1906) and these were differentiated into
bipolar ones when the cells migrated progressively from the
juxtaventricular location towards the neuroblast layers.   At
that stage, the axon rapidly increased in length and its tip
thickened.   Most of these findings were confirmed by Cajal
(1929).

Following detailed study of neural tube cells in Pig and
Chick, F. C. Sauer (1935) challenged the old concept of neurogenesis
when he showed that spongioblasts (radially arranged columnar
cells - His, 1899) and the rounded cells in mitotic stages near
the lumen i.e. the germinal cells of His, were not two different
types of cells but instead were the interkinetic and mitotic
stages of the same cell.   He reported that the cells of the

epithelium, when about to divide, underwent a change of form
by which the nucleus migrated to the lumen and the cytoplasm
assumed a rounded form.   After division, the nucleus migrated
from the lumen.   The movement of nuclei in the wall of the
neural tube was confirmed by the recent exhaustive studies of
M. E. Saüer and Chittenden(1959);   M. E. Saüer and Walker
(1959);   Sidman, et. al. (1959) and Sidman (1961);   Fujita
(1960 and 1962) etc.

 With the use of Colchicine on neural tube cells in
mitosis, Watterson, et. al. (1956) reported, in chick
embryos, the presence of several layers of mitosis and the
absence of a true germinal layer.   Their results supported
Saüer's hypothesis.

 Microspectrophotometric measurements of DNA in the
nuclei of the chick neural tube were made by M. E. Saüer and
Chittenden (1959) to test the hypothesis of the inter-mitotic
migration of nuclei.   Their results supported the hypothesis
of F. C. Saüer (1935).   M. E. Saüer and Walker (1959) used
radioactive tritium - labelled thymidine, which is known to
be incorporated only into DNA, on the chick neural tube, to
investigate the mode of differentiation and migration of
neuroblasts.   These authors reported that synthesis of DNA
occurred only in the nuclei of the peripheral part of neural
wall and not in the juxta-nuclear zone.   These findings

confirmed the hypothesis of interkinetic migration of nuclei.

As is already apparent, an important new development is the attempt to investigate the mode of differentiation and proliferation of the cells of the neural tube. Study of the mode of cell differentiation and proliferation may throw light on the mechanisms involved in regeneration. In a histological study of the mitotic pattern in the neural tube Fujita (1960) reported that the germinal cells were rounded and were found directly beneath the internal limiting membrane: the nuclei of germinal cells showed mitotic changes whose direction was always parallel to the internal limiting membrane. He postulated that the resting cells in the mantle layer, i.e. ependymoblasts and the primitive spongioblasts, were nothing but the germinal cells in the resting state. Similar autoradiographic studies by Sidman et. al.(1959) and Sidman (1961) showed cell proliferation and migration in the primitive ependymal zone. These results confirmed Fujita's work.

Fujita (1962) has continued his earlier study on the pattern of mitosis and migration of matrix cells (Germinal cells which include the germinal cells of His and Spongioblasts) in the retina and neural tube of chick embryos using autoradiographic techniques. He reported that resting cells or Spongioblasts were cells preparing for mitosis, and the characteristics of matrix cells were definitely different from

ependymal cells. Furthermore, with the cumulative labelling technique, Fujita (1963) reported that the percentage of labelled matrix cells increased linearly with time and reached 100% in one hour or so in the chick embryo and he suggested that the matrix cell population was homogenous: cyto-differentiation was determined within the matrix layer while the undifferentiated cells were in the post-mitotic resting period. Differentiation of neuroblasts became recognizable after the cells migrated from the matrix layer. Based on these findings, the author proposed a simple scheme for cytogenesis in the central nervous system, somewhat different from his previous hypothesis, namely that the nerve cells, neuroglia and ependymal cells were all derived directly from the matrix cells at successive stages of development: the first migratory cells formed the neuroblasts (with no further incorporation of DNA): cells in the final stages of migration ceased mitotic activity and eventually differentiated into neuroglia and ependymal cells.

Smart (1961) reported that in the subependymal layer of the mouse, a distinct layer of actively dividing cells between the ependymal and mantle layers, there was a collection of undifferentiated migratory active cells which played an important part in the histogenesis of the cerebral cortex. The author suggested that the formation of the subependymal

layer provided a large reservoir of undifferentiated neuroblasts
which were able to migrate actively and thus produced new neurons.
This layer decreased in later life and the ciliated appearance of
adult ependymal cells was formed.   At that stage, the neuro-
blasts of the subependymal layer lost their histological
characteristics (light muclei, clear cell sap, prominent nucleolus
and lightly staining fibrillar material).   These findings were
based on autoradiographic analysis of the developing mouse brain.
More information is required about the structural differences
during these stages; particularly electron microscopical analysis.
Unfortunately, little has been done in this direction as yet.

ELECTRON MICROSCOPIC STUDY OF THE DIFFERENTIATION OF THE NEURAL TUBE

Electron microscopic studies are necessary in order to
understand the fine structural characteristics of the matrix
cells, as most of the complex structures are beyond the resolution
of light microscope.   Moreover, light microscopy does not allow
us to follow the fine structural changes occurring during the
differentiation and maturation of matrix cells.   During the
past few years some workers have used the electron microscope
to study changes in the early neural tube.

Fujita (1964) described the fine structural changes occurring
during early embryonic stages and followed them up to adult stages
in white Leghorn fowl, using electron microscopy and autoradiographic
techniques.   He reported that up to the 4th day of incubation, the

wall of the parietal portion of the optic lobe consisted solely
of matrix cells.   During the 5th day of incubation a mantle
layer appeared in the external zones of the optic lobes.   The
cells of this layer (neuroblasts) were not labelled by thymidine
when injected on the 5th day.   These cells became the primitive
neuroblasts.   Matrix cells had densely packed oval nuclei and
each cell was bounded by distinct cytoplasmic membrane and they
appeared similar in shape: scanty cytoplasm: oval nuclei with
long axis directed outwardly, nucleus with double membrane:
many nucleoli: cytoplasm with denselypacked ribosomes and several
mitochondria.   There was no endoplasmic reticulum or inter-
cellular membrane system and also an inconspicuous Golgi-apparatus.

Primitive neuroblasts have numerous cytoplasmic processes
with mitochondria; small vesicles and ribosomes but little
endoplasmic reticulum.   The author laid emphasis on the importance
of the appearance of endoplasmic reticulum and suggested that it
was the characteristic feature of differentiating neuroblasts.
The cytoplasm and nucleus enlarged in developing neuroblasts and
the endoplasmic reticulum developed rapidly with increase of small
vesicles and vacuoles in the cytoplasm and with increase in the
number of ribosomes.   At 10 days stage of incubation, most of the
developing neuroblasts resembled adult neurons.

The neuroblast body has never been shown to be present in
the matrix layer by any worker.   However, the cytoplasmic
processes of the neuroblast were seen in the matrix layer of a

very young chick embryo (Bellairs, 1959).

Tennyson and Pappas (1964) and Tennyson (1965) studied the fine structural changes in the development of neuroblasts of the dorsal root ganglia of rabbit embryos. Most of their findings confirmed Fujita's (1963) report. Furthermore, they described in detail migratory neuroblasts (bipolar phase), where one process resembled an axon and the other contained granular endoplasmic reticulum which is typical of dendrites. Details were also given of the process of differentiation of neuroblasts into neurons.

The pattern of structural changes during the early differentiation of neuroblasts is fairly well established now (Eschner and Glees, 1963; Meller and Wechsler, 1964; Pick, Gierdin and Delemos, 1964 and Meller, Eschner and Glees, 1966). However, no definite answer is available, as yet, about the origin of the endoplasmic reticulum and other intracellular components of the neuroblasts. The differentiation of glioblasts from matrix cells was studied by Fujita (1964); Glees and Sheppard, (1964), and Glees and Meller, (1964). No difference between glioblasts, ependymoblast and neuroblast was found at the matrix level except that the matrix cells lying at the inner surface of the neural tube differentiated into ependymoblast and the deeper ones into glioblasts and the latter migrated into the mantle layer.

The development of cilia in the matrix cells was considered
to be the sign of differentiation of ependymoblasts from the
matrix cells.

Further studies at high resolution are needed before
filaments in perikaryon and the process of earliest neuroblasts
can be compared meaningfully with neurofilaments and neurotubules
of adult neurons - along with other possible changes which may
occur during development. With the present available techniques,
it should be possible to achieve a fairly thorough understanding
of the development and function of intracellular organelles.
The pattern of origin of impulse conduction and synaptic
transmission in the individual cell is not known. New
experiments should be devised to investigate the participation
of the endoplasmic reticulum and neuro-filaments, in specific
neuronal activities. It is quite possible that specific
activities of neurons are based on the pattern of differentiation.

MIGRATION OF NEUROBLASTS

Tilney (1933) described the migration of neuroblasts, into
their definite positions in the cortex, in three distinctive
waves, in a detailed histological analysis of the developing
cortex of the Rat. During the first migration wave (16-17
days of gestation) the neuroblasts formed a lamina below the pia
called the primary granular lamina which gave rise to the 2nd
and 3rd layers. The second migration (18-19 days of gestation)

formed a secondary granular lamina and gave rise to the 4th layer of cortex. In the 3rd migration, neuroblasts established a deeply situated lamina. A mechanism has been suggested by Bergquist and Kallen (1954) to explain the formation of migratory zones from the grannular lamina as seen in urodeles. A migratory area was formed by lateral migration of matrix layer cells. The migratory cells formed a mass which was connected with the matrix layer. The cell mass separated from the matrix layer and formed a migratory layer. The migratory layer could be further divided by this process to form a separate layer. New proliferation processes followed by new migratory processes took place in the matrix layer. Thus a secondary migratory layer was formed ventral to the first one.

Agnevine and Sidman (1961) studied cell migration during histogenesis of the cerebral cortex of the Mouse, using autoradiographic techniques. They reported that the pattern of migration of neuroblasts was the reverse of the one proposed by Tilney (1933) and showed that cells do migrate extensively in the developing mammalian cerebellum. Cells labelled first in the early stages were later found in the fifth and sixth layers, whereas, the cells labelled later were traced in the fourth, third and second layers.

These findings were confirmed by Berry and Rogers (1963), using the X-ray irradation technique on the developing cortex and more recently by Berry and Rogers (1965) using autoradiographic methods.

Berry, Rogers and Eayrs (1964 a and b); Berry and
Rogers (1965) studied the detailed migratory pattern of
cortical neuroblasts, and the mechanisms underlying the
processes of histogenesis and differentiation in the
cerebral cortex, using various techniques, e.g. auto-
radiography, Nissl preparations, silver methods, Golgi-Cox
techniques and phase contrast study of fresh preparations.
These authors put forward a scheme for the mechanism of
differentiation and migration of neuroblasts.

They suggested that the processes of the ependymal
cells never became dissociated anatomically from the
surface of the cerebral cortex, at least during histogenesis.
The nuclei of the ependymal cells divided near the ventricular
surface but the cytoplasm did not divide.   "While the one
nucleus remains near the ventricular wall, the others migrate
towards the cortical surface within the cytoplasm of the
process of the ependymal cell.   Reaching the zonal layer,
cytoplasmic division occurs and nucleus with scanty cyto-
plasm becomes independent.   It is thought that while the
nuclei are within the ependymal process, they are oval

but when independent, they become rounded, which would

explain the distribution of these two shapes of nuclei during

the early stages of development".
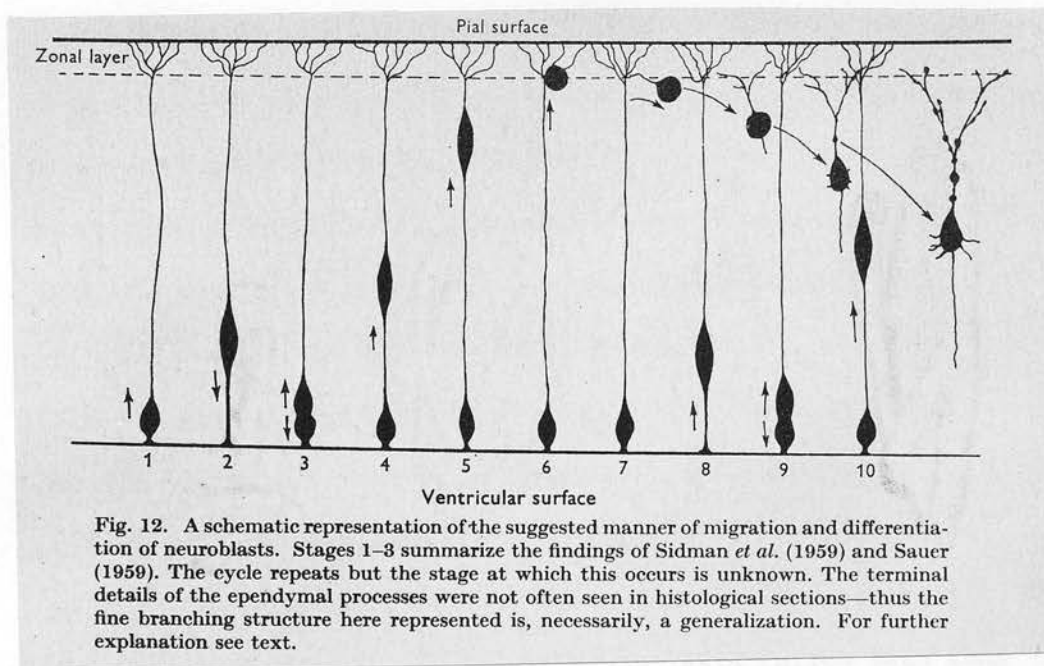
Fig. 12. A schematic representation of the suggested manner of migration and differentiation of neuroblasts. Stages 1–3 summarize the findings of Sidman *et al.* (1959) and Sauer (1959). The cycle repeats but the stage at which this occurs is unknown. The terminal details of the ependymal processes were not often seen in histological sections—thus the fine branching structure here represented is, necessarily, a generalization. For further explanation see text.

This diagram has been taken from Berry and Rogers (1965).

It is possible that the daughter nuclei of the migratory cells which are pushed outward form in fact the growing tips which are known to carry scanty cytoplasm. In this case the exploratory tips after attaching themselves to the basal part of zonal layer may suck the nuclear and cytoplasmic material through filamentous attachments from the mother cells and finally detach themselves and form separate cells. Movements of the primary mesenchyme cells in the developing Sea Urchin larvae have been investigated by Gustafson and Wolpert (1961). The organisation of a coherent pattern on the ectoderm of the gastrula was shown to be achieved by the exploratory extension $(0.5 \mu$ or less in thickness) of pseudopods in the larval wall. After making sufficient contact with blastocoele wall, the pseudopods of several cells fused to form a common pseudopod. With the help of this common pseudopod the mesenchyme cells migrated towards the appropriate direction. The end of common pseudopod was highly branched. Behaviour of these pseudopods may have relevance in neuroblast migration as well.

No experimental evidence is available to support Berry and Rogers (1965) hypothesis of the mechanism of neuroblast migration. Moreover, why should neuroblasts follow such long routes and settle down in areas far removed from the one where they underwent early differentiation. In order to understand these mechanisms, it would be necessary to develop methods to

trace out single cell movements during neuroblast migration.
The methods obviously include the culturing of neural tube
before migration starts and following the movements of a
single neuroblast by time lapse photography.   Electron
microscopy and ordinary histological techniques would not be
very useful for this purpose as it is impossible to follow
the movement of a cell.

Cellular Segregation:-

There are many instances of cells moving directionally and
segregating in specific regions, e.g. neural crest cells move
to their destinations along defined routes.   The directional
movement of cells is obviously a widespread phenomenon of
considerable developmental and functional significance.   Cells
could be attracted chemotactically by diffusible chemical agents.
If cells are selectively eliminated elsewhere, they might also
become concentrated at a given locus after completely random
movements.   Cells may be trapped by some mechanism if they
reach the proper places.   The possible mechanisms could be
selective adhesion or "selective fixation" (Weiss, 1947) or
cessation of movement, etc.

When the direction of movement of cells is influenced by
a gradient in concentration of substances in solution, chemotaxis
is involved.   A cell must be able to detect a concentration
difference over a distance corresponding to its own diameter and

move parallel to the gradient. There is no conclusive evidence
of Chemotaxis for animal cells and information is lacking on the
mechanisms involved in Chemotactic responses. Wandering cells
of neural tube cease movements and adhere to particular cells in
particular regions of the embryo, i.e. there is selectivity in
adhesion which could be the possible means of trapping cells
during development. Selectivity has been demonstrated by the
behaviour of aggregates of dissociated cells.

Townes and Holtfreter (1955) demonstrated that amphibian
cells may move within an aggregate of several different embryonic
cell types. Segregation in the aggregate was according to cell
type; the cells re-established their former associations. The
re-aggregation pattern of suspensions of freshly dispersed chick
embryonic cells in liquid culture medium was studied by Moscona
(1960). He observed that these cells frequently "appear to
follow each other in clearly descernible rows" and that the cells
moved within fine strands of highly transparent slimy substances,
evidently an exudate of cellular origin. Based on these
observations, Levi-Montalcini (1964) suggested that the extra
cellular matrix might act as a cell integrating system with
specific cell directing activities. Also, we have to take into
consideration the effects of chemical agents present in the medium
where cells would migrate. Levi-Montalcini (1964) showed the
presence of such agents in the medium throughout the life cycle

and pointed out that the migratory cells were highly receptive to these agents. The nature and origin of these chemicals is not known - neither do we know anything about the reactive mechanisms of these agents on migrating neuroblasts.

Tissue specific segregation has been shown in retinal pigment cells when these were dissociated and mixed with cell suspensions from a variety of organs (Trinkaus, 1965). The immobility of cells in tissues and the topographic stability of the tissues may rest on the selective adhesion of cells to each other.

It seems likely that the sorting out involves random cell movements followed by qualitatively specific adhesion between similar cells.

Steinberg (1964) assumed that sorting out of cells resulted entirely from random mobility and quantitative differences in the general adhesiveness of cells; and that an aggregate containing cells of more than one kind may be treated as if it were a multiple phase of immiscible liquids. He further assumed that thermodynamic relationships which govern miscibility and surface spreading in liquids would apply to the cells in the biological system. There must be maximal adhesion of cell surfaces. Steinberg (1964) further suggested that the surface of various kinds of cells must be encoded with characteristic differences which play a part in the cellular adhesive differentials. Differences in the adhesive sites of quantity, of order and of

kind were the three main types of adhesive codes which have
been postulated.

It is possible that in the developing nervous system,
cells become specified with relation to each other in terms
of a system of crossed gradients (Harrison, 1921, in developing
and regenerating amphibian limbs; Harrison, 1936 and 1945, in
the development of amphibian ear; Sperry, 1965, and Gaze et. al.,
1963 and 1965, in compound eyes). There is no evidence so far
on the nature of the gradients involved in the developing nervous
system.

De Haan (1964) described the migration of four day chick
heart cells; these cells extruded filamentous processes and
formed firm attachment to the substrate. These processes became
enlarged when the ruffled membranes of the cells flowed into them.
De Haan suggested that if such activity is associated with
differential adhesiveness, it could account for the directed
migrations observed in these cells. It seems highly likely
that this sort of behaviour must also underline not only the
development but also the ability of regenerating optic nerve
fibres to find their appropriate terminations.

Histogenesis of the Optic Tectum

Relatively little attention has so far been given to the
histogenesis of optic tectum of vertebrates, particularly the
lower vertebrates. The histogenesis of the optic tectum of

the domestic fowl was worked out with electron microscope and autoradiographic techniques (Fujita, 1963), details of which have already been given.

Leghissa (1951) studied the histological development of the optic tectum of the trout (Salmo fario). He divided the development of the tectum into twelve different stages, starting from eight days before hatching up to ninety days after hatching, by using various histological techniques. Remarkable changes in thickness of outer white and inner cellular zones of the tectum were reported. Gray (cellular) and white (outer zone) matter increased progressively up to the seventh stage, (twenty-five days after hatching). From the seventh stage onwards there was a reduction in thickness in the grey periventricular zone and a progressive increase in thickness in the outer white zone. The only explanation given to account for this change was that a few neuroblasts probably migrated from the grey periventricular zone. Another possible explanation could be a change in packing density before and after migratory phases of the grey periventricular zone. The phenomenon of cell death during normal development could be another reason for the change in size of grey and white zone in the tectum. Neuroblast differentiation and migration in Salmo fario were not considered at all. Study of these aspects may aid investigations of regeneration of the central nervous system.

The relationship between histogenesis and biochemical growth during the development of the optic tectum in chick embryos was described by Grayet and Bonichen (1961). A rostrocaudal gradient of cholinesterase in frogs was reported by Boell, et. al. (1955) where they showed that cholinesterase first developed at the rostral pole of the tectum. Similarly, Herrick (1942) and Kollros (1953), have shown the stratification of tectum which was first seen in the rostral pole extending caudally as more optic fibres innervated the tectum. However, nothing is known as yet about the maturation of electrical activity, particularly visual, in the tectum in relation to innervation by the optic nerve fibres; this information would help us to determine whether functional connections have been established. In this aspect it is important to know the behavioural pattern during development particularly optokinetic responses.

REGENERATION IN THE CENTRAL NERVOUS SYSTEM
(Excluding Optic Nerve)

Regeneration in its strictest sense is the restoration of organisation of lost parts of the body. Regeneration in the embryonic nervous system involves the capacity of the embryonic nervous tissue to heal the wound by cellular multiplication with the possibility of restoring normal structure, whereas, in adult nervous tissue, regeneration is usually restricted to restoration

of the lost part, e.g. axon or dentrite, as adult neurons in
higher animals have lost the capacity to multiply.

Spinal Cord Regeneration

Maron (1959) and Hibbard (1964) have shown regenerative
capacity in the spinal cord of the larvae of Lamprey and
Petromyzon respectively.   Koppanyi and Weiss (1922) were
able to trace histologically the fibre tracts after spinal
cord regeneration in teleosts (Carassius vulgaris), whereas,
Hooker (1930 and 1932) and Nicholas (1930) were unable to trace
any morphological reconstitution in Fundulus and goldcfish, when
the spinal cord was transected at various larval stages.   These
conflicting observations led Tuge and Hanzawa (1937) and Kirsche
(1950) to verify the previous findings and they reported that in
young adult teleosts, anatomical and physiological restitution of
the transected spinal cord could occur.   Return of co-ordinated
movements was reported in these animals.   (N.B. Ten Cate, 1935,
and Hooker, 1930-31, reported that in goldfish it became
progressively more difficult for a fish to hold its body in the
normal position the further forward the section is made.
Swimming was supposed to be brought about by pectoral fins and
the body musculature of the anterior end of the animal.) Adult
animals took longer to recover than did young ones.   Kirsche
(1950) reported the presence of a growth cone at the tip of
regenerating fibres and showed movements in the tail fin following

electrical stimulation above the lesion, thus suggesting functional regeneration in the spinal cord. Piatt and Piatt (1958) have shown significant spinal cord regeneration in post metamorphic and adult frogs. Butler and Ward (1966) reported the reconstitution and return of function of 2 mm ablated spinal cord in the postbrachial region in larvae of Ambystoma maculatum. They observed the growing nerve fibres from both ends with terminal vesicles on their tips which bypassed the ablated area and joined the opposite end to form a bridge. Recovery of the use of hind limbs for walking and of trunk and tail muscle for swimming was correlated with the extent to which nerve fibre bridges were established. Larvae which walked and swam normally, possessed fibre bridges. Migration of ependymal cells to close the canal and the movement of cells of the gray matter over the cut surface were shown to be responsible for regeneration.

Amphibia seem to possess remarkable properties of neural regeneration. Numerous workers have shown the regenerative capacity of the central nervous system in different stages of the life history of Amphibia. In excellent reviews on this subject Piatt (1955) and Clemente (1964) have pointed out the outstanding capacity for regeneration of spinal cord and different parts of the brain. Most of these studies were restricted to larval amphibians. Because of the enormous amount of literature

available on this subject, a few recent studies only will be
considered.

REGENERATION IN THE BRAIN OF LOWER VERTEBRATES

After unilateral extirpation of medulla and mesencephalon
in Amblystoma embryos (stage 21-22), Detwiler (1945, 1946)
reported the gradual restitution of the lost half from the
contralateral intact wall of the neural tube.   Restoration
was accompanied by proliferation and migration of cells mainly
from the dorsal part of the normal side of the brain.

Morphological regeneration in animals (after metamorphosis)
was reported in different brain parts of urodeles (Sibbing, 1953).
In a series of experiments in recently metamorphosed Xenopus, the
regenerative capacity in the adult end brain, after excision, was
compared with that in tadpoles.   It was reported that post
metamorphosed animals took longer to regenerate than the larvae.

Looking for the scource of regeneration Srebro (1957) and
Jordan (1958), while working on end-brain regeneration in larval
and adult frogs, described mitotic activity in ependymal and
subependymal cells.   They considered this mitotic activity as
one of the scources of regenerating tissue.   However, Sibbing
(1953) thought it to be cells migrating after division from
various parts of the brain.   Unfortunately he did not mention
which areas.   Kwiat-Kowski (1961) transacted the nerve
connections at various levels (i.e. between end-brain and

diencephalon and transection of the posterior part of the brain)
in Xenopus laevis, thirty days after metamorphosis, and reported
that the front sector of the brain unites more rapidly after
separation than the posterior one. Maron (1963) excised the end
brain hemisphere at the border of the diencephalon and separated
it from the olfactory nerves in four days old Lebistes reticulatus.
A complete morphological restitution of the hemisphere was reported,
one hundred and twenty days after operation. Active division and
migration of ependymal cells, which assumed an oblong shape, was
suggested to be the source of regeneration. The embryonic ability
of the ependymal cells to divide and differentiate into neurons was
presumably still present in these animals.

Using 3-7 cms and 15-17 cm long Carassius carassius - L.,
Kirsche (1960)examined the regenerative ability of the optic
tectum. A small surgical lesion in the tectum was made. Most
of the gap caused by incision seemed to be filled up with new
cells, twenty seven days after the operation. He reported the
presence of matrix cells (undifferentiated neuroblasts) near the
middle and lateral edges of tectum in association with the
ependymal layer. The origin of the new cells was thought to be
these undifferentiated cells (matrix zone) which divided to give
rise to new cells. A process of migration of new cells was
suggested, comparable to the neuroblast migratory movements during
development. In another series of experiments, a big area of

tectum was removed, leaving the tectal poles and the medial

dorsal matrix zone intact.   Mitotic activity was reported in

the remaining intact matrix zones.   One hundred and twenty

five days after the operation the extent of mitotic activity

was reduced, and the building up process of the lost area

started.   Tectal layers gradually re-appeared along with the

restitution of fibre tracts.   Even three hundred days after the

operation the tectum did not return to its original shape.   No

regeneration was reported in the case where the whole tectum of

one side was removed, yet in other cases, when the matrix zones

were kept intact, some regeneration was observed.

After removal of one telecephalon in 6-7 cms long

Amblystoma, incomplete regeneration was observed (Kirsche and

Kirsche, 1964).   Three different sources of regeneration were

mentioned:

    (a) The sensory epithelium of the organ of smell

    (b) The place of resection in the area of the

        primordium hippocampi

    (c) The place of resection in the border area

        between the telencephalon and diencephalon.

The regenerated *telecephalon* remained small in comparison with the normal

side, even five hundred days after operation.   As a result of

incomplete regeneration, compensatory hyperplasia of the unoperated

side was reported. A significant increase in the number of cells in the bulbus-olfactorius resulted in a marked enlargement of the normal half of telencephalon.

Segaar (1965) reported regeneration in the telencephalon of Stickle-back (Gasterosteus aculeatus) after unilateral extripation of the hemisphere, studied by Bodian-Ziesmer and Golgi- Cox techniques. Mitosis observed in the ependymal cells was supposed to originate new cells. Regeneration was observed eighty four days after the operation. Recent detailed analysis of regeneration of the optic tectum in a fish (Leucaspius delineatus) was reported by Richter (1965). Three days after incision of the tectum, formation of an incision canal (a canal formed due to incision and filled with meningeal tissue) was observed at the site of the lesion. Mitotic activity in the matrix zone was followed by migration of newly formed neuroblasts and glioblasts. The newly formed cells were orientated outwardly through the incision canal towards the upper layers. The area around the site of lesion developed traumatic degeneration and phagocytes and degenerating cells were observed. New capillaries emerged in the traumatised area five days after the operation. The formation of normal layering started from the basal layers. A fibrous bridge extended from Torus-longitudinalis and Torus-semicircularis and facilitated the spread of the basal cells from the Tori towards the tectum. Tectal restoration to a normal

architecture took four hundred and twenty seven days after the lesion. The regenerative ability was smaller in Leucaspius than in Carassius carassius (Kirsche, 1960).

In view of these findings, one would expect that anatomical regeneration be followed by the return of physiological function in the operated part of the brain. Unfortunately, the physiological aspect of regeneration was not considered at all. Kirsche and Kirsche (1961) studied the behavious of Goldfish after tectal manipulations and reported the return of normal behaviour. (Twisting movements were replaced by normal ones). It may be necessary to perform additional experiments to verify Kirsche's result.

Compensatory hyperplasia as seen in the forebrain of Ambolystoma (Kirsche and Kirsche, 1964) was not reported in one Goldfish tectum when the other failed to regenerate after removal. Moreover, most diagrams of the regenerating tectum by the above mentioned authors are not very convincing, as they concentrate mainly on the cytoarchitecture. They have ignored the fibrous pattern of the tectum. A detailed histological analysis is required in order to confirm the above findings.

Richter (1965) suggested that the reformation of the pattern of the tectum, during regeneration after excision, was similar to that of normal development. However, considering the pattern of normal development of tectum (Fujita, 1963; Berry and Rogers, 1965),

where the outermost layers are formed first, it appears that regeneration is not similar to development. Moreover, conditions in the brain during regeneration are entirely different from those during development. Unless the migration of newly formed neurons (if any) is traced, either autoradiographically or in tissue culture and the differentiation of matrix cells into neurons is compared to that during normal development, it would be difficult to compare normal development and regeneration. Electron microscopic observations in normal adult tectum are necessary to identify the possible presence of matrix cells - as it is beyond the resolution of the light microscope to distinguish matrix cells in the cellular periventricular zone.

Abraham and Tury (1965) claimed the presence of a mitotic figure 200-350 mμ away from the site of experimental lesion in the outer layers of the tectum of Rana ridibunda. The presence of such mitotic figures in the outer layers has never been observed by any other worker so far. The authors did not mention its exact origin. This cell could be glial or a neuron.

Considering the varied capacity for regeneration in the central nervous system of lower vertebrates, it is quite clear that the time taken by regeneration during larval stages is less than during adult life. Moreover, larval brain has the capacity to restore the lost part completely, whereas, adult brain never regenerates to normal; although mitotic division and proliferation

have been seen in the adult brain after lesions.   This last
observation clearly suggests that adult brain does retain the
power to regenerate and possible embryonic cells are still
present in adult life, which in response to injury activate
and proliferate to give rise to new cells;   or adult cells
re-acquire the ability to divide.   When this capacity for
brain regeneration in adult animals (fish) is lost, is not
known.

In order to study functional regeneration in the optic
tectum, new experiments have been devised.   After incision,
removal and/or reimplantation of the excised tectum - point to
point mapping of the retina on the optic tectum has been under-
taken.   It is also the purpose of the present study to investigate
the normal development of tectum with various available techniques
and to look for the pattern of regeneration after experimental
lesions in the optic tectum.   Studies have been undertaken to
compare the course of regeneration with that of development.
With the advancement of histological techniques during the past
fifty years, and the use of Marchi's staining for degenerating
fibres, it has been clearly established that the retina projects
topographically onto the lateral geniculate body, superior
colliculus and the optic tectum.   Functional confirmations of
the spatial organisation of the visual system have been provided
by electrical recordings from the visual centres.

The Sub-mammalian optic tectum has a structural complexity
comparable with that of mammalian visual cortex. The optic
axons terminate in the optic tectum which is the chief visual
centre. The optic tectum receives fibres from many centres
and it is truly a centre of correlation.

ANATOMICAL EVIDENCE OF TOPOGRAPHICAL PROJECTION
OF THE RETINA

With histological techniques it has been shown that the
optic axons terminate in a retinotopic order on the optic tectum
and its mammalian homologue, the superior colliculus, but under-
standing of the function of the different strata and individual
cells of the optic tectum has advanced little. After enucleation
of one eye followed by degeneration of the optic nerve it was found
that primary optic fibres decussate completely at the chiasma in
Amphibians and Fish, and Marchi degeneration could be found on the
contralateral side only. (Bellonci, 1888; Wlassak, 1893;
Ramon Y Cajal, 1952-1955).

Cowan et. al. (1961) have traced degenerating fibres after
unilateral enucleation of the eye in adult pigeons using different
silver techniques. They reported that apart from the main termin-
ation of optic fibres in the tectum, there was a direct projection
to the hypothalmus as well; they further reported the presence of
centrifugal fibres to the retina in this visual system.

Armstrong (1950, 1951), however, has shown uncrossed optic

fibres in the Snake and Lizard, which appeared to terminate
in the ipsilateral geniculate body and not in the optic
tectum.

In Mammals, the optic nerve fibres have been traced
to the Superior Colliculus of the Opossum (Tsai, 1925;
Bodian, 1937), the Ferret (Jefferson, 1940); Rabbit
(Pavlov, 1900; Loepp, 1912; Minkowski, 1920; Brouwer,
Zeeman and Houwer, 1923; Brouwer and Zeeman, 1926;
Overbosch, 1927); Rat (Lashley, 1934; Jefferson 1940;
Nauta and Van Straten, 1947); Sheep (Nichterlein and Goldby,
1944); Goat (Minkowski, 1920); Cat (Frobst, 1900; Barris,
Ingram and Ranson, 1935; Hoessly, 1947); Dog (Probst, 1900);
Monkey and Apes (Bernheimer, 1899; Minkowski, 1920; Brouwer
and Zeeman, 1925, 1926; Crosby and Henderson, 1948); and
Man (Leonowa, 1896; and Minkowski, 1920). Ipsilateral
fibres to Superior Colliculus have been traced in almost
all the mammalian groups studied so far.

Study by Marchi's method of degeneration from a more
localised lesion in the retina has been made in Fish (Lubsen,
1921; Akert, 1949, a,b,; Stroer, 1939 and 1940); Pigeon
(Kapper, Huber and Crosby, 1936) Rabbit (Brouwer, Zeeman and
Houwer, 1923; Brouwer and Zeeman, 1925, 1926); Rat (Lashley,
1934), and Opossum (Bodian, 1937). The studies of these
authors were not sufficiently detailed to show a point to

point projection hypothesis, though they frequently
supported it.

Lubsen (1921) studied the degeneration following
localised retinal lesions in a teleost fish (Leuciscus
rutilus). His findings were confirmed by Akert (1949,
a,b,) on another fish (Salmo-iridens). They showed that
retinal quadrent lesions resulted in specific localised
degeneration in the contralateral tectum. They further
reported that the nasal retinal quadrānt projected to the
caudal part of the contralateral optic tectum, the temporal
retinal quadrānt projected to the rostral part of the tectum;
the dorsal retinal quadrānt projected to the lateral part of
the tectum and the ventral retinal quadrānt projected to the
medial part of the tectum. The above mentioned findings
were further confirmed histologically by Leghissa (1955) in
Goldfish and a few other teleost fish, and Stroer (1939) in
Salmo, Salar, Clupea, Lebistes and Clarias - all teleosts.
A specific selective projection regenerating from partially
ablated retinae in Goldfish has been shown by the Bodian
protargol technique - (Attardi and Sperry, 1963). When the
dorsal half of the retina was removed, surviving ventral
fibres became segregated beyond the nerve scar and selectively
entered the medial tract to connect with the dorsal tectum.
Conversely, when dorsal retina remained intact, the regenerating

fibres filled selectively the lateral tract and the
ventral tectum.   Fibres from the temporal retina invaded
the anterior portion of the tectum and did not extend into
the posterior regions.   Conversely, those fibres from the
anterior hemiretina bypassed the anterior zones to innervate
the appropriate layer in the posterior zones of the tectum,
these fibres also bypassed the plexiform layer in the margin
to connect only in the central zone.   The plexiform layer in
the marginal zone was innervated only when fibres were avail-
able from the peripheral retina.   These authors suggested
that specific chemical affinities govern neuronal connections
including the patterning of central fibre pathways.

Only a gross projection of retinal quadrants onto the
specific areas in the tectum has been repeatedly shown.   No
attempt has been made to study histologically the point to
point projection.

Herrick (1941 a and b), using a silver nitrate technique
was unable to find any evidence of retino-topic projection
onto the tectum in Necturus and Catfish (Ameiurus nebulosus).
The optic nerve fibres in Amphibia retain a fasciculation
according to retinal quadrents for a very short distance and
become randomly interlaced in the optic nerve and appear to
terminate in the tectal neuropile (Herrick, 1942 and 1948;
Maturana, 1958).   That is why Herrick (1941 a and b, 1942

and 1948) could not possibly trace the optic nerve fibres in Necturus and Amblystoma.

As there is very little anatomical evidence in support of point to point topographical representation of the retina onto the tectum, detailed histological analysis of this problem is required. Moreover, there is not any anatomical evidence produced so far for a bilateral representation of the part of the retina concerned with binocular vision.

PHYSIOLOGICAL EVIDENCE OF TOPOGRAPHICAL PROJECTION
OF THE RETINA

The technique of mapping the visual field by recording the action potentials from positions on the visual cortex evoked in response to a small light in the visual field, was developed by Talbot and Marshall (1941). Employing this technique, Apter (1945) mapped the projection of visual fields on the superior colliculi of the cat. It has been shown by similar techniques that there is a topographical representation of the visual field on the optic tectum of the Carp and Tench (Buser and Dusardier, 1953); Goldfish (Jacobson and Gaze, 1964; Cronly-Dillon 1964); a few fresh water fish (Schwassmann and Kruger, 1965); Xenopus (Gaze and Jacobson and Székeléy, 1963); Newt (Burgen and Grafstein, 1964 and Cronly-Dillon, 1967); Alligator (Herric and Kruger, 1965); Pigeon (Hamdi and Whitteridge, 1953 and 1954);

Superior Colliculus of Rabbit (Hamdi and Whitteridge, 1953; Chaudhary and Whitteridge, 1965); Rat (Siminoff et. al., 1966; Forrester and Lal, 1967); Goat (Cooper, Daniel and Whitteridge, 1953; Hamdi and Whitteridge, 1953); Cat (Vejbaesya, 1967) etc.

From the catfish, Tench and Carp, Buser and Dusardier, (1953), recorded evoked potentials in the contralateral tectum when they stimulated the retina with bipolar electrodes 1 m.m. apart. A separate projection of the retinal quadrents was found which confirmed the findings of Lubsen (1921) and Akert (1949 a and b), in a Teleost studied by Marchi's method.

Apter (1945), recorded action potentials from the Superior Colliculus of the cat, using a spot of light as stimulus. She reported that for any position of the electrode, the evoked response was of minimum latency and maximum amplitude only at a localised position of light in the visual field. Apter was able to map point to point projections of the visual field in detail. She reported further that on each Superior Colliculus the representation of the ipsilateral temporal half field and of the contralateral nasal half field were superimposed in such a manner that corresponding points in the homonymous half fields were in register.

The number of microns of visual areas devoted to the

representation of each degree of the visual field when
measured radially from the fixation point is known as the
magnification factor.   The measure of the amount of tectal
representation for different parts of the retina has been
made for the visual areas of certain mammals (Talbot and
Marshall, 1941; Daniel and Whitteridge, 1959; Vejbaesya,
1967); in frogs (Jacobson, 1962); in Xenopus (Gaze,
Jacobson and Székély, 1965).

Hamdi and Whitteridge (1953, 1954) determined the
representation of the visual field on the optic tectum of
the pigeon.   Using a steel microelectrode on the tectum,
they recorded the responses evoked by a neon flash subtending
at an angle of 0.5 degrees in the contralateral visual field.
They reported that the superior half of the visual field was
represented on the dorsal surface of the tectum and the
inferior half field on the ventrolateral surface.   These
responses which were obtained from the tectum were evoked from
the positions of the visual field, approximately symmetrical
about the horizontal meridian.   The horizontal meridian ran
along the lateral edge of the tectum, whereas, the vertical
meridian ran transversely across the tectum with the nasal half
field represented rostrally and the temporal half field
caudally.   McGill, Powell and Cowan, (1966 a and b) have shown
in detail the point to point projection of the retina on the

optic tectum of the pigeon using histological and electro-

physiological techniques. They reported that the superior

retinal quadrant is represented inferiorly in the tectum

and the anterior quadrent posteriorly. It still remains to

be seen whether or not there is any visual representation

on the ipsilateral optic tectum of the pigeon - as reported

by Gaze and Jacobson (1962 a) in the frog, and Gaze, Jacobson

and Székély, (1965) in Xenopus.

Hamdi and Whitteridge (1953) mapped the representation

of the retinal quadrants on the Superior Colliculus of

rabbit. Their findings confirmed the results of Bouwer and

Zeeman (1925 and 1926). Work on the rabbit was extended by

Kerr and Seneviratne (1963) and Seneviratne (1963) who

reported that visual field represented on the collicular

surface was of the shape of a narrow band. Cooper, Daniel

and Whitteridge, (1953) and Hamdi and Whitteridge, (1953)

found that the pattern of retinal projection on the Superior

Colliculus of the goat was identical to that of the monkey

and cat etc.

Gaze (1958 a and b) showed the point to point

projection of the visual field on the optic tectum of the

frog. The naso-superior, naso-inferior, temporo-inferior

and temporo-superior quadrents of the visual fields are

represented on the rostral, lateral caudal and medial quadrants

of the optic tectum.   He was unable to find evidence of an
area centralis retinae.   These findings were confirmed by
Maturana et. al. (1960) and Jacobson (1962).   The latter
author further reported that area centralis projected to
the lateral surface of the tectum.

Gaze and Jacobson (1962) reported that the central
region of binocular field in frogs was represented through
both eyes on both ipsilateral and contralateral tects.
The left side of the field from the central region projects
through both eyes to the right tectum, while the right side
of the field projects through both eyes to the left tectum.

The topography of the visual field onto the optic
tectum of goldfish has been mapped by Jacobson and Gaze (1964);
Cronly-Dillon (1964) and Schwassmann and Kruger (1965).
Different types of visual responses were recorded from single
units in the optic tectum.

REGENERATION OF THE OPTIC NERVE

The regenerative capacity of the optic nerve has only
been investigated in the lower vertebrates, especially in
Amphibians and fish.   This subject has been exhaustively
reviewed by Clemente (1964);   Gaze (1960 and 1967);
Jacobson (1967 c);   Koppanyi (1955);   Sperry (1951 a,b, and c;
1955, 1965);   Stone (1953 and 1964);   Székély (1966).   The
main findings are as follows;   After simple section of the

optic nerve, it regenerates in Fish (Sperry, 1948; Jacobson
and Gaze, 1965); Amphibians - Urodeles (Mathey, 1926 and
1927; Stone and Chace, 1941; Sperry, 1943; Gaze et. al.
1963 and 1965). Anurans (Sperry, 1944; Maturana, 1958;
Gaze, 1959 and 1960; Maturana et. al. 1959, Jacobson, 1960;
Gaze and Jacobson, 1963). Matthey (1926 and 1927) cut the
optic nerve in newts and for the first time observed that
some months later, the animals could see again through the
operated eye. His animals then behaved like normal ones.
He was able to prove that true visual recovery had occurred
by removing the normal eye and showing that the animal still
followed a small lure moved outside its tank. This could only
happen if the spatial relationship between the retina and the
brain had been restored by the regenerating optic nerve.

Matthey's experiments were followed by Sperry (1944),
where he cut the optic nerve in a frog and rotated the eye
by 180° about the visual axis. He reported the return of
vision but with the development of reversed visuomotor
behaviour in case of the reversed eye. These maladaptive
responses have never been known to be corrected by experience
(Sperry, 1944, 1951 a and b; Stone, 1953). He proposed that
the regenerating fibres grow back to their proper places in
the brain.

Stone (1953), reported maladaptive motor responses four

and a half years following rotation of the eye. Recovery
of the normal optokinetic responses and proper localization
of a lure occurred immediately after the eye was rotated
back to its normal position. These experiments suggested
that the normal topographical representation of the retina
on the optic tectum was restored, irrespective of the
position of the retina.

The restoration of the retinotectal projection to the
optic tectum after regeneration of the optic nerve has been
shown electrophysiologically (Gaze, 1959, Gaze and Jacobson,
1959; Maturana, 1959, Jacobson and Gaze, 1965; Jacobson,
1962), histologically (Attardi and Sperry, 1963) and
behavioural i.e. return of optokinetic responses (Sperry,
1943, 1945 b and 1951 b and c, and Stone, 1944, 1953.)

Seeking an explanation for the normal restoration of the
retinotectal projection after optic nerve regeneration, one
has to consider the hypothetical mechanisms put forward by
various workers, which may determine the selective central
connections of the optic nerve.

Harrison (1910) postulated the selection of paths by
outgrowing nerves and the specificity of nerve end organ
connections. This original concept was thoroughly worked
out separately by workers such as Weiss, Hamburger and Sperry.
These investigators have modified the basic suggestion into
many sophisticated working hypotheses.

(I) NEURAL SPECIFICITY: Sperry (1943-1945) while working
on the visual system of various animals devised a hypothesis
accounting for the selective central connection of regenerat-
ing optic nerve fibres. According to him each optic nerve
fibre or each ganglion cell in the retina would be uniquely
specified in some manner and each appropriate region on the
tectum also specified in a similar way; when the optic nerve
fibres regrow, they would be able to sort themselves out by
finding the specific point on the tectum. Sperry (1956)
elaborated this idea by suggesting that each ganglion cell
with its axon which presumably was uniquely labelled,
sprouted out many branches on reaching the tectum. These
branches grew widely over the tectum until one of them
found tectal cells with similar labelling. The remaining
branches retracted.

The strongest evidence supporting this hypothesis came
from the work on Goldfish by Attardi and Sperry (1963).
These authors showed selective reconnection of the optic
nerve fibres following nerve regeneration from partly
ablated retinae in the Goldfish. With the dorsal half
of retina destroyed and the optic nerve cut, the regener-
ating optic nerve fibres selectively entered the medial
tract and formed connections with the medial tectum.
However, when the dorsal retina only remained intact, the

regenerating fibres grew into the lateral tract of the optic

nerve and finally innervated the ventral tectum.   After the

removal of temporal retina, nasal fibres entered both tracts

of the optic nerve and after by-passing the rostral part of

the tectum, they terminated in the caudal part of the tectum,

whereas, the temporal part in the other case grew into the

rostral tectum.   These findings strongly suggest that a

chemoaffinity mechanism is at work during the regeneration

of optic nerve fibres; and also that this mechanism is

effective both during pathway selection and termination

selection.   These authors used Bodian's Protargol technique

to differentiate between regenerating and normal fibres.

Unfortunately, this technique does not show the individual

fibre connections and terminals in detail in the tectum.

    Furthermore, Arora and Sperry (1962), reported that if

the medial and lateral optic tracts were interchanged

surgically, the regenerating nerve fibres recrossed the

tectum to end in their original position which also

supported Sperry's original hypothesis.

    In order to verify these findings electrophysiologically,

Jacobson and Gaze (1965) adopted two manoeuvres to change the

size and relationship between the retina and the tectum in

adult Goldfish.   In one series of experiments, immediately

after cutting the half optic nerve close to the retina, a

sharply defined area of the tectum did not give any
retinotopic projection.   In another group of experiments
the optic nerve was half cut along with optic nerve crush
proximal to the cut.   The regenerating crushed optic nerve
fibres from this series connected only with those parts of
the tectum with which they normally connect.   The remaining
half of the tectum was left unconnected.   In a second series
of experiments, half of the optic tectum was removed (either
medial or lateral) and the optic nerve was crushed.   The
retinotectal projection was mapped after optic nerve
regeneration.   The regenerating fibres connected only with
their appropriate position in the tectum.   These authors
concluded that regenerating optic nerve fibre connections
with the tectum are fully determined and the relative change
in the number of optic nerve fibres or tectal cell has no
effect.   They further suggested a very rigid form of
"place specificity" which controls the selective reformation
of connections.

Similarly, Westerman (1965) removed different quadrents
of retina in adult Goldfish and cut the optic nerve.   After
regeneration of the optic nerve, he recorded electrophysiol-
ogically the retinotopic projection and discovered that the
projection from the retinal quadrent which was left intact
was restored with no extra quadrantic overlap on the tectum,

suggesting that there were no widespread tectal arborizations amongst regenerating axons.

After the removal of retina in newts, Cronly-Dillon (1967) reported that the restoration of retinotectal connections did not proceed in a uniform manner for all points of the retinal projection. The tectal representation of the central part of the retina was found to be normal, five months after the operation, whereas, the regenerating optic nerve fibres from the peripheral retina were more scattered but distributed roughly to the same position and showed larger multi-unit receptive fields. The author suggested that fibres coming from the peripheral retina branched over fairly extensive areas of the tectum as compared with the fibres projecting from the centre of the retina. Moreover, he further suggested that fibres and tectal cells which constitute the projection from the centre of the retina possessed a greater degree of specificity than those serving the peripheral region of the retina. The pattern of branching which was observed during early period of regeneration had gradually evolved to give more spatially restricted pattern of specific local connections when the animals were left to recover for a year. The last observation strengthens Sperry's (1963) prediction of branching nerve fibres during the early period of regeneration.

The observations of Stone (1930 and 1964) and Stone and
Ellison (1945); Twitty and Schwind (1931) do not support
Sperry's hypothesis directly.   These authors transplanted
the larval eyes between different species of Salamander e.g.
between Amblystoma triginum and Amblystoma punctatum or a
small eye from Triturus viridescens transplanted in place
of a big eye of Amblystoma punctatum.   Most of the animals
used were between 30-90 mm in length and some of them were
just prior to metamorphoses.   The replacement of a normal
eye with a large one resulted in the enlargement of
contralateral tectum of the host and the substitution of
an eye by a small eye of different species resulted in the
shrinkage of contralateral tectum (Harrison, 1929 and Twitty,
1932).   The size of optic tectum became adapted to the size
of the transplanted eye.   A return of vision has been
reported in these animals.   From these observations it
appears that regenerating fibres from the transplanted eyes
grew in larger or smaller numbers and that they made
connection with the tectum, resulting in normal vision.
These authors pointed out that visual activity in these
animals was not appreciably different from that of normals.
In those cases where a small eye was grafted in place of a
big one, one would expect a loss of efficiency in these animals
as the small eye will have a smaller number

of ganglion cells in the retina than will the big one. On
the contrary their results suggested that the optic nerve of
the grafted salamander eye selectively finds the proper brain
which must be similarly organised to restore vision.

To seek an explanation for these results, one should
re-investigate these findings electrophysiologically with
particular reference to any change in the magnification
factor (microns tectum per degree visual field). It is
quite possible that the tectum in some of these animals
was not polarised at the time of eye transplantation and the
new optic nerve fibres formed specific connections in a manner
similar to that in a normal animal. Another possibility is
that these results are a manifestation of central nervous
system plasticity in these animals.

Moreover, it is difficult to say with certainty what
would happen to the regenerating nerve fibres if they were
selective or predetermined to terminate at particular loci
in the tectum; would they bypass the experimentally ablated
area to terminate at their proper places?
GRADIENT HYPOTHESIS: Sperry's (1945 a), prediction that
the nasotemporal (anteroposterior) gradient might be shown
to be fixed separately and prior to the dorsoventral
gradient, has since been confirmed by Székely (1954);

Stone, (1960) and Jacobson, (1967). Székély (1954) showed
that although normal vision was found after the rotation of
the eye field in the medullary plate stage of Triturus,
reversed visual fields developed when the eye rotation was
done immediately after the closure of the medullary plate.
After rotating the anterio-posterior and dorso-ventral
axes separately, it was found that the functional specificity
was determined first along the antero-posterior axis.

Credence to the gradient interpretation has been given
by the recent experimental studies of Gaze et. al. (1963,
1965). These workers surgically formed double nasal or
double temporal retinae in Xenopus laevis embryos, which they
called "compound eyes". Embryos were operated between stage
30-32 (Nieuwkoop and Faber) when the eye was polarised in
nasotemporal axis and not polarised yet in the dorsoventral
axis (Székély, 1954 and Jacobson, 1967). Double nasal
compound eyes were made by replacing the excised temporal
half of the eye by a nasal half taken from another embryo.
Similarly, double temporal retina was formed by replacing
the excised nasal half by a temporal half. The visual
projection from the operated eye was mapped when the larvae
had grown into adults. These authors reported that in the
case of double nasal retinae, fibres from the nasal and
temporal poles projected to the caudal half of the lobe,

whereas, fibres from the middle part of the eye occupied the
rostral part of the lobe i.e. at a place where temporal fibres
normally terminate.   But in the case of double temporal retina,
the rostral part of the tectum was innervated by fibres from
the retinal poles and the fibres from the middle part of
the eye terminated in the caudal part of the lobe.   Each
hemiretina projected in this fashion over the whole tectum
covering a common area in mirror-image fashion.   The
magnification factor along the horizontal and vertical meridia
was calculated in both normal and compound eyes and it was
observed that the ratio of horizontal to the vertical factor
for the normal eye was 0.9 whereas, for the compound eye it
was 1.7.   There was a normal ipsilateral projection from
                                                 the
compound eyes; however, the ipsilateral projection in/normal
eye was abnormal which remained to be explained.   These
authors postulated the existence of a system of cross grad-
ients which are supposed to be active in the development of
retinal and the tectal specificity.   These results are com-
patible with the hypothesis that in embryo the pattern of
retino-tectal connections appear to be determined by a
system of gradients across the retina and the tectum in
which each retinal ganglion cell acquires an unique value
in the gradient system which is matched by a comparable value
in the tectal cells with which it connects.   If the retina

is reduced in size in the embryo at a stage before the gradients system is set up, a complete gradient is nevertheless established across the reduced retina and since the tectal gradients are normal, the fibres across the reduced retina will spread out to connect with the whole tectum. However, once the gradients have become established, halving the retina or tectum would not produce this result since each cell in the retina or the tectum had already acquired its unique values. If this is the process of spreading out of fibres over the whole tectum in the compound eye experiments, it could equally be possible that half of the tectum which receives optic nerves fibres develops to twice its normal size while the deafferenated half atrophies. (Gaze et. al., 1965, Sperry, 1965, and Jacobson, 1967).

In order to test this assumption, one would have to manipulate the tectum prior to its polarisation i.e. either to make a compound tectum or to reduce its size. However, one would not expect either total failure of development or atrophy of half the tectum after removal of half retina - as the tectum has other afferent connections besides the optic nerve terminations and the tectum does not totally degenerate after the eye removal in embryos (Larsell, 1931; McMurray, 1954).

It should be possible to transplant these compound eyes into normal animals and see what sort of pattern they formed.

This would probably tell us whether tectal overgrowth or
optic nerve spreading is occurring in the animals with
compound eyes.

Gaze and Jacobson (1963) mapped the retinotectal
projection of Rana temporária at various intervals from
23 to 247 days after the section of optic nerve with the
idea of looking for the time sequence of events during
regeneration. Four types of regenerated retinotopic
projections were classified in these experiments. The
earliest recordings gave a disorganised anomalous
regeneration i.e. grouped stimulus positions projecting
to large areas of tectum but with each half field
projected to the appropriated half of the tectum.
Animals which gave this type of projection were grouped
as Pattern I. Four animals gave a partially organised
projection in the anteroposterior axis of the retina only
and not in the other axis. This was called Pattern II.
In Pattern III there was a virtual restoration of the normal
retinotectal projection. Pattern IV projection was
obtained at longer intervals after nerve section. There
was partial or complete restoration of the normal map
together with an anomalous but retinotopically organised
projection to the wrong side of the tectum. The internal
order of the projection was similar to that of normal

ipsilateral projection. Based on the above results these authors suggested a certain sequence in the formation of connections by the regenerating optic nerve fibres in which Pattern I and Pattern II were stages in the process of recovery to normal pattern. To prove their hypothesis, these authors did try to record several times from the same animal but without any apparent success.

The differentiation and maturation of tectal layers proceeds in an anteroposterior direction (Kollros, 1953) and the first optic nerve fibres enter from the anterior pole of the tectum (Herrick, 1942 and 1948; and Leghissa, 1951). Moreover, enzyme activity is formed first at the anterior pole during development (Boell et. al., 1955). It is clear from these observations that the rate of growth is not simultaneous in every region of the tectum. The first optic fibres form connections on the rostro -lateral side of the tectum.

From these observations it appears that the pattern of regenerating optic nerve fibres resembles that of development. More data on optic nerve regeneration and developmental sequences is required before one can say with certainty how far this supposition is true.

There are discrepancies in the recent report of Burgen and Grafstein (1962) and Grafstein and Burgen (1964).

These authors studied visual responses electrophysiologically
to determine the pattern of retinal projection to the optic
tectum in newts, following regeneration after the removal of
retina, accompanied by removal of the pigment layer at the
back of the eye.   In four animals in which pigment layer
was not removed and the cornea was replaced in the original
position, the retinotopic projection was essentially normal.
In another set of experiments, the cornea was rotated at
different degrees, and a wide range of variation in the
pattern of projection was reported.   In some of these the
representation of the central part of visual field was
located in an abnormal position on the tectum.   In most
of these cases, the orientation of the representation of
peripheral part of the visual field was consistent with that
of the central field, regardless of whether the latter was
normal or abnormal.   In some cases, within a rotated
projection pattern they found a double localization of the
central area - one of these was in the normal position and
the other corresponded to the rotated projection.   These
authors suggested that their results were incompatible with
the hypothesis that a given retinal cell can make connections
only with a specific region of the tectum.   It has been
suggested (Gaze, 1967) that some of the peculiarities in
the results of Bergen and Grafsteins experiments may be due

to the mutual inductive effects between adjacent parts of
the regenerating retina.   Mutual inductive effect of two
parts of the retina was apparent in cases when after
rotation of peripheral part of the eye, there was not a
single case in which the peripheral field representation
was rotated while the centre was not.   Could these results
be dynamics of gradient organisation?

De Long and Coulombre (1965) demonstrated that
embryonic optic fibres in the chick terminate on specific
local areas of the tectum indicating that the local
topographic specificity of the tectum arises independently
of the retina or optic fibres.

The authors made a lesion in the retina, removing one
quadrant by electrocoagulation methods in chick embryos of
three, four and five days incubation.   By histological
techniques, these authors showed the presence or absence of
optic fibres in the tectum.   Retinal fibres cover the tectum
by twelve days of incubation, having started to enter the
tectum at six days.   Retinal ablation at the four or five
day stage showed a localised abnormal area without optic
fibres on the tectum, but no change in the tectum was
noticed if the retinal quadrants were ablated at the three
day stage.   These results indicated that the tectum becomes
specified at four or five days of incubation, at which stage

the optic nerve fibres have not yet entered the tectum; and
thus that the tectal specificity developed before the arrival
of optic nerve fibres. These authors further suggested that
"if either the retina or tectum, or both, lacked local
specification or were mapped by only quantitative gradients,
the remaining optic fibres would be expected to spread out
over the entire tectal surface".

The above mentioned observations are opposed to the
findings of Crelin (1952), Wiemer (1955) and Kirsche (1960)
where they demonstrated that the optic tectum did not become
functionally polarised before the optic nerve fibres grew into
it. Crelin (1952) succeeded in rotating the tectum on one
side in Amblystoma embryos before Harrison's stages 30 and
the animals recovered normal vision. However, beyond stage
thirty, it was found impossible to obtain a normal reimplan-
tation of the rotated tectum. Haemorrhage was a further
hazard. At this stage, the motor connections of the tectum
were visibly effected by the operation when the tectum was
examined several weeks later. Rotation of the lobe upto
stage 35 however, did not result in reversed vision. Thus
the lobe was not polarised before stage 35. Crelin concluded
that 'the differential properties possessed by optic axons
from the different retinal loci are believed to have served
as an organising influence on the formation of synaptic

connections within the rotated tectum'. He believed that
these properties were biochemical specificities carried by
each optic fibre into the tectum. As has already been
suggested, if the retinal fibres are prevented from entering
the tectum until an adult stage of development, the tectum
may remain undifferentiated with respect to the optic nerve
projection - but this was not the case in chick (De Long and
Coulombre, 1965).

Stone (1944 to 1964) observed that optic cups of
Amblystoma could be rotated or transplanted without
affecting normal vision if the transplantations were done
long before the optic nerve fibres reached the tectum. He
did not mention the stage of development at which rotations
were done. It was clear from his further observations that
the polarity of the retina was fully expressed in both axes
before the eye was connected with the tectum. Polarisation
and specification of the retina has been shown in detail in
Xenopus larvae (Jacobson, 1967 a). A normal retinotopic
projection was found when the eyes were inverted dorsoven-
trally and anteroposteriorly if the operations were done at
stage 28-29. Eye cup rotation at stage 30 resulted in
anteroposterior inversion of the retinal projection and the
rotation stage at 32-35 resulted in inversion of anteroposterior

and dorsoventral axes. These studies showed that the ganglion
cells were unspecified at stage 28-29; anteroposterior
polarisation of retina occurred at stage 30 and dorsoventral
polarisation occurred between stage 30 and 32.

The presence of such a stage in the chick when the retina
was not specified has been observed by De Long and Coulombre
(1965); this was the four days of incubation stage.

The difference between the results of Gaze et. al.
(1963 and 1965) and those of De Long and Coulombre (1965),
may be due to a difference in the embryology of the species,
e.g. a toad in adult life can regenerate optic nerve fibres
but the chick cannot; or it could be assumed that gradients
work in toads and not in chicks. The entire question of
gradients versus mosaic specifications cannot as yet be
decided. It is valid to assume on a gradient hypothesis
that a partial retinal ablation will result in no bare patch
in the tectum. A mosaic concept of specificity would presumably
be genetically determined (e.g. eye transplantation experiments
of Stone).

MODULATION OR RESPECIFICATION THEORY:-

Weiss (1924) proposed a hypothesis that all motor fibres
might conduct coded messages for all the muscles, with each
motor end-plate then picking out its appropriate component
according to its own specificity. Weiss (1936) argued that

most physiological concepts of central nervous system actively
assume that all neurons are alike, and that the significant
variations are in the electrical state of the membrane.   He
postulated (Resonance Theory) that all motor fibres carry a
wide variety of motor messages and that the individual muscle
selected their appropriate messages.   This statement was
disproved by Wiersma (1931), who demonstrated that any one
motor nerve only gave impulse when its particular muscle was
contracting.   Therefore, selection had to take place further
proximally, in the centres and if the ganglion cell could not
know in advance precisely with which muscle it would eventually
be connected, it had to learn after it had connected.   The
necessary information had to be transmitted directly through
the motor neuron itself.   Weiss modified his old concept of
resonance i.e. only those connections were functionally effective
which coincided with the original pattern, to a new process
of retrograde specification which he called modulation. Support
for 'modulation' came from the various experiments mentioned
below.

Weiss (1942) transplanted an extra eye near the ear of a
newt larva.   When this region in the normal animal is touched,
it does not give any blink response.   On the other hand, when
the extra grafted eye was touched, the normal eye gave a lid-
closure reflex.   Weiss postulated that axons growing into

the transplanted eye to innervate cornea had become respecified or modulated by the specific character of the corneal tissue and this allowed them to form the appropriate central connections for the reflex.

However, Székély (1959) showed that specific modulation by corneal tissue in Weiss's experiment was not a necessary postulate. Székély transplanted a limb near the gill in an urodele larva (Pleurodeles and Triturus). After metamorphosis, no corneal reflex was observed on stimulating the limb. However, after repeated cutting of the limb in the larval stage a regeneration blastema was formed and on its stimulation a corneal reflex was observed. After complete regeneration of the foot, the corneal reflex disappeared. Székély suggested that the pattern of discharge sent by the regenerating blastema may be similar to that of the cornea as both have a similar type of innervation.

Following electrical stimulation of the cornea, Kornacker, (1963), recorded post-synaptic potentials in the lateral part of the medulla at the level of abducens nucleus. He concluded that impulses of the finest fibres were monosynaptically transmitted to the dorsal end of the abducens dendrites. When two electrical stimuli were applied within short intervals to the snout nerve, similar

responses were recorded.   He postulated that the activity
of a sufficient number of fine fibres was essential to evoke
a corneal reflex and the diameter of fibres innervating an
extra cornea or a regenerating blastema could be reduced and
so resulted in a corneal reflex.

Miner (1956), described the mis-directed wiping responses
after rotation of the skin in Frogs - suggesting that this was
due to acquired specificity.   A Frog wipes his back with his
hind leg when the back is stimulated and wipes the belly with
his forelegs when the belly is stimulated.   Miner cut a flap
of trunk skin in midlarval stage tadpoles thus severing every
nerve connection.   After rotating the skin 180° she replaced
it back to front so that the dorsal end of the flap covered
the belly and the belly skin i.e. ventral end, covered the
back.   After metamorphosis, the frog wiped with the fore leg
on the belly after stimulating the back and with the hind leg
on the back after stimulating the belly.   Even the colour of
the grafted dorsal skin was like that of the belly and vice
versa.   Miner suggested that there was a local differentiation
within the covering skin and the neurons innervating the skin
had the specific quality of skin imprinted on them.   The
central processes of these neurons were postulated to
terminate selectively on central neurons with corresponding
specifications so as to permit these reflexes to occur.

However, one is not sure about the nature of the neural mechanisms involved in corneal reflex and in the skin graft experiments. Electrophysiological techniques are required to obtain information about the nature of these mechanisms.

It is important here to mention the findings of Sperry and Arora (1965), which suggest preferential peripheral reinnervation rather than myotypic respecification or modulation in the regeneration of nerve-muscle connexions in the oculomotor system of the cichild fish. These authors observed the rapid full recovery of normal eye movements after complete section of the main nerve trunk of cranial nerve III of Astronotus ocellatus either within the orbit or intracranially. These results indicated co-ordinated activation of the four extrinsic oculomotor muscles. After sectioning the individual muscular branches of the nerve and the transplantation of these into foreign denervated oculomotor muscles in different combinations, the recovery of abnormal muscle contractions was observed. These muscle contractions tended to be weaker than normal and were timed according to the original muscle from which the nerve branch was taken - and produced abnormal eye movements. These authors suggested that myotypic respecification effects failed to appear when individual nerve branches were separately inserted into foreign muscles. Less than normal vigour of

contraction obtained from the crossed nerve-muscle combin-
ations, when these did become functional, suggested peripheral
selectivity.   They inferred from their findings, the presence
of some kind of preferential chemoaffinity in the oculomotor
system that favoured restoration of the original over foreign
nerve-muscle connexions was suggested.

Restoration of the fin movement following regeneration
of the brachial plexus after surgical section has been shown
in a cichlid fish - Astronotus ocellatus (Mark, 1965).
After sectioning the brachial plexus completely on one side,
fin movement was restored three weeks later.   When the nerve
trunks from the plexus were sectioned close to the muscle they
supply and were implanted into antagonistic muscles, neuro-
muscular transmission was re-established but with unco-
ordinated fin movements which remained so.   It was shown
that implantation of the crossed nerve was successful and that
the adductor and abductor muscles were in fact supplied by
the antagonistic nerves.   These results suggested the
unlikeliness of central change or modulation in the return
of co-ordinated muscle action when regeneration of the
brachial plexus was allowed to occur unhindered.   Mark
suggested that the restoration of muscular co-ordination
must depend primarily on the selective re-establishment in

the periphery of a majority of correct nerve muscle

connections - and that specific selective reconnection of

nerve sprouts and muscle fibres can occur in cases where

each muscle cell is normally innervated by several nerve

endings. These results of Sperry and Arora (1965) and

Mark (1965) bring into question the validity of the con-

cept of myotypic respecification. It appears necessary

to reinvestigate the old findings concerned with res-

pecification or modulation. Evidence for the modulation

process has been shown to exist for those types of afferent

neurons which, unlike those of the retina, do not have their

cell bodies in the receptor periphery. (Eccles et. al. 1962).

This process of modulation which is an example of adaptive

adjustment, has never been shown to exist in the visual

system.

Considering all theories on the mode of connection of

optic afferents we may infer the existence of specific

differences amongst the retinal neurons and that these are

instrumental in establishing and restoring their orderly

projection and representation on the optic tectum.

To approach the question of selectivity in regeneration

of the optic nerve, instead of interfering with the input, it

is possible to interfere with the receiving end i.e. the

tectum. New experiments have been devised e.g. excision of

the tectum in young adult goldfish with immediate

reimplantation in normal orientation. If the specificity

mechanism lies in the tectal tissue, what would happen if

the tectum was rotated? Would it form a rotated map after

regeneration, if there was any regeneration at all?

In this way the early phase in the course of regeneration

of the optic nerve in the graft could be followed electro-

physiologically to see whether there were exploratory branching

fibres. Other answers to be expected from these experiments

will be:

1. How soon after the excision and reimplantation of the
   tectum does function return in the graft?

2. In which region does the first functional connection
   return and what is the mode of spreading of optic
   nerve fibres?

3. How precisely is the point to point retinotopic
   projection restored?

4. What is the fate of the tectal graft i.e. its anatomical
   architecture - is there any change in it? If so, does
   the graft restore itself to a normal morphological
   structure before function is returned? How much
   time does anatomical and functional regeneration take,
   if there is any?

METHOD OF MAPPING THE PROJECTION OF THE VISUAL FIELDS
ONTO THE OPTIC LOBES

Goldfish (Carassius auratus) from 6 to 10 cm. in length were used to map the visual field on the optic tectum. The animal was anaesthetized by immersion for a few minutes in aqueous solution (1/2,000) of MS222 (tricaine-methane) and then set up in a supporting dish of acrylic dental repair material moulded to the shape of its body. The gills were continuously perfused with fresh water, which was kept aerated in the fish's mouth throughout the experiment. The cornea was coated with liquid paraffin to prevent it from drying. The skin on top of the skull was removed with a scalpel and a window in the cranium was made with a dental drill to expose the optic tectum and cerebral hemisphere. The skull flap was removed. The membranes covering the tectum were removed by suction. The optic lobes were kept moist in a pool of liquid paraffin. After demedullation, 0.01 ml. tubocurarine was injected intramuscularly. The optic lobes were drawn

on co-ordinate graph paper with the help of a camera lucida. One cm. distance on the graph paper corresponded to 0.2 mm on the tectum.

The fish was positioned on the platform of a micro-manipulator with its left eye centred on an Aimark projection

EXPERIMENTAL METHODS

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and cerebral hemisphere. The skull flap was removed. The
meninges covering the tectum were removed by suction. The
optic lobes were kept moist in a pool of liquid paraffin.
After decerebration, 0.01 cc. tubocurarine was injected
intramuscularly. The optic lobes were drawn
on centimetre graph paper with the help of a camera-
lucida. One cm. distance on the graph paper corresponded to
200μ on the tectum.

The fish was positioned on the platform of a micro-
manipulator with its left eye centred on an Aimark projection

perimeter with a radius of 33 cm. (U.K. optical, Bausch and Lomb). By means of this perimeter a spot of white light of variable diameter (1-3-5-10 mm. in diameter on the arc) and intensity could be projected to any position in the fish's visual field.

The eye was centered on the perimeter by means of a narrow beam of light projected from the centre of the perimeter through a hole in the centre of the plane mirror lying on the axis of the perimeter and inclined at 45° to it. The position of the eye was adjusted so that the light beam entered the eye and was reflected back and could be seen reflected in the mirror as a glow emerging from the pupil. For mapping the retinotectal projection, the 10° spot was used and the luminance of light spot was kept at approximately 13 millilamberts. A separate light source illuminated the entire perimeter arc, when necessary, providing a maximum luminance of 6 millilamberts which could be reduced to zero by means of a rheostat.

As there is a pattern of blood vessels on the surface of the tectum the electrode could be positioned with reference to these and the centimeter grid of the drawing. Under vision, through a dissecting microscope, a

microelectrode could be guided by means of a micromanipulator
to each of the positions on the tectum corresponding to the
intersections of the grid on the drawing. The electrodes
used were made from glass micropipettes filled with an alloy
of Wood's metal and indium. The electrodes were tipped with
a ball of platinum 2-8 μ in diameter. The electrode was
lowered onto the surface of the tectum until electrical
contact was made.

Action potentials in the tectum, evoked in response
to a spot of light in the visual field, were recorded between
the microelectrode on the tectum and an indifferent electrode
in the body of the fish. The indifferent electrode was a
metal clamp holding the dorsal side of the body. The evoked
potentials were led through a cathode follower and a Tektronix
type 122 pre-amplifier to a Tektronix type RM565 oscilloscope
and to an audioamplifier and a loudspeaker. The H.F. cut-
off at the pre-amplifier was 10,000 c.p.s. and the coupling
time constant was 2 msec.

The position of the light spot on the perimeter was
adjusted to give the maximum response for each electrode
position. The shutter of the perimeter was operated by hand
and the duration of each stimulus flash was approximately
0.5 sec. Under these conditions the response consisted of
a burst of action potentials from several units. This

process was repeated with the microelectrode at 100 micron
intervals on the tectal grid. In this way it was possible
to obtain responses from 40-50 positions on one side of the
tectum.

Localised electrical lesions in the tectum were made to
test the accuracy with which the electrodes were placed.
The position of the lesions was checked histologically. The
electrical lesion was made by simply switching off the cathode
follower momentarily and then switching it on again. This
procedure was repeated 2-3 times till the visual response was
no longer heard. The current passed through the electrode
tip by this technique was sufficient to make a spherical
lesion 25-50 μ in diameter. The experiments were performed
in darkness with the fish's eye in the air.

TECTAL EXCISION AND REIMPLANTATION WITH OR WITHOUT ROTATION

After the fish has been anaesthetized with MS222, a
window in the skull was made with the help of a dental drill
and a scalpel so as to expose right side of the tectum.
Most of the dorsal side of the tectum was cut away with
ircidectomy scissors, the cuts extending down to the ventricle.
The excised tectum was lifted up and then reimplanted with
normal orientation or with 90° and 180° clockwise rotations.
The skull flap was replaced and a drop of "New Skin" (St.
Helens Lancs. England) or physiological adhesive-Isobutyl

Cyanoacrylate Monomer (Ethicon U.S.A.) was applied which formed
a thin film and kept the skull flap in position. The animals
were released in water and were allowed to recover from the
anaesthetic. These fish were then kept for a period ranging
from 12-368 days before they were used to map the projection
from the left retina to the right optic tectum.

In another series of experiments, most of the caudal
half of the right tectum was removed and the left optic nerve
was crushed. In some of the tectal reimplants, the optic
nerve of the contralateral side was also crushed. The left
optic nerve was approached via the orbit and transected with
a fine pointed pair of forceps. The outer sheath of the optic
nerve was left intact. Care was taken not to disturb the
blood supply of the nerve.

The method for mapping the projection of the visual field
after experimental alterations was as already described for
normal animals.

After completing the experiments, the brain was fixed in
Heidenheins' Susa. Serial sections were cut at 15 microns
and were silver impregnated by Holmes' method.

HISTOLOGICAL METHODS FOR THE DEVELOPING TROUT TECTUM

The eggs were obtained from brown trout (Salmo-fario)
and were fertilized by milt from brown trout at the fresh
water fisheries laboratory, Pitlochry, Perthshire, by Dr. Marr.

The fertilized eggs were brought to the laboratory immediately
and were released in a running fresh water tank, where they
were kept till 4 months after hatching. From 50 days after
spawning, eggs were fixed in following solutions daily and the
process was continued 120 days after hatching:-

(1) Nissl preparations

Whole heads were fixed in either formol-saline or
Bouin's fluid for 6-12 hours, embedded in paraffin wax,
serially sectioned at 2-5 μ and the sections stained with
cresyl violet or Gomori's haematoxylin - phloxine or
Heidenheins iron haematoxylin.

(2) Silver preparations

Heads were fixed in various modifications of Cajal's
fixative or in Heidenhein's Susa fixative for 12-24 hours
and were later impregnated by Cajal's Silver impregnation
techniques or in Holmes' silver method.   In each case brains
were embedded in paraffin wax and sectioned at 10-15 microns.

(3) Golgi-Cox preparations

Various modifications of this technique were applied
and the one found most suitable was that of Ramon-Moliner
et. al. (1964).   Best results were obtained when the tissue
was impregnated for 25 days.

(4) Fresh preparations

The optic lobes, freshly removed from the embryos, were

carefully dissected out in goldfish ringer (Robertson, et. al. 1963) to separate the tectum. Small pieces of tissues were then washed in this ringer solution and disintegrated by gentle pressure applied to a cover slip placed on the slide. The sides of the cover slip were smeared with paraffin wax to avoid drying. The preparations were examined immediately with a high power phase contrast microscope.

METHODS FOR ADULT TECTAL HISTOLOGY

Apart from Holmes' silver technique used for histological studies of the tectum of normal and experimental animals, various Golgi-Cox and Golgi-rapid techniques were employed. The successful Golgi-Cox technique has already been mentioned. After various trials with different proportions of Golgi-rapid fixative, the following technique has given the best results.

In anaesthetized fish, Goldfish Saline was perfused through the heart, quickly followed by the perfusion mixture (fixative) which consisted of 0.25% Osmium-tetroxide and 1.75% Potassium dichromate @ P^H 6.8 - 7.4 and 2.5% Sodium Chloride. The process was continued for 15-20 minutes. The brain was taken out and 3 mm. thick pieces were cut. An alternative method which also gave equally good results was by immersing the brain directly in the fixative. Impregnation of the tissue was done at 20-25° C., and the

volume of fixative was at least 50 times that of the tissue.

The fixation and or impregnation was carried out for 2 days.

The tissue was taken out and briefly washed with 0.75%

aquous Silver nitrate and was immersed in it for 2 days.

The whole process was repeated with 2 days in fixative and

2 days in Silver nitrate. Triple impregnation was also

tried out but double impregnation gave satisfactory results.

After a brief washing with distilled water, tissues were

dehydrated directly in a mixture of absolute alcohol 1 part

and ether 2 parts. The tissues were left in this solution

for 24 hours with 4 changes of fresh solution. Blocks of

the tissues were then transferred for embedding to the first

celloidin or LVN solution (celloidin - 30 gms; ether - 250 cc.;

absolute alcohol - 250 cc., after stirring add absolute alcohol

100 cc. and ether 450 cc.) for 10 days at -10° C. Second

Celloidin solution was made with 50 gms. of celloidin where

the tissues were left for five days followed by a third

Celloidin solution (with 80 gms. of Celloidin) for five

days, both at -10° C. Blocks were made in a cardboard

caster and hardened by immersion in Chloroform. 50-100 μ

thick sections were collected in 70% Ethanol and transferred

to 95% alcohol for 10 minutes followed by two changes of 10

minutes each in 100% Butanol. The sections were cleared in

cedarwood oil and were rinsed in Toluene before mounting.

Other methods employed for studying histology of tectal transplants at different stages after operations were as follows:-

Glees' modifications (Marsland, et. al., 1954) of Silver impregnation for paraffin and frozen sections was applied. Heidenhein's Iron Haematoxylin method was also used on the operated animals. Fresh preparation with a phase contrast microscope were also studied. An attempt was made to study mitosis, if any, in the tectal grafts by the following technique:-

0.2 cc. of 0.02%colchicine was injected peritonally into the experimental animal six hours before sacrifice. The tectal graft was taken out and dissected into small pieces. Tissue was disintegrated in a hypotonic solution (To 1 cc. of stock Isotonic solution added 7 cc. of distilled water) and left in it for fifteen minutes. After spinning down the tissue in a centrifugue, the hypotonic solution was drained off. The tissue was fixed in 3:1 absolute alcohol and glacial acetic acid for ten minutes. A drop of this preparation was poured on a pre-cooled slide and a drop of 2% aceto-orcein in 45% acetic acid was poured on the tissue and left for ten minutes. The tissue was squashed under thumb pressure applied on the coverslip. The sides of the

coverslip were smeared with vaseline.   Preparations were
studied immediately.

METHOD FOR TESTING THE OPTOKINETIC RESPONSES IN THE
DEVELOPING TROUT EMBRYOS

Optokinetic responses consist of the movement of the
fish's tail in the reverse direction, and sometimes of its
whole body towards the direction of the movement of a series
of vertical stripes passing horizontally across the animals
visual field.   The embryos were placed in a petri dish half
filled with water, on a stationary platform in the centre of
the drum 40 cm. in diameter which could be rotated horizontally
around the embryo.   Inside of the drum was painted with
alternate white and black vertical stripes 4 cm. broad.   The
drum was rotated manually at different speeds between 10-40
cm. per second.   One embryo from the stock was tested daily
after hatching until the first optokinetic response was
observed.

A few days before and after the appearance of opto-
kinetic responses, the embryos were used to study the appearance
of first visual responses by electrophysiological. techniques
already described.

COMPLETE CHRONOLOGICAL LIST OF EXPERIMENTAL ANIMALS

USED FOR MAPPING

(Approximate length for all Goldfish used was 7 cms.)

Abbreviations used :

Right tectum excised and reimplanted without rotation — A

Right tectum excised and reimplanted with 90° clockwise rotation — B

Right tectum excised and reimplanted with 180° clockwise rotation — C

Right tectum (caudal half) removed and Left Optic Nerve crushed — D

Right tectum excised on three sides only — E

Experimental No.	Type of Operation.	Days between Operation and Recording.
TT1	A	172
TT2	A	172
TT3	A	172
TT4	A	175
TT5	A	86
TT6	A	98
TT7	A	99
TT9	A	107
TT17	A	76
TT18	A	28
TT19	A	42
TT20	A	36
TT21	B	61
TT22	A	66
TT23	A	12
TT24	A	93

Experimental No.	Type of Operation.	Days between Operation and Recording.
TT25	A	64
TT26	A	82
TT27	B	28
TT28	A	87
TT29	A	88
TT30	A	112
TT31	A	76
TT32	B	79
TT33	A	89
TT34	B	104
TT35	A	94
TT36	A	148
TT37	A	182
TT38	B	168
TT39	E	39
TT40	B	177
TT41	E	44
TT42	D	52
TT43	D	79
TT44	B	194
TT45	A	239
TT46	A	94
TT47	A	108
TT48	A	366
TT49	A	167
TT50	A	144
TT51	A	145
TT52	A	111
TT53	B	171
TT54	B	172
TT55	D	114
TT56	A	115
TT57	C	124
TT58	E	181
TT59	A	92
TT60	B	77
TT61	D	90
TT62	D	97
TT63	A	97
TT64	A	98
TT65	C	91
TT66	C	91
TT67	D	91

EXPERIMENTAL RESULTS

ANATOMICAL STUDIES ON THE OPTIC TECTUM

The cortical region of optic lobe is called the optic tectum and the optic nerve fibres arborize in it. Stratification of the optic tectum is apparent on sections stained with Holmes' Silver or any other technique. The scheme of stratification had been rather confused because the earlier workers differed in their opinions as to the number of strata in the optic tectum of teleosts. Stieda (1873) recognised five layers, Van Gehuchten (1894) described six, Fusari (1887) and Neumayer (1895) described seven; P. Ramon and Ramon ɤ Cajal (1911) mentioned ten zones or strata beginning from the ventricular surface and counting towards the outer surface of the tectum. Huber and Crosby (1934) described six layers because it fitted into the description of tectal layers as seen in other vertebrates and because it was based on a functional interpretation of the layers. Leghissa (1955) divided the optic tectum into seven layers from an extensive study of teleosts. The terminology used in this study is adopted from Huber and Crosby (1934) and Leghissa (1955). Reference is made to Plate 4.

Stratum Opticum, A. (Zone 10 of P. Ramon and Ramon ɤ Cajal and Retinal strata of Leghissa). Right under the pia a thin layer contained the optic fibres which can be seen with the

silver or sometimes with Golgi preparations. In Golgi

preparations, optic terminals were observed. The optic

terminals resembled the endings found by P. Ramon (1891)

in the Lizard, and Lázar and Székely (1967) in the optic

tectum of Frog. The optic nerve fibres run horizontally

along the most superficial layer. These fibres start

breaking up into an inverted umbrella or a tuft-like

arborization (11). These terminal arborizations are seen

as deep as layer D. Superficial arborizations are smaller

in diameter than deeper ones. With vital staining, this

area is not seen at all. The vertical ascending axonal

terminals of the pear shaped neurons reach this layer.

The brush-like terminations of certain other afferents have

also been observed in this layer. All this mesh work was

stained particularly well in modified Golgi rapid preparations.

The diagonal fibres, which run from this stratum to the

lowermost layer and eventually end up in tori-longitudinalis

(Leghissa, 1955), have not been observed, either in Goldfish

or Trout.

The plexiform External plane (B).

This corresponds with the description given by Cajal.

Most of the unmyelinated retinal afferents terminate here.

The dendritic branches of the underlying pyramidal neurons

expand horizontally in this layer. A few dendritic terminals

of the pyriform periventicular cells also reach this stratum.
Horizontally orientated pyramidal cells, with branching axons
which run horizontally, have been observed in this layer, (9).
Their axons run horizontally for a short distance and bifurcate,
then further divide into finer branches with swollen endings.
The axon arises directly from the soma.   Leghissa did not
mention these cells at all, whereas, Cajal's diagram shows
their presence.   The presence of another type of cell has
been observed in this layer (10).   An inverted pear-shaped
cell whose soma is present in this layer, sends down a
vertical dendrite from which the axon arises and terminates
horizontally in layer D.   The axon breaks up into many
terminal arborizations.   However, the dendrites take a
peculiar course.   The vertical dendrites are rather short
and have been seen as far as layer D, whereas, the horizontal
dendrites run quite a distance.   A few branches of the
horizontal dendrites run apically and reach the outermost
layer.   The presence of this cell has not been observed by
any other worker.

Stratum gréisum Superficiale, C. (Seventh and eighth layer of
P. Ramon and Ramon y Cajal).   This zone constitutes a main
receptive layer of the tectum as it receives terminal fibres
of the optic nerve.   A few large cells can be found in the
innermost part of this layer including the large pyramidal

neurons (12). The dendrites of these neurons do not have spines. The apical dendrite goes down towards the ventricle and from the main dendrite, quite near the soma, arises the axon which after penetrating into the deeper layers, enters layer F and can be traced in a lateral direction for a considerable distance. These axons form the efferent fibres from the tectum. Relatively large basal dendrites with up to four main branches, leave the cell at its base, entering the outermost layers. They run up to the surface giving off several secondary and tertiary branches which bear spines.

Bipolar neurons (8) have also been observed in this layer. The axon emerges from the ascending and descending dendrite and reaches layer (F). The main descending dendrite gives off primary and secondary branches which ramify in layer E. The ascending dendrite reaches the outermost layer and has typical spines.

A few fusiform neurons (14) have been observed in this layer. The axon arises from the cell body and descends into layer (D) where it turns horizontally. A few collaterals were seen at the terminal end. The axon could not be traced for a longer distance. The dendrites have many secondary and tertiary branches.

Internal plexiform stratum, D. (Layer 6 and 5 of Cajal).
This layer is formed by the numerous terminations and

ramifications of the optic afferents and the dendrites
of deeper neurons.   Moreover, dendritic ramifications of
various tectal neurons terminate here.   The dendritic
arborizations of the pear-shaped periventricular neurons
(1) and the pyramidal neurons of different layers terminate
here.   A few branches of the deeper multipolar neurons (6)
and terminal bushes of the optic nerve fibres arborize here.
A few bipolar and pear-shaped neurons (8 and 13 respectively)
are also seen.   The axons in both cases arise from the cell
body and run deep vertically down to the level of layer F
and finally run horizontally.

Internal grey layer, E. (Central grey zone of Leghissa, and
zone 4 of Cajal).   Pyramidal and multipolar neurons are found
in this layer.   The main dendritic tree of the pyramidal
neuron goes towards the outer layers.   A few dendrites expand
in layers C and D.   Bipolar bidendritic type of neurons (7)
are seen in this layer.   Most axons of these neurons leave
the tectum as efferent fibres (layer F).   Multipolar neurons
(6) have varied forms.   Most voluminous ones are provided
with a few strong dendrites, and others have numerous thin
dendritic branches which stop at short distance and ramify
in layer E.   Some of the dendrites of the big multipolar
neurons reach as far as layer C.

Stratum album centrale, F. (Zona bianca profunda of Leghissa
and Zone 4 of Cajal). These fibres are the axons of
pyramidal and pear-shaped multipolar and periventricular
neurons. In transverse sections, these fibres appear to
run laterally and form intertectal comminures. Laterally
orientated pear-shaped monopolar neurons (5) are seen in
this layer. The axon arises from the main apical dendrite,
running deeper and parallel to the outer extent of layer G.
A few dendritic branches go as far as layer E. They have
T-shaped bifurcations. A small pear-shaped vertically
orientated neuron (7) extends its dendrites up to layer E.
The axon arises from the main apical dendrite which bends
downwards and runs deep. At the level of its cell body it
bends laterally and joins the efferent fibres.

Grey periventricular zone, G. (Zone 2 of P. Ramon and Ramon
y Cajal). This layer consists of numerous small rounded
pear-shaped and pyramidal cells which are loosely packed.
In the small pyramidal neurons (1) the axon arises usually
from the cell body, opposite to the apical dendrite, and
after a short run terminates in the same layer or sometimes
it is observed running laterally. The apical dendrites run
vertically and reach layer D where the collaterals ramify.
The small pear-shaped neurons also extend their apical dendrites
outwardly up to layer C. In both these cases the apical

dendrites are stout and do not divide till they reach the
outer layers. Most of the axons form bundles of fibres
which leave the tectum at the lateral edge. Lining the
optic ventricle are small fusiform shaped cells which are
seen very rarely with Golgi techniques. A long straight
process extends out from their opposite pole which
bifurcates into three or four branches in the outer zones
of the tectum. They have a feathered appearance in the
Golgi sections. Their surface is covered with thick tiny
pointed twigs. These are the ependymo-glial cells (4).
In plate 4, these have been shown to start in the middle
of layer G. Presence of this layer has recently been
shown with Golgi and electron microscopical studies of the
teleost optic tectum (Kruger and Maxwell, 1966).

DEVELOPMENT OF THE TECTAL NEURONS

The brown trout (Salmo-fario) has been used for the
present study. This section will deal only with neurons
stained with Golgi-Cox techniqe. No stratification of the
tectum can be seen at the time of hatching (which started
71 days after spawning, when the embryos were kept in
running water at about 8° - 10° C. in the laboratory).
In 80 μ thick Golgi stained sections of recently hatched
embryos, a few cells are seen (Plate 1, 1,2,3,4). These
cells lie in the white zone which is very thin compared to

the underlying cellular zone. No optic nerve fibres have
been observed at this stage. A few of the stained cells
are either circular and without any processes (Plate 1,3),
or with a few processes which are difficult to designate as
dendrite or axon (Plate 1, 1 and 2). Very few cells have
been observed in which dendrites were easy to identify
(Plate 1,4). The dendrites are coarse and twisted. Near
the distal ends of dendrites there are occasionally nodular
swellings (pseudo-spines). At this stage the cell body is
oblong and spindle shaped (Plate 1, 1 and 2).

Seven days after hatching, many cells have developed
dendrites and axons and have started taking particular forms
and shapes. A small pyramidal neuron (Plate 1,5) extends
its apical dendrite towards the outer margin of the developing
tectum. The apical dendrite divides into two near the outer
surface and bears typical dendritic spines. A very small
axon arises from the perikaryon. Another pyramidal cell
(Plate 1,6) whose body lies a little bit deeper that the
previous one extends the apical dendrite in the same way as
No. 5 and its axon, which is thin and smooth, runs downwards
into the deeper layers. Both these cell types have basal
dendrites without secondary branches. A few ependymo-glial
cells have been seen (Plate 2,17). A bipolar cell body
(Plate 1,7) present quite near the inner cellular zone, extends

its main dendrite outwards towards the outer layer where it gives secondary branches laterally which form the exterior plexiform layer. The axon arises from the main apical dendrite and runs laterally. The basal dendrite goes towards deeper layers. A multipolar cell (Plate 1,8), situated quite near the ventricle, shows dendrites which have many secondary branches. The axon arises from the cell body. A few optic nerve fibres can be seen at this stage, reaching the outer layers. Their mode of expansion is difficult to trace as yet.

Fourteen days after hatching, most of the dendrites look smaller. A few cells with thick dendrites can still be seen (Plate 1, No. 9 and 10). A laterally orientated cell body (Plate 1,9) which extends its dendrite laterally, is seen embedded in the outer retinal fibrous layer. The axon arises from the opposite pole and ramifies laterally. A small pyramidal cell with thick dendrites (Plate 1,10) lies beneath the retinal fibrous layer. Another laterally orientated cell (Plate 1,11) with comparatively thin dendrites lies beneath the retinal fibrous layer and extends its axon towards upper layers.

Twenty five days after hatching, a pear-shaped cell (Plate 1,12), situated in the deep cellular layer, extends its dendrites to the surface. The axon arises near the soma.

A large pyramidal neuron (Plate 2,13) in the outer white
zone, quite near the surface, extends an apical dendrite
outwardly which gives off secondary branches laterally.
The apical dendrite then extends further outwardly and
gives off another pair of lateral branches which give rise to
secondary and tertiary branches.   The axon arises from the
primary lateral branch and runs towards the deeper layers.
A pyramidal cell in the middle of the white zone (Plate 2,
14) gives of many stout basal dendrites near its perikaryon.
The apical dendrite gives off secondary branches.   The axon
which arises from the cell body, runs obliquely towards the
deeper layers and has been traced for quite a long distance.
Just near the surface lies a horizontally orientated cell
(Plate 2,15) whose soma is serrated.   The axon runs
obliquely.   Lying in the same plane as No. 14, is another
horizontally orientated cell (Plate 2,16) with very thick
dendrites, one of which runs laterally.   The axon is
very thin and arises from the opposite pole of the apical
dendrite.   Deeply situated in the lower layer is a large
ganglionic cell (a cell with numerous dendrites arising from
its soma; Plate 2,18) which extends its dendrites inwardly
and towards the surface.   A few dendrites bear typical
pseudo-spines.   The axon is thin and arises from one of the
ascending dendrites.   It turns round and goes into the deeper

layers.

Thirty days after hatching, a small ganglionic cell (Plate 2,19) has been seen in the outer layers of the tectum. Dendrites around the cell body are thick to start with and then abruptly break up into a large number of complicated terminal branches. The axon is small and runs towards the deeper layers.

Fifty days after hatching, many optic nerve fibres are seen entering the tectum and a few terminal branches have also been observed. From the middle of the outer white zone arises a small pear shaped neuron. (Plate 2,20). From the cell body an apical and enormously developed dendrite, thick at its base, extends for a long distance towards the surface and breaks up into finer secondary and tertiary branches. The axon is thin and arises from the opposite pole, runs into the deeper layers.

Fifty six days after hatching, many optic nerve fibres are seen on the outer surface of the tectum (Plate 3,21) where a number of dendrites of different neurons reach. A small pyramidal neuron of the outermost layer, orientated dorsoventrally (Plate 3,22) extends its thick dendrite towards deeper layers. The axon arises from the deeper branches of the dendrites and ramifies in layer D. A few basal dendrites give secondary branches and have "Pseudo-spines". The feather-like ependymoglial cells can be seen

at this stage.

Seventy two days after hatching, there is a tremendous increase
in the length of dendrites.   The cell body nearly attains its
maximum size, the terminal arborizations increase in number
and the lateral branches are greatly extended.   A vertical
bipolar neuron (Plate 3,24) has all the characteristics of a
mature neuron.   Most of the dendrites have secondary and
tertiary branches and many pseudo-spines on their collaterals.
The axon arises from the main apical dendrite, bends sharply
and goes into the deeper layers.   Even at this stage there
are a few maturing neurons (Plate 3,23).   This is a small
pear-shaped neuron with thick apical dendrites which suddenly
divide into finer branches.   A few pseudo-spines are seen.

At hatching and up to fifty six days after hatching,
the contour of the cell body and dendrites is uneven.   The
dendrites are comparatively simple but thickened at the basal
portion and their course is twisted.   Near the distal end
there are a few branches and nodular swellings.   In matured
neurons, the cell bodies are smooth and have a specific shape,
depending upon the types of neurons.   Their dendrites are
increased in number and length and their course becomes
comparatively straight.   On their surface numerous small
lateral buds or "pseudo-spines" appear.

THE DEVELOPMENT OF THE OPTIC TECTUM OF A TELEOST (SALMO-FARIO)

Starting from thirty two days after spawning, trout eggs were immersed in different fixatives and sections were stained with methylene blue, Heidenhein's Iron haematoxylin and Gomori's haematoxylin and Phloxine for histological analysis. A day-to-day study of the development of optic tectum in Trout was made. There was no sign of the tectum 46 days after spawning. 48 days after spawning or 22 days before hatching (Plate 4A,1), the first sign of the outer white zone of the tectum appeared on the lateral side of optic lobes. During development the tectum undergoes important morphological changes. The total thickness of the tectum increases tremendously along with the thickness of the grey inner cellular and the outer white zone./ The Table shows how the thickness of these various zones becomes modified during development.

TABLE

Age of the animal in days	Total Thickness of the tectum (IN mms.)	Thickness of the outer white zone (IN m ms.)	Thickness of the inner grey zone (IN m ms.)
Before hatching			
22	6.2	0.5	5.7
12	8.1	1.1	7.0
9	9.0	1.7	7.3
7	9.7	1.9	7.8
5	9.0	2.0	7.0
2	9.6	3.0	6.6
After hatching			
0	9.6	2.1	7.5
6	11.0	3.5	7.5
13	10.0	3.5	6.5
22	9.5	4.0	5.5
27	11.0	4.0	7.0
32	10.5	4.5	6.0
37	11.0	5.5	5.5
47	11.2	5.4	5.8
68	10.0	5.3	4.7
77	10.5	6.0	4.5
91	10.8	6.7	4.1
98	11.0	7.2	3.8

Thickness was measured in wms. from the photographs whose enlargements were identical. Slight variations in the total thickness of the tectum is attributed to the different growth rate of individual embryos. The measurements were made from the transverse sections, at the level of the 4th cranial nerve. There is a progressive increase in the thickness of the white zone. Between 32 and 37 days stage, a tremendous decrease in the grey zone is noticed. The thickness of the grey and the white zone is almost equal in the 37 days embryo (Plate 6,13). From this stage onwards, the white matter grows rapidly, whereas, grey matter goes on decreasing. 98 days after hatching (Plate 6,18) the tectum is almost like a normal adult one, with all the layers visible. From these findings, it seems that 22 days before hatching, the tectum is composed of undifferentiated neuroblasts with practically no white zone. The white zone appears 48 days after spawning. Few mitoses are seen in the grey zone during development. Decrease in the thickness of the grey periventricular zone is attributed to the constant movement of differentiating neuroblasts outwardly towards the white zones.

Twenty two days before hatching, the outer white zone
is hardly distinguishable; no nerve cells are seen in it
(Plate 4A,1).   Twelve days before hatching, a few migratory
neuroblasts have started invading the outer white zone (Plate
4A,2).   The right hand side of figure 2 (Plate 4A,2) shows
a cell (presumably bipolar) which is horizontally orientated.
A few other cells are also pushing themselves towards the
surface forming the outer layer; this becomes quite evident
five and two days before hatching (Plate 4A,5 and 6 respec-
tively).   All these cells are orientated towards the white
zone.   Nine days before hatching, the first optic nerve
fibres are seen entering the tectum in the anterior region.
Five and two days before hatching, the number of nerve cells
which have migrated into the white zone has increased
tremendously, although stratification of the tectum is not
very clear except for the outermost layer.   The number of
neurons in the outer white zone is even greater in the
embryos which were fixed on the day of hatching (Plate 5,7).
The thickness of the outer layers of the tectum increases
not only due to the different cells migrating into it, but
also from the different afferent fibres reaching in it i.e.
optic nerve fibres etc.

Six days after hatching, the neurons of the deeper layer
of the tectum are seen forming different layers.   Some

multipolar and pyramidal neurons of the deep white zone were
identified lying quite near the grey periventricular zone.
Coming out from the underlying grey periventricular zones
are small white fibrous extensions which, presumably are
the neurites of these cells.   Figure 9 (Plate 5) indicates
the presence of deep pyramidal cells which become very clear
and distinct in figure 10 - (twenty two days after hatching).
It appears that the layers of the outer white zone start
differentiating on about the 22nd day after hatching (Plate
5,10).   The number of neurons in the external white zone
goes on increasing continuously and they take their definitive
positions by 32 days after hatching.   Most of the layering of
the tectum is evident at this stage.   Thirty-two days after
hatching (Plate 5,12) many fibrous bundles are seen emerging
from the underlying cellular area.   Thirty-seven days after
hatching, when the outer white zone is almost equal to the
inner grey periventricular zone in thickness (Plate 6,13),
cell migration was not observed (see section on neuroblast
migration) and it is presumed that cells in the outer zone
mature in their shape and size progressively from this stage
onwards.   Figures 14, 15, 16, 17 and 18 (Plate 6) show the
progressive decrease in the thickness of the grey periventricular
zone.   It is clear from figure 18 (Plate 6) that the size of
the outer cells over a period of time have increased
significantly.

Various changes in the thickness of the tectum and that of
the outer white and inner grey zone are shown in the graphs
1 and 2.

In order to see whether or not there was any increase
or decrease in the cell population of grey periventricular
zone during development, relative cell counts in different
stages were done.   The results are tabulated below.   Cells
were counted, under a standard grid.   All the cells within
one strip of the grid were counted.

Photograph No.	Days before hatching.	No. of Cells per Unit Area, of the grey periventricular zone.
1	22	74
2	12	76
3	9	85
4	7	74
5	5	65
6	2	76

Days after hatching.

7	0	91
8	6	95
9	13	90
10	22	78
11	27	88
12	32	85
13	37	72
14	47	88
15	68	69
16	77	78
17	91	89
18	98	71

It appears from these results that although the width
of grey periventricular zone varies in a certain way, the

packing density in this zone does not differ considerably
during development. The thickness of the grey zone
decreases during development, partially because the cells
differentiate and move out into the white.

MIGRATION OF NEUROBLASTS IN THE DEVELOPING OPTIC TECTUM

Phase contrast study: Tissues, freshly taken from the
developing optic tectum of a Trout (Salmo fario), were
studied directly under the phase contrast microscope.
Embryos were examined weekly from seven days after hatching
to thirty days after hatching. It was clear from the
preparations which were examined that the tectum was com-
posed of a great number of fine processes, lying roughly
parallel to each other (Fig. 21). These processes were
followed for a long distance. They originate from ovoid
cells (Fig. 21). In the early stages, these processes
seem to originate from bipolar cells, lying near the grey
cellular zone (Fig. 19). These processes often contained
an oval cytoplasmic mass (Figures 19 and 21). In the later
stages of development, the oval mass extends its processes
only in one direction (Fig. 21). In this particular case,
the oval mass was smaller in size. The process extended for
quite a long distance beyond the small oval mass. Between
these processes, there were present other big rounded cells,
but they did not have any connection with the processes.

Some of these latter cells have their own processes which are very small. Twenty two days after hatching (Fig. 20), the outer white zone contained migratory cells in which the differentiating cell was still attached to its parent cell. The differentiating cell has developed a number of dendrites. The nucleus of the parent cell as well as that of the differentiating cell was quite clear. The parent cell was oval in shape.

OPTOKINETIC RESPONSES IN THE DEVELOPING TROUT EMBRYOS

The earliest body movements which were observed, even before the embryo was hatched, became well-integrated swimming movements of the body after hatching. The embryos did not give any optokinetic response till later during the development. Weekly tests for optokinetic responses were performed. The first optokinetic responses in the developing trout were observed between thirty seven and forty two days after hatching. The occurrance of optokinetic responses at this stage corresponds to a stage in the development of the tectum where the size of the outer white zone was equal to that of the underlying grey periventricular zone (Graph 2).

These embryos did not give any visual responses at this stage, when tested electrophysiologically by placing an electrode on the tectum and stimulating the eye. Weekly

tests were performed to note the onset of first visual responses. It was in the later stages of development (i.e. between 75-80 days stage after hatching) that visual responses were first observed electrophysiologically.

RESULTS OF MAPPING THE PROJECTIONS OF VISUAL FIELDS ONTO THE
OPTIC LOBES OF NORMAL GOLDFISH

The representation of the normal visual field on the optic tectum was successfully mapped in ten Goldfish. In four Goldfish, the electrode positions were not placed in serial topographical order and the person who found the visual field positions did not know the electrode position on the tectum. This "blind" mapping was done mainly to check the accuracy and repeatability of the normal map where the electrode is placed in a particular sequence. The electrode was lowered to the tectal surface and advanced into the superficial layers by means of a micromanipulator to a depth at which maximum responses were encountered to movement of a flash light in front of the eye. Then the optimal stimulus position was sought, using a 2° spot of light projected out on the perimeter arm. For each electrode position on the tectum there was an area 2° - 20° in diameter in the visual field, from within which the 2° stimulus evoked a response. Within this receptive field there was a position at which the response was clearly maximal. This position was located by varying the size of

the stimulus and the depth of the electrode. The position
of maximal response was determined partly be viewing the
oscilloscope trace, but mainly by subjective evaluation of
the loudness of the audio-amplified responses. Records were
made only of the position of the stimulus in the visual field.
Most of the responses consisted of a group of action potentials
from many units. No particular attention was paid to latency,
amplitude or duration of the responses.

PROJECTION OF THE LEFT VISUAL FIELD ONTO THE RIGHT OPTIC TECTUM
OF A NORMAL GOLDFISH

The retino-tectal projection from the upper half of the
left visual field to the dorsal surface of the right optic
tectum is shown in Fig. 22 (From Jacobson and Gaze, 1964).
The projection from the lower half of the visual field to
the lateral surface of the tectum has not been mapped in
any case.

The electrode positions on the right optic tectum and
their corresponding receptive fields for the left eye, when
the electrode was not placed in a systematic order, are shown
in Figs. 23 and 24. The numbered points on the left visual
field are shown corresponding with the positions of the
electrode on the surface of the right optic tectum.

The topographical scheme of this projection is as
follows:

The superior visual field projects to the medial part of the
tectum, the nasal field to the anterior part of the tectum,
and the temporal field to the caudal part of the tectum.
All receptive fields are approximately of fairly uniform
size.   The distance between two points in the visual field
is relatively regular.   Thus there is no evidence of change
in the magnification factor from one part of the field to
another.

The topographic relationship of the left visual field
to the contralateral optic tectum in non-sequential or
"blind" mapping is essentially similar to that of sequential
mapping (Fig. 22).   At the end of the experiments, the
electrode was placed on any position and the position in the
visual field found was always the same.

RESULTS OF MAPPING THE LEFT VISUAL FIELD ON THE RIGHT OPTIC
TECTUM FOLLOWING EXCISION AND REIMPLANTATION OF THE OPTIC
TECTUM WITH NORMAL ORIENTATION

A part of the right optic tectum was excised and
reimplanted with normal orientation in over 500 young adult
Goldfish (Size 3"). Most of the rostral half of right
optic tectum was removed with the help of iridectomy
scissors; the cuts extended down to the ventricle. The
animals were allowed to recover from the anaesthetic.
After some 1-12 months the fish was then used to map the

retino-tectal projection to the operated tectum. Most

such experimental animals did not survive. Almost 25%

of them died within a week of the operations, probably due

to haemorrhage and brain injuries, but most deaths occurred

3-4 weeks following the operations, due mainly to a fungal

growth on the replaced skull. However, 67 animals which

survived this period were used for mapping the projection

on the graft.

After mapping the retino-tectal projection in these

animals, three months after the operations, the brain was

fixed in Susa for histological studies. 15 μ sections

were impregnated by Holmes' Silver method. A detailed

histological analysis of the serial sections of the right

tectum was made to find out the exact extent of the tectal

graft. A reconstruction of the whole of the tectum was

made by plotting every section on graph paper. Each

section of the tectum was divided into four regions.

Fig. 25 shows the details of the tectal and graft recon-

struction. The distance from the sagittal line to the

medial edge of the tectum was measured (a), also the distance

between the medial edge of the tectum and the beginning of

the graft (b), the width of the graft (c) and the distance

between the lateral edge of the graft and the lateral edge

of the tectum (d).

The area of the reconstructed graft (Fig 25 bottom) and the tectum was then adjusted to make it conform to the area of the tectum as drawn at the time of recording. In this way, the exact extent of the graft in relation to the tectal area was reconstructed (Fig 26 left). This particular reconstruction of the graft is of the animal TT9 (Fig. 26) which was used for mapping the retino-tectal projection in the grafted region, 107 days post-operatively. There is a restitution of the normal visual projection over the area of the graft. Electrode positions on the tectum are marked (1-28). A composite diagram of the reconstructed graft is seen on the upper half of the Fig. 26. The stimulus positions of the visual field, shown in the lower circle, correspond to the tectal electrode positions.

Tectal graft responses to visual stimulation were recorded in almost all experimental animals included here, showing that some degree of optic nerve regeneration had occurred. In five fish, no tectal response was recorded. In both normal and experimental animals the responses consisted of bursts of action potentials from many units.

As the degree of restoration of retino-tectal map differs greatly from one experimental animal to another, it was not possible to categorise them in different groups.

The earliest mapping done was 36 days after tectal

reimplantation (Fig. 27A TT20). Very few points in the
grafted tectum gave definite visual responses. Small
circles on the right optic tectum represent the electrode
positions which did not give any visual response.
Electrode position 15 which is within the graft and is encircled,
represents the position at which an electrocoagulated lesion
was made. This lesion was detected in the histological sect-
ions and helped in reconstructing the graft. Electrode
positions such as 14, 15, 20 and 34 which are situated near
the lateral edge of the graft, indicate clearly that
regeneration or visual restoration starts from the lateral
towards the medial edge of the tectum. The orientation of
the positions in the visual field which gave responses is
similar to that of normal projection pattern.

Fig. 27B (TT19) represents a retino-tectal map, 42
days after tectal reimplantation. This was the earliest
occasion after operation when all electrode positions in the
graft gave visual responses. The electrolytic lesion (17)
was traced histologically. There is a slight indication of
abnormal projection between two rows particularly at the
medial edge of the graft. The rows slightly overlapped
one another instead of being separate at the peripheral end
of the dorsal visual field.

Fig. 28A (TT25) also shows a slight overlap of rows in

the dorsal visual field. The projection in the naso-
temporal axis of the field showed normal organisation.
This animal was recorded 64 days after the tectal reimplant.
A few points within the graft were not localizable. An
electrolytic lesion at electrode position 15 was traced
histologically. Fig. 28B (TT22) showed the normal
organisation of the projection in both nasodorsal and
temparo-dorsal axis of the visual field. This animal was
recorded 66 days after the operation. A few points in the
tectum did not respond to the stimuli. An electrolytic
lesion at position 7 was seen histologically.

In an animal recorded seventy six days after the
operation, very few positions of the electrode gave visual
responses, (Fig. 29A, TT17). An electrolytic lesion at
position 14 was traced histologically. It appeared that
the retino-tectal projection map from the graft did not
recover at all. The histology of the graft showed lack
of organisation. The retino-tectal projection outside the
graft was normal.

Eighty two days after the tectal operation, most
positions in the graft gave responses which were normally
organised. An electrolytic lesion placed in the tectum
was found. (Fig. 29B, TT26).

Eighty six days after the reimplantation (Fig. 30A, TT5),

where most of the tectum was involved, the graft did not give any satisfactory map. The antero-lateral region of the graft gave a few responses. No response was picked up from the posterior part of the graft. Slight overlap of lines at the dorsal visual field was seen.

Eighty seven days after the operation (Fig. 30B, TT28) the visual projection was nearly normal, although a few points in the graft did not respond to the visual stimuli. It is very difficult to account for the position 6 in the visual field.

With the "Blind" mapping techniques, the retino-tectal projection in an animal 89 days after operation was more or less normal (Fig. 31A, TT33). Although the lines in the visual field were not exactly parallel to each other, the orientation of these lines was nearly normal. All points recorded in the tectal graft gave visual responses. Four electrolytic lesions were made which were traced histologically. Electrolytic lesions were made after the mapping was finished, at the maximum response depth.

Ninety four days after the operation, a similar type of pattern to that in the previous animal was found. (Fig. 31B, TT35). Only one electrolytic lesion was made which was seen histologically.

Figure 32 (A and B) shows the results of 2 animals recorded

94 and 111 days respectively (TT46 and TT52) after the tectal reimplantation. Virtually normal retino-tectal projection is seen in both cases. One crooked line in Fig. 32A (TT46) could be due to an occasionally misplaced stimulus position, which caused the first row to cross over the second one. An electrolytic lesion in Fig. 32A was seen histologically. These results could have been obtained from normal animals. Comparing the lines in the visual fields, it appears that lines passing through the graft and outside are almost alike.

One hundred and forty four days (TT50) post - operatively, a few points in the retino-tectal projection overlapped the normal rows (Fig. 33A). Apart from points 15 and 23, the projection was like normal. No electrolytic lesion was made. On the other hand, 148 days after operation (Fig. 33B, TT36), very few points in the graft gave visual responses. A few points on the lateral edge and the rostral side of the tectum gave responses which indicate that regeneration in this case probably started very late. Outside the graft the retino-tectal projection was normal.

One hundred and sixty seven days after the operation (Fig. 34A, TT49), a few crooked lines with slight overlap are still evident, otherwise a normal visual projection is

present. Figure 34B (TT44) represents an almost exact normal projection and so far the best, within and outside the tectal graft. No better projection could be expected. An electrolytic lesion made at electrode position No. 5 was traced histologically. This projection was mapped 194 days after the operation.

Figure 35 (A and B) represents the results of long term experiments in which most of the available dorsal tectum was removed and reimplanted. These animals were recorded 239 and 366 days after the operations respectively. In the animal recorded 239 days post-operative, many points in the tectum did not give visual responses. The orientation of the visual projection was similar to normal but very few points were recorded (Fig. 35A). In the animal recorded 366 days after operation, although most of the points were recorded, the projection was still not normal. Twisting and crossing over of lines was evident. The size of the graft at the time of recording was very much reduced as compared to that at the time of operation. An electrolytic lesion was checked histologically.

Three things emerge clearly from these observations. Firstly: that most such operations do not work i.e. even after three months, tectal grafts do not give visual responses. Secondly: depending upon the size of the graft, where-ever

the graft forms connections with the normal area, normal
retino-tectal projection returns in the graft.

Thirdly: if the graft is very large, regeneration takes
much longer for the restoration of the retino-tectal map.
Finally, it is evident that regeneration of retino-tectal
projection within the graft starts mostly from the antero-
lateral side towards the medio-caudal.

Do these responses which appear to come from the graft,
actually come from it or is it the activity of units recorded
over a distance coming from the surrounding normal tissues,
especially in the smaller grafts? In order to test this the
following experiments were performed.

Recordings were done immediately after making a three
sided cut in the tectum, medial, rostral and lateral and
also in other orientations, and immediately after tectal
reimplantation. No responses were obtained from the
reimplants or within the three sided cut although the tissue
surrounding the graft and the three sided lesion behaved
normally. A few animals with three sided lesion were
recorded 39 and 44 days after the operation. In these
experiments, only a cut extending down to the ventricle was
made and to ensure that the tissue was separated from the
surroundings, the flaps were lifted up at the edges. The
animals were allowed to recover in the way already described

and the retino-tectal mapping was done later. When the
retino-tectal projection was mapped 39 days after the
operation (TT39), the situation was very different from that
of the reimplants. Almost no visual responses could be
recorded within the affected rectangle. The area of no
response extended further back posteriorly. Only two
points within the affected area were visually localized
and they were situated on the anterior most edges of the
lesion suggesting that regenerating optic nerve fibres have
started growing into the affected part. The area surrounding
the lesion gave normal localizable responses (Fig. 36A).

In another animal of this series, the retino-tectal
projection returned almost to normal, when recorded 44
days after the lesion (Fig. 36B, TT41). Except two points
(6 and 22) in the visual field, most of the points were
properly organized. Electrode positions at points 18,
23 and 27 did not give any response. This last observation
can be compared with the tectal grafts where regeneration
within the graft appears to start from the lateral towards
the medial edge of the tectum.

If visual recordings from the graft were actually coming
from outside it, one could not account for the normality of
the map that was obtained. Hence it is concluded from these
observations that responses actually were recorded from the

grafts and that grafts, some how or other, have formed

connections with the surrounding normal tectum.

RESULTS OF MAPPING THE LEFT VISUAL FIELD ON THE ROTATED OPTIC

TECTUM FOLLOWING REGENERATION

If part of the specificity mechanism lies in the tectal

tissue, what happens if the tectal graft is rotated?   Do

the ingrowing nerve fibres form a rotated map over the graft?

Two different sets of experiments were performed to answer

these questions.

1.   Optic tectum was excised (in similar manner to normal

     reimplantation experiments) and rotated 90° clockwise

     and replaced in the tectum.

2.   Following similar procedures as above, the tectum was

     rotated 180° clockwise.

Thirty animals in the first series and ten in the second

series were operated.   In animals where the tectal lesion was

not symmetrical, the tectal graft tended to return to its

original position, due to pressure from the surrounding tissue.

However, a few animals with rotated tectum were recorded which

gave interesting results.

Figure 37A shows the predicted results of graft rotation.

On the basis of results of the normal projection (Fig. 22),

a theoretically reconstructed projection was drawn for the

90° clockwise rotation of the tectum. Altogether nine points were taken into consideration. These results are compared with the 90° rotated tectal maps.

A 90° rotated graft when recorded 61 days after the operation (Fig. 37B, TT21) shows at least four lines which are slightly twisting. The nasal and temporal extremities of the map approximate to normal. There is a striking deviation from normal in the centre. There are certain similarities to the predicted result (Fig. 37A). An electrolytic lesion made at tectal point 29, was seen histologically. These results are further compared with two other 90° rotated maps. When recorded 171 and 172 days after the operation (Fig. 38 A and B, TT53 and TT54 respectively), the rotated maps have again a certain elusive similarity to the predicted one (Fig. 37A).

All points were fairly localizable within the graft and the receptive field area did not extend more than 25° for most of these points. In the animal recorded 172 days after the operation (Fig. 38B), a few points immediately caudal to the graft did not give any response to visual stimuli. In this particular case, fibre bundles from the anterior part of the tectum were seen to follow a curious and twisted pathway as compared with the normal fan-like projection of the optic fibres over the normal tectum (Fig. 38C).

Although these fibres were very clear when observed, before
recording in the living animal, the photograph was poor, due
to lack of contrast on the fish tectum: the colour of the
tectum and that of blood vessels is nearly the same.

Recording after 180° clockwise rotation of the graft,
the visual map became chaotic in the centre although the
nasal and temporal extremities of the map approximated to
normal. Two animals of this series survived long enough
to be useful for mapping experiments. Figures 39 A and B
show the results of these manoeuvres. These animals were
recorded 121 and 91 days post operatively respectively (TT57
and TT66). In the animal recorded 121 days after the
operation, most of the points were localized. Receptive field
areas were not wider than 25° within and outside the graft.
Only one point in the tectum (34) was not localized, although
this was again repeated after the experiments. Single unit
activity at tectal point 16 was recorded (Fig. 39A). These
were mostly sustained 'off' units. The map, within the
graft, was definitely twisted.

In another animal (Fig. 39B, TT66), recording was done
91 days after operation, only three rows fell within the
grafted tectum. One of these rows definitely showed a
marked deviation from its normal position in the visual field.
In the last row, two central points (26 and 27) did not

respond well to visual stimulation. They were too faint to be localized. The nasal and temporal extremities of the map approximated to normal.

It was not possible to draw any useful conclusions from 180° rotation experiments, as very few results were available. One can say with certainty that definite bending or twisting of the map occurs which could be due to rotation.

RESULTS OF RETINO-TECTAL MAPPING FOLLOWING REMOVAL OF POSTERIOR TECTUM AND CRUSH OF THE LEFT OPTIC NERVE

Of the twenty fish with right caudal tectum removed and left optic nerve crushed, five survived long enough for recording. No responses could be recorded in 3 (95, 98 and 120 days after the operation). Restoration of the retinal outline and localized responses were evoked in tectal positions projection across the surface of the remaining anterior tectum had occurred in two (114 and 97 days after the operation), and the retino-tectal projections are shown in Figs. 40 A and B.

In the first case (Fig. 40A, TT55) responses were evoked in anterior most row of positions on the tectum from the naso-superior quadrent of the left visual field, whereas, the responses in the posterior most row (points 18-23) were evoked from naso-inferior to superio-temporal quadrent of the visual field. The remaining two rows were recorded from within the visual field of the first and second row. It appears that the whole visual field which would have

expanded over the whole dorsal tectum has compressed
itself onto the remaining half tectum. Receptive fields
of the regenerative fibres were localized to the place shown
in the visual fields.

In the second animal (Fig. 40B, TT62), recorded 97 days
after the operation, only two points gave responses in the
first row of tectal positions. Even these two positions
(2 and 3) appear to deviate slightly from normal. Responses
evoked from second row (points 6-10) were mostly from the naso-
superior quadrant of the left visual field with slight deviation
at point 8 which fell in the superio-temporal quadrant.
Responses from the third row were evoked half from the naso-
superior and half from the temporo-superior quadrant. Quite
defined and localized responses were evoked in tectal position
14 from two different areas in the left visual field. One
of these areas was in the naso-superior and other in the
temporo-superior quadrant of the left visual field. These
areas were about 70° apart from each other. Visual proj-
ections from tectal points 11-14 and also from 14-17 were like
normal projection but each of these groups was situated in a
different quadrant of the visual field. The last row
projection from the temporo-superior quadrant and the projection
was normal. Another peculiar thing observed was that these
rows intersect each other in the visual field in a fashion

comparable to that seen in the early regeneration stages

in tectal reimplants. Most of the receptive field were

localizable. To check the fate of right visual projection

onto the left i.e. unoperated tectum, the same animal after

the first mapping was turned around and the right visual

projection was mapped from the responses evoked at different

positions on the left tectum. A normal retino-tectal

projection across the surface of the left optic tectum was

obtained (Fig. 40B).

LEVEL OF SIGNIFICANCE OF THE PROJECTION MAPS

Considering the normal projection maps (Figs. 22, 23 and

24) for each row of electrode positions there is a correspond-

ing row of stimulus positions. The rows of field positions

pass upwards to the superior part of the visual field.

The pattern of these rows is identical in both quadrents

i.e. naso-superior (NS) and temporo-superior (TS). Thus a

normal progression from one stimulus position to the next

in the row can be derived. Allowing 90° latitude for the

accuracy of this progression, then in the naso-superior

quadrent the row should run
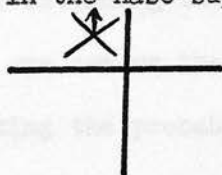
while in the temporo-superior quadrent they should run
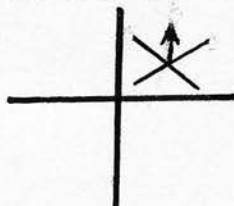
One can evaluate the rows of field positions by scoring the
direction from each position to the next, in terms of the
co-ordinate system.   Thus in the fifth row (Fig. 22) there
are eight joining lines between positions 25 and 33.   These
all fall in the appropriate co-ordinate quadrent.   If the
positions in the field were randomly distributed, one should
expect the vector joining the two positions to fall with
equal frequency in each quadrent.   So the expectation of
its falling in the correct quadrent is $\frac{1}{4}$.

For the row considered, one may use λ^2 to test the observed
frequency with which the joining line falls in the correct
quadrent, against the frequency expected on the basis of random
progression.

$$\text{Observed correct} \quad (O) \ = \ 8;$$
$$\text{Expected correct} \quad (E) \ = \ 8/4;$$
$$O-E \ = \ 6// \text{ and } \frac{(O-E)^2}{E} = 18.00$$

Thus here, $\lambda^2 = 18.00$ for one degree of freedom.   The
probability of this happening on the basis of chance is
0.001.   This is the probability of obtaining the observed
distribution of field positions in one row on the basis of
random progression.   After calculating the probabilities of
each row, the results were summed to give an overall λ^2 for the
series of rows.

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The $_\lambda 2$ test when applied to the normal projection gives
a probability of occurrence of less than .000001 for the
regular rows of stimulus positions.

This application of the $_\lambda 2$ test has been adopted from
Gaze and Jacobson (1963). The test have been applied to all
the projection maps mentioned earlier.

After summing the results of individual rows in each
map, the total result gives the overall probability of
occurrence of less than 0.000001 for the regular rows of
stimulus positions. Typical cases were as follows:-

Experiment TT44, Fig. 34B.

Row	Observed Correct (O)	Expected Corrected (E)	$\frac{(O-E)^2}{E}$ χ^2	Probability on the basis of chance
(1)	3	3/4	6.75	0.01 <
(2)	4	4/4	9.00	0.001 <
(3)	5	5/4	11.25	0.001 <
(4)	5	5/4	11.25	0.001 <
(5)	4	4/4	9.00	0.001 <
(6)	4	4/4	9.00	0.001 <

Experiment TT52, Fig. 32B.

(1)	4	4/4	9.00	0.001 <
(2)	4	5/4	6.05	0.01
(3)	5	6/4	8.165	0.001 <
(4)	4	6/4	4.17	0.02
(5)	4	6/4	4.17	0.02
(6)	5	6/4	8.165	0.001 <
(7)	5	6/4	8.165	0.001 <

The maps in which individual rows gave low significance were as follows:-

Experiment TT36, Fig. 33B.

(1)	2	4/4	1.00	0.30
(2)	2	2/4	4.50	0.02
(3)	3	4/4	4.00	0.02
(4)	2	3/4	2.08	0.10
(5)	3	3/4	6.75	0.01

Experiment TT5, Fig. 30A.

(1)	1	2/4	0.50	0.50
(2)	1	1/4	2.25	0.10 <
(3)	3	3/4	6.75	0.01 <
(4)	1	1/4	2.25	0.10 <
(5)	1	1/4	2.25	0.10 <

Quite apart from the probability tests, in most cases the orderliness of arrangement is apparent on inspection of these projection maps.

RESULTS OF THE HISTOLOGICAL STUDIES OF THE
EXPERIMENTAL ANIMALS

At the end of mapping experiments, every animal's head was fixed in Susa. Sections at 15 μ were cut and stained by Holmes' silver method. Most of the histological results are based on this technique unless otherwise mentioned.

Figures 41 A and B give the low and high power photograph of the optic tectum of a normal Goldfish. The normal layering and uniform contour of the tectum is apparent. These pictures are to be compared with those of the reimplanted and rotated grafts.

A photomicrograph of a transverse section through the whole of the right optic tectum, in which part of the tectum had been reimplanted, shows the location of the graft (Fig. 42.). Two deep depressions at the site of the incision are present. There is a gross abnormality in the histological structure of the grafted tectum. At the site of the lesion, no stratification of the tectum could be seen and most of the fibres in that area formed a mesh-like structure (Fig. 43). The histology of the middle region of the graft shows no normal layering except for the lowest fibre layers, which consist mainly of the afferent and commisural fibres. The cells in the grey periventricular zone are quite distinct.

Very few cells in the outer area can be located and it is
extremely difficult to trace their axons or dendrites as
this area formed a totally disorganized structure (Fig. 44).

Loss of stratification in the outer white area is
evident in all the animals studied. In some cases the
graft seems to have sunk down slightly into the ventricle.
In a high power photomicrograph of the grafted tectum
(Fig 45. TT49), there is no normal layering in the graft
region but there are abundant nerve fibres here. The lower
cellular zone or grey periventricular zone has a number of
cells which extend numerous fine processes towards the outer
white zone. A conspicuous ∪ shaped fibrous bundle is shown
in this photograph. Near the right hand end of this bundle,
numerous nodules or swellings are seen. The chain of
swellings or nodules can be traced easily for a distance.
Both ends of this bundle are approximately at the same level
in the outer zone of the tectum. At the left hand end of
this bundle, numerous fine branches are noticed which expand
laterally in both directions. Efferent or commisural fibres
lying immediately above the periventricular cellular zone are
present. The inner plexiform layer on the left side of the
picture is clearer than the right. The outer white zone has
conspicuously fewer cells. The outermost fibrous layers are
not separately detachable. It was not

possible to say with any certainty what was the origin and fate of this peculiar bundle present in the graft.

In animals with 90° rotated grafts, the histological structure was not very different from that of the reimplants. Figure 46A and B show the histology of the middle and posterior region of the grafted tectum (TT53). There is no normal layering in the graft region, but abundant nerve fibres are present. In Figure 46A, a few fibres are seen at the site of lesion which join the outermost fibrous layers of the graft with the lower most efferent and commisural fibrous layer of the normal tectum. These fibrous connections have been observed in a number of cases both in the reimplant and rotated tectal grafts. The significance of these fibres is difficult to assess. The histological structure of the posterior region of the graft became slightly different than the middle region (Fig. 46B). In this case, the lower most efferent fibrous layers become very prominent and form thick fibrous bundles. It was difficult to trace these bundles at the site of the lesion. The presence of u shaped fibrous bundles are seen frequently in the rotated grafts as well. These bundles are always found lying deep in the periventricular cellular zone (Fig. 46C).

To determine the fate of the tectal graft from rostral to caudal end, every 10th section from one animal was drawn

with a camera lucida.   15 µ transverse sections, stained

with Holmes' silver technique were used in this study

(Fig. 47, TT52; 111 days stage).   The extent of the lesion

can be seen easily in over 50 sections.   There are two

cuts in the sections showing the graft.   The most anterior

and posterior sections show the normal structure of the

tectum and the graft does not extend thus far.   At the site

of the lesion there is generally a depression in which

meningeal tissue is seen.   Deeper fibre bundles from the

normal area of the tectum seem to extend into the tectum at

the site of the lesion, bending up parallel to the lesion and

passing in the superficial zone of the graft.   In caudal

sections, where the rostral extension of the cerebellum

lies just beneath the tectum, a few fibres are seen forming

a bridge between the tectal graft and the cerebellum.   In

more caudal sections, fibre bundles which emerge from the

tectal graft go deeper into the tegmentum as in the normal

tectum.

In another series of experiments, the histology of

the early stages of the graft was studied by Holmes' silver,

Glees' and Heidenhein's iron haematoxylin techniques.   Brains

were directly fixed with different fixatives at various stages

after tectal reimplantations.   No electrophysiological

observations were made from these animals.   The earliest

animal studied was 2 days after tectal reimplantation.   The
grafted tectum at this stage showed total degeneration of the
upper half of the outer white zone (Fig. 48), whereas the
lower white zone lying just above the grey periventricular
zone still has a number of cellular elements present mostly
in the deeper areas.   The deeper parts of the tectum are
still more or less intact.   The formation of an incision
canal at the site of lesion is evident.   This canal is
filled with meningeal tissues and blood cells.   When
studied at high power, the 2 day graft shows degenerating
processes from the deeper pyramidal cells.   Swellings of the
outer dendritic extension with hyaline cytoplasm surrounding
the nucleus are characteristic features of most of the
cells and their processes at this stage (Fig. 49).

Vacuolation of the upper white zone becomes very
evident in 3 day grafts (Fig. 50).   A very clear incision
canal is present filled with blood cells and meningeal
tissue.   Slight degeneration of the surrounding intact
tectum is also evident at this stage.

Within the main cellular layers bordering the
ventricle i.e. grey periventricular zone, mitotic activity
is noticed (Fig. 50A, 3 day stage).   Late teleophase, in
the centre of the picture, is seen.   In a phase contrast,
living tissue study of a 3 day graft, a few cells (Fig. 50B)

are seen which have a cytoplasmic bridge between two
separate lobes. They look like migratory cells, containing

In a 12 day graft, total degeneration of the outer zone
is seen except the underlying periventricular and efferent or
commisural fibrous layers which still are intact, (Figs. 51A
and B). The sections were stained with Holmes' silver
method in Fig. 51A. A few fibres from the normal tectum
on the right side are seen entering the graft. Numerous
degenerating cells along with blood and meningeal cells on
the outermost region and near the incision site are noticed.
The outer region of the tectum becomes vacuolated and these
vacuoles are bigger in the outer zones.

The sections were stained with Glees' method in Fig.
51B. A fibrous bridge is formed by new fibres entering
from normal tectum. These fibres in the graft could be
traced for some distance.

Looking at the site of lesion in a 20 day graft (Fig.
52, Glees' staining), many new fibres have entered the graft
from the adjoining normal tissue of the tectum. Although
the lower fibre bundles are still separate, the outer zones
are connected with new fibres. Most fibres of this bridge
are seen coming from the lower fibrous layers of the normal
tectum which are seen bending near the site of lesion running
upwards and entering into the graft near its outer zones.

Within the graft, the lower cellular zone along with the efferent fibrous layer seem more or less intact, retaining its normal shape. All these stages mentioned above were also examined for cell division by a chromosome staining technique. Very few mitoses were seen, and all of them were in the lower periventricular zone. No mitosis has been observed in histological sections.

Living tissue phase contrast study of the later stages of grafts has been made and migratory cells as seen in development, were observed.

Photograph of the apparatus showing the projection perimeter
on the right. The microscope on the left could be swung into
position so that the tip of the electrode could be seen as it
was driven by the micromanipulator to the correct position on
the optic tectum. Micromanipulator with electrode is in the
centre just above the fish holder. The fish, with its left
eye centred on the perimeter, was held in position with a U
shaped clamp. The fish is shown sitting in a fish holder.
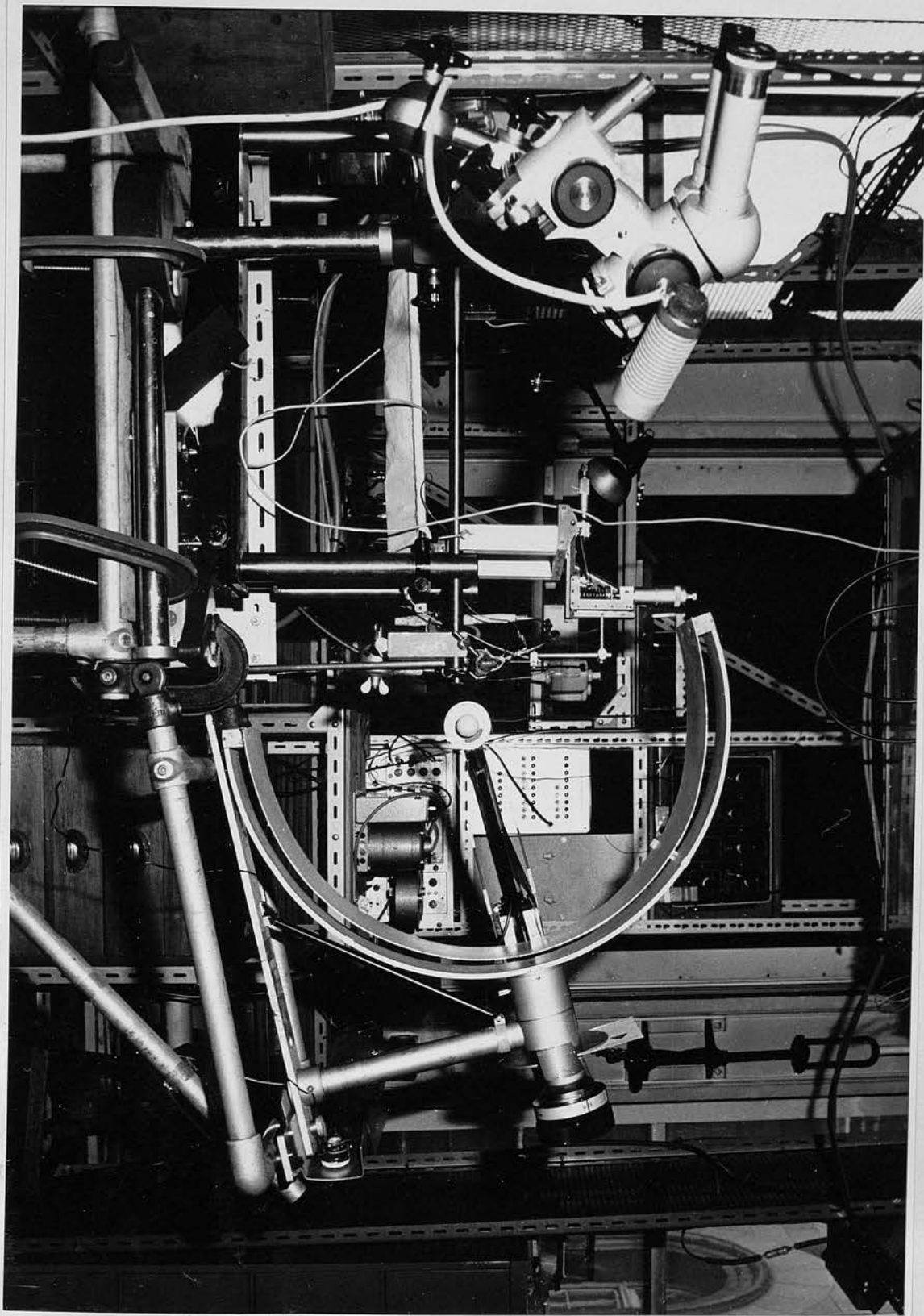
PLATE I.

DEVELOPMENT OF THE TECTAL NEURONS OF SALMO-FARIO

Camera-lucida diagrams, of various stages of development of tectal neurons, drawn from 80μ thick Golgi-cox impregnated sections. Orientation of cell bodies with their processes is the same as seen in the transverse sections. Arrow indicates axon.

Figs. 1-4   Show the neurons in recently hatched embryo

Figs. 5-8   Show the neurons at 7 days stage after hatching

Figs. 9-11   Different stages at 14 days after hatching

Figs. 12   A pear shaped neuron at 25 days after hatching

1 0.01mm 2 0.01mm 3 0.01mm 4 0.01mm 5 0.02mm 6 0.02mm 7 0.02mm 8 0.02mm 9 0.02mm 10 0.02mm 11 0.02mm 12 0.1mm
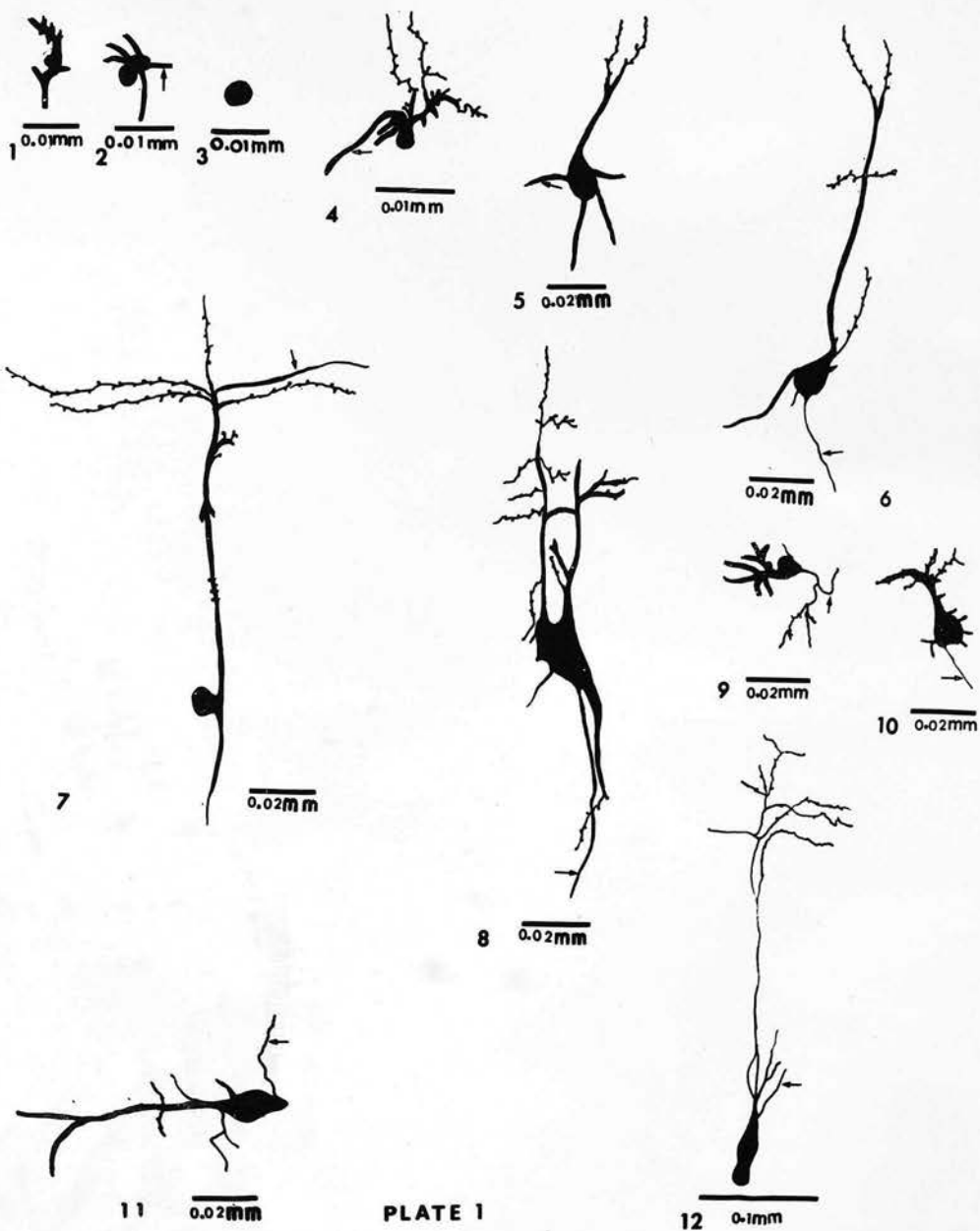
PLATE 1

<u>PLATE 2.</u>

<u>DEVELOPMENT OF TECTAL NEURONS OF SALMO-FARIO</u>

Figs. 13-16 & 18   Different stages of neurons during the
                    development of tectum at 25 days after
                    hatching.

Fig. 17   An ependymo-glial cell.

Fig. 19   A small multipolar cell with its processes
                    at 30 days stage after hatching.

Fig. 20   A small pear shaped neuron with its apical
                    dendrites at 50 days after hatching.

These are Camera lucida diagrams from Golgi-cox impregnated
sections.

Arrows indicate axons.

13

$\overline{0.02\,mm}$

14

$\overline{0.02\,mm}$

15

$\overline{0.02\,mm}$

16

$\overline{0.02\,mm}$

17

$\overline{0.02\,mm}$

18

$\overline{0.02\,mm}$

19

$\overline{0.02\,mm}$

20

$\overline{0.02\,mm}$
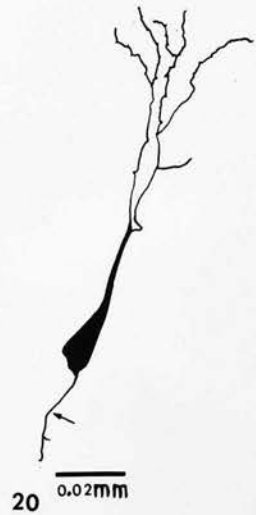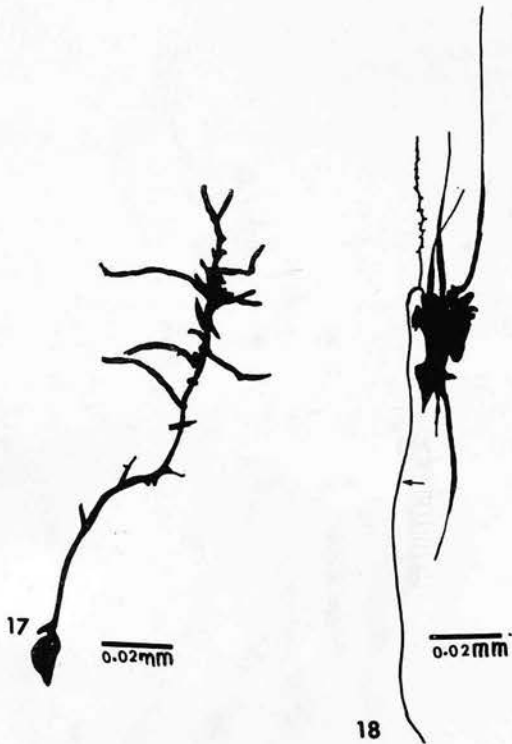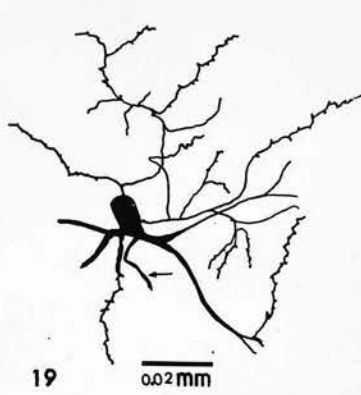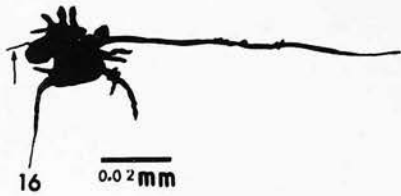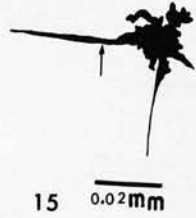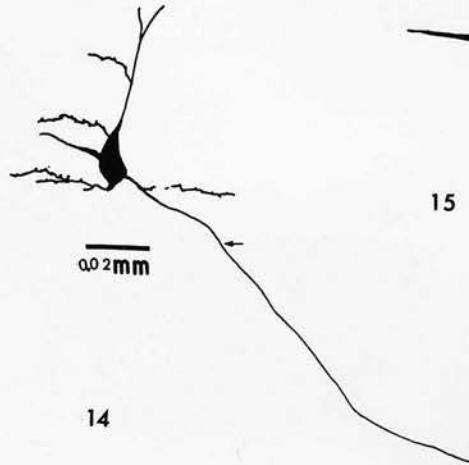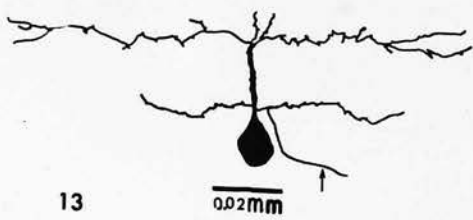
PLATE 2

PLATE 3.

DEVELOPMENT OF TECTAL NEURONS OF SALMO-FARIO

Camera-lucida diagrams drawn from Golgi sections at different stages of tectal development.

Figs. 21-22   Show horizontally orientated optic axons with dendrites of pear shaped neurons intermingling. The optic axons could be traced from the optic nerve.

Figs. 23-24   Show 2 neurons at different stages of development. Fig. 24 shows a bipolar neuron with extensive dendritic branches which are generally seen in adult animal.   Arrows indicate axons.

21

0.02 mm

PLATE 3

22　0.02 mm　23

0.02 mm

24
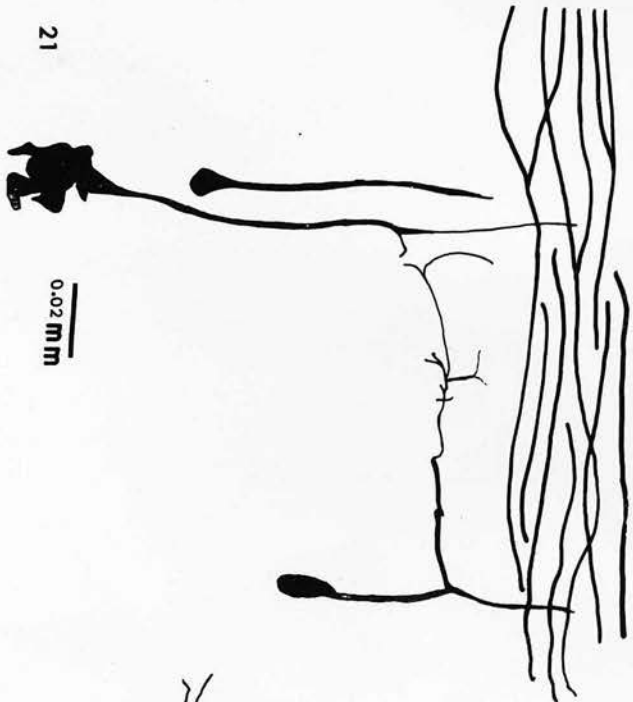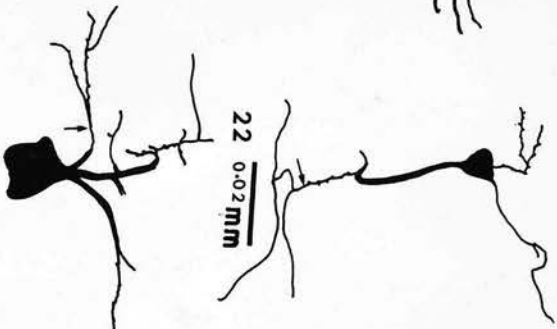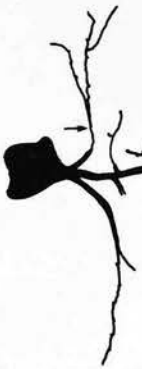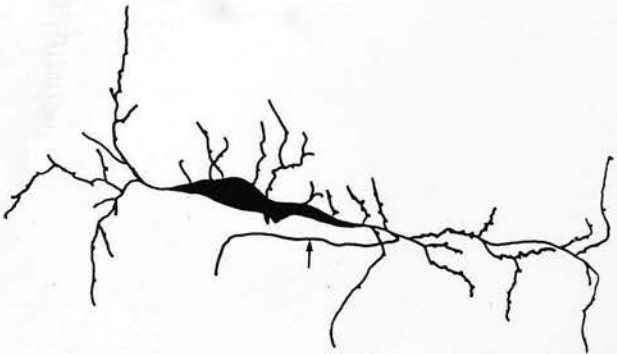
0.02 mm

THE OPTIC TECTUM OF GOLDFISH AND TROUT

Camera Lucida reconstruction of the structure of the optic
tectum of trout and goldfish - based on Golgi rapid specimens.
Arrows indicate the axons.   Nos. 1-14 are types of neurons
described in the text.   A-G represent the different layers
of the tectum.   'Pseudo-spines' of dendrites are indicated
in the upper layers only.   On the extreme right, an ependymo-
glial cell is shown.
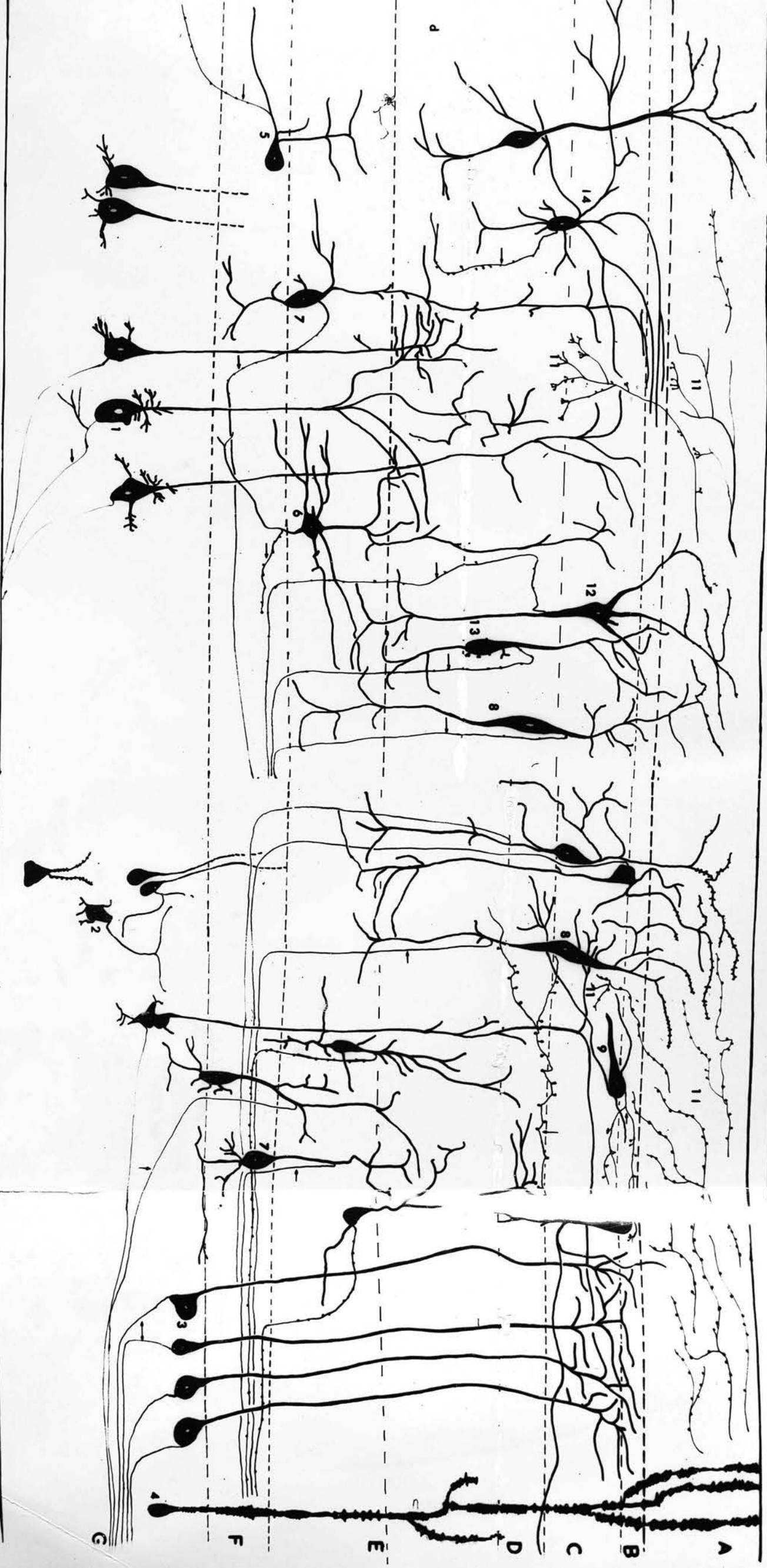
PLATE 4

DEVELOPMENT OF THE OPTIC TECTUM OF SALMO-FARIO

Fig. 1   Photomicrograph of the transverse section of optic
         tectum 22 days before hatching, showing hardly
         distinguishable outer white zone.

Fig. 2   Photomicrograph of optic tectum, 12 days before
         hatching.   Appearance of a distinct outer white zone
         which is invaded by few cells.

Fig. 3   9 days before hatching.

Fig. 4   7 days before hatching.

Fig. 5   5 days before hatching.

Fig. 6   2 days before hatching.

These photomicrographs were taken at the level of 4th
cranial nerve.   Photographs illustrate the growth of outer
white zone with various cells and the beginning of the strati-
fication of the optic tectum.

0.1 mm

Plate 4 A
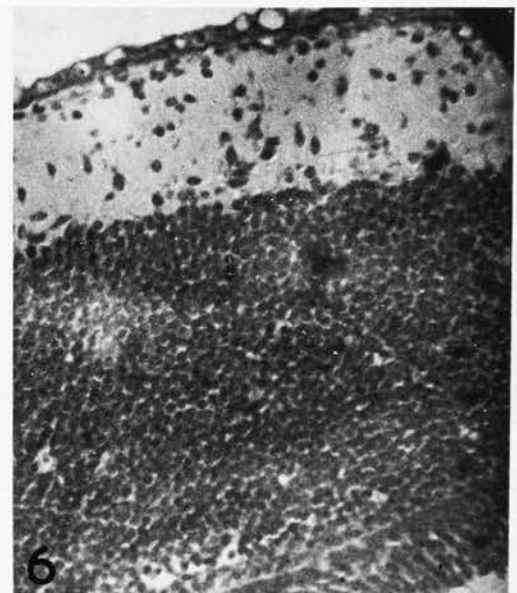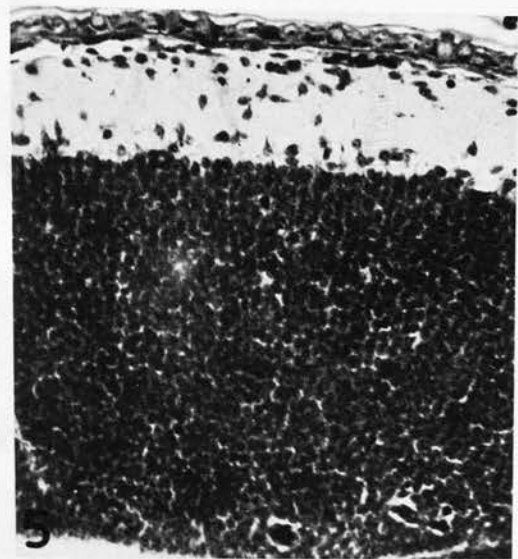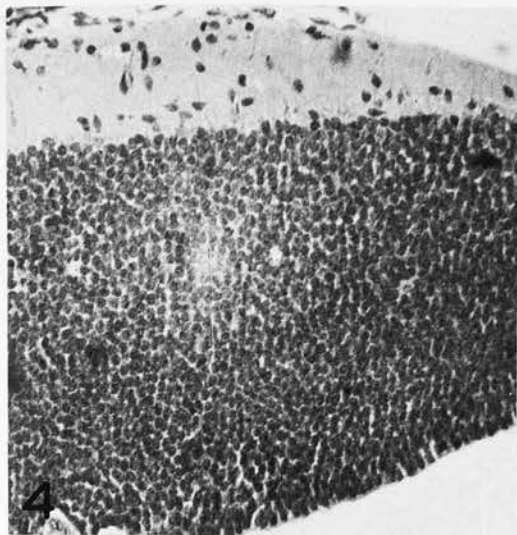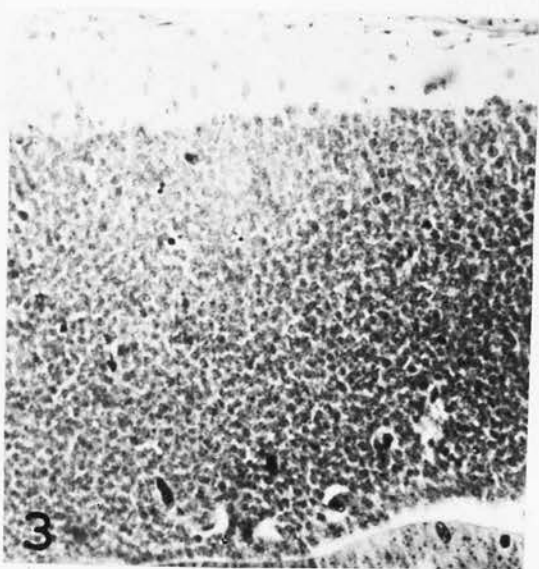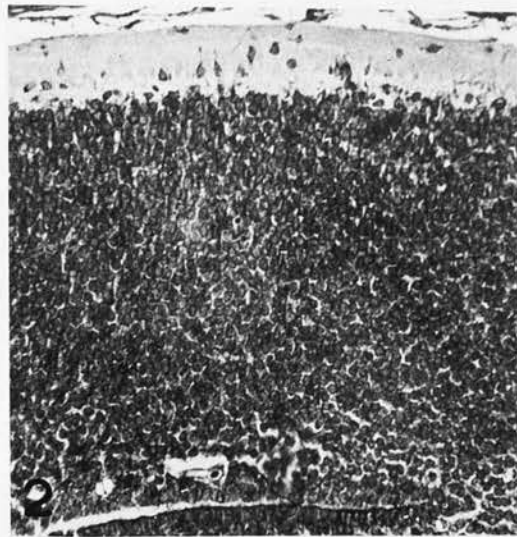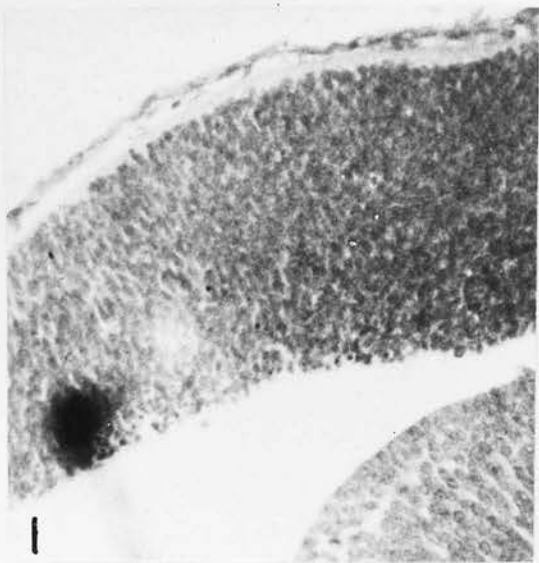
PLATE   5.

DEVELOPMENT OF THE OPTIC TECTUM OF SALMO-FARIO

Fig. 7   Hatching day stage.

Fig. 8   6 days after hatching.

Fig. 9   13 days after hatching.

Fig. 10   22 days after hatching.

Fig. 11   27 days after hatching.

Fig. 12   32 days after hatching.

These photomicrographs were taken from the transverse
sections at the level of the 4th cranial nerve.   Different
stages illustrate the growth and development of various layers
in the outer white zone.   Details of figures are described in
the text.   Fig. 12 shows the stage where outer white and
inner grey periventricular zones are almost equal in thickness.
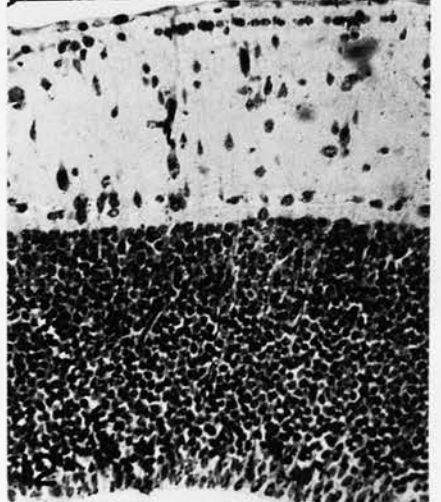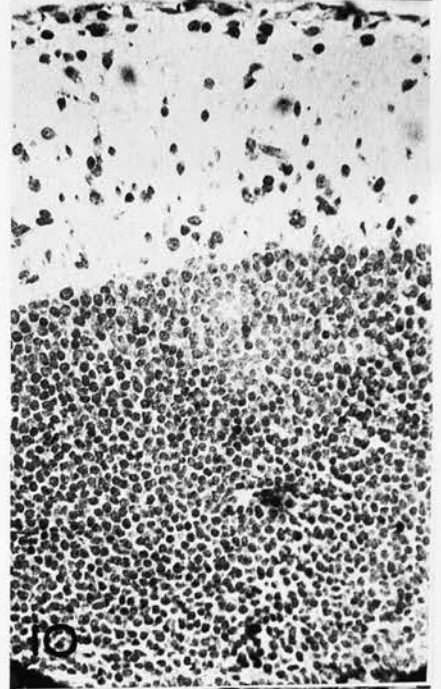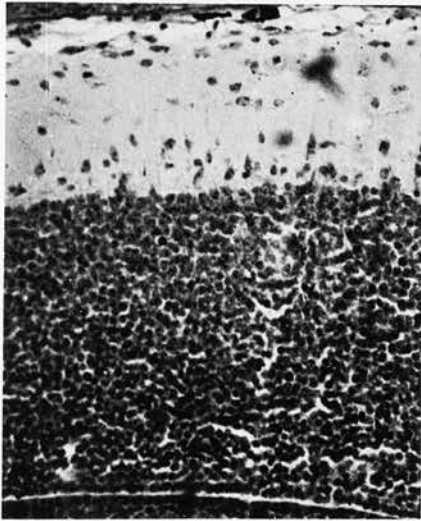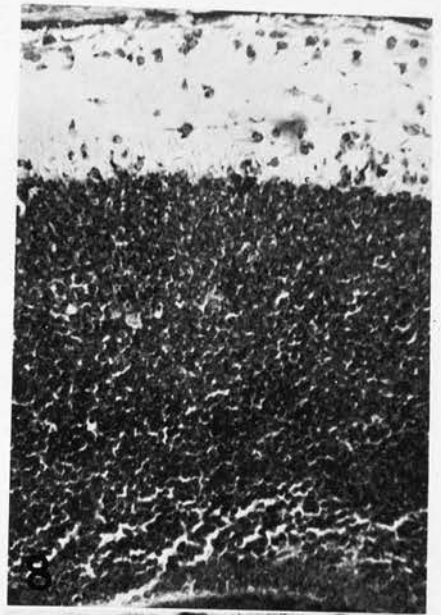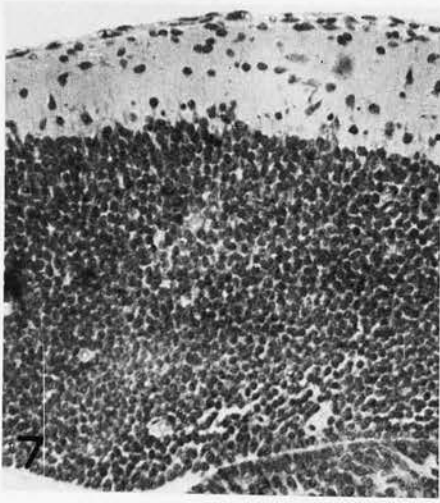
0.1 mm

Plate 5

DEVELOPMENT OF THE OPTIC TECTUM OF SALMO-FARIO

Fig. 13   37 days after hatching.

Fig. 14   47 days after hatching.

Fig. 15   68 days after hatching.

Fig. 16   77 days after hatching.

Fig. 17   91 days after hatching.

Fig. 18   98 days after hatching.

These photomicrographs show a number of cells arranging themselves into different layers.   Details of figures are described in the text.   A progressive decrease in the grey periventricular zone is evident.

13

14

15

16

17

18
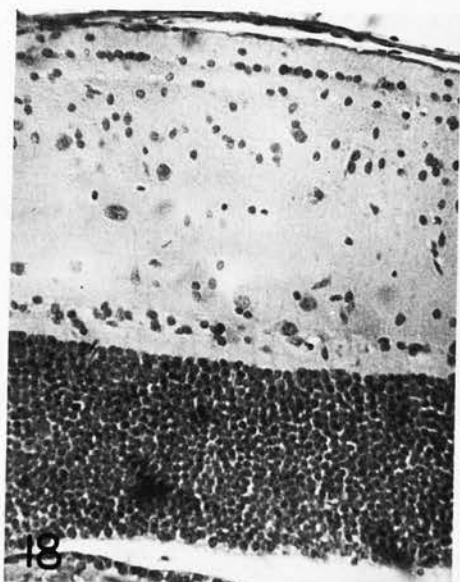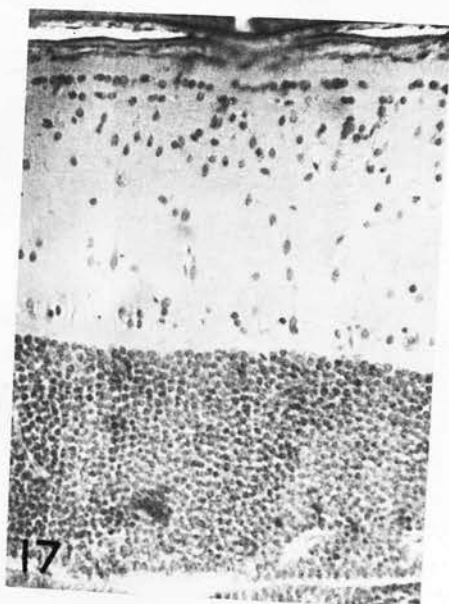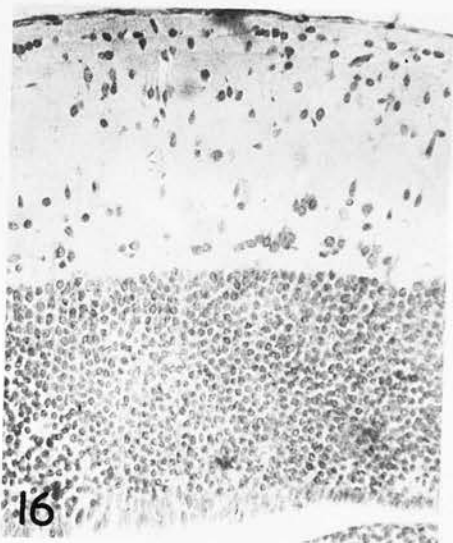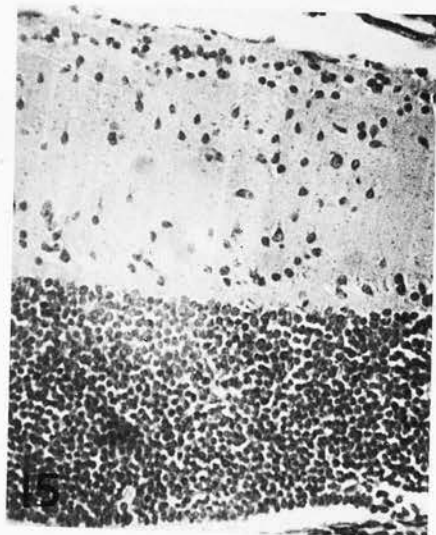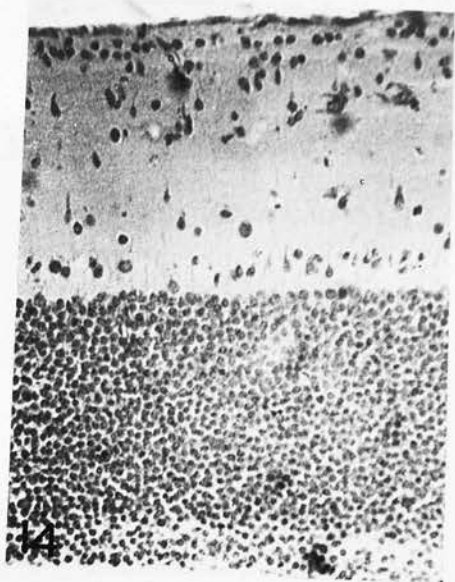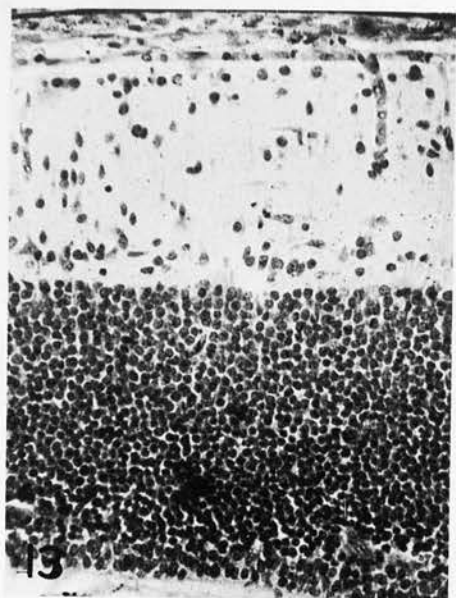
0.1 mm

Plate 6

GRAPH   I

The graph shows the rate of growth of the tectum in relation
to the growth of its white and grey zones before hatching.
The hollow squares indicate the total width of the tectum.
Black circles indicate the width of inner grey periventricular
zone and hollow circles indicate the width of outer white zone.

THICKNESS OF THE TECTUM

12
10
8
6
4
2

22   12   9   7   5   2

AGE IN DAYS (BEFORE HATCHING)
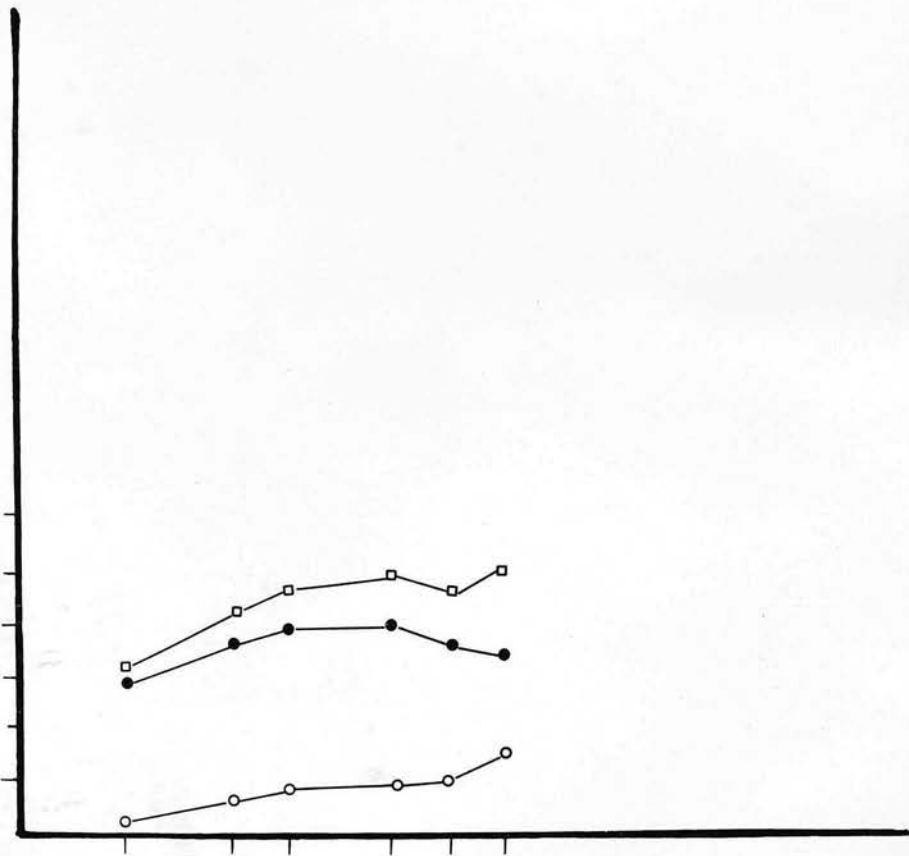
GRAPH II

The graph shows the relationship between the grey and white
zones of the tectum during development, after hatching.
Bar at 37 days stage indicates equal thickness of grey and
white zones.

THICKNESS OF THE TECTUM

□ = TOTAL WIDTH

○ = OUTER WHITE ZONE

● = INNER PERIVENTRICULAR ZONE

AGE IN DAYS (AFTER HATCHING)
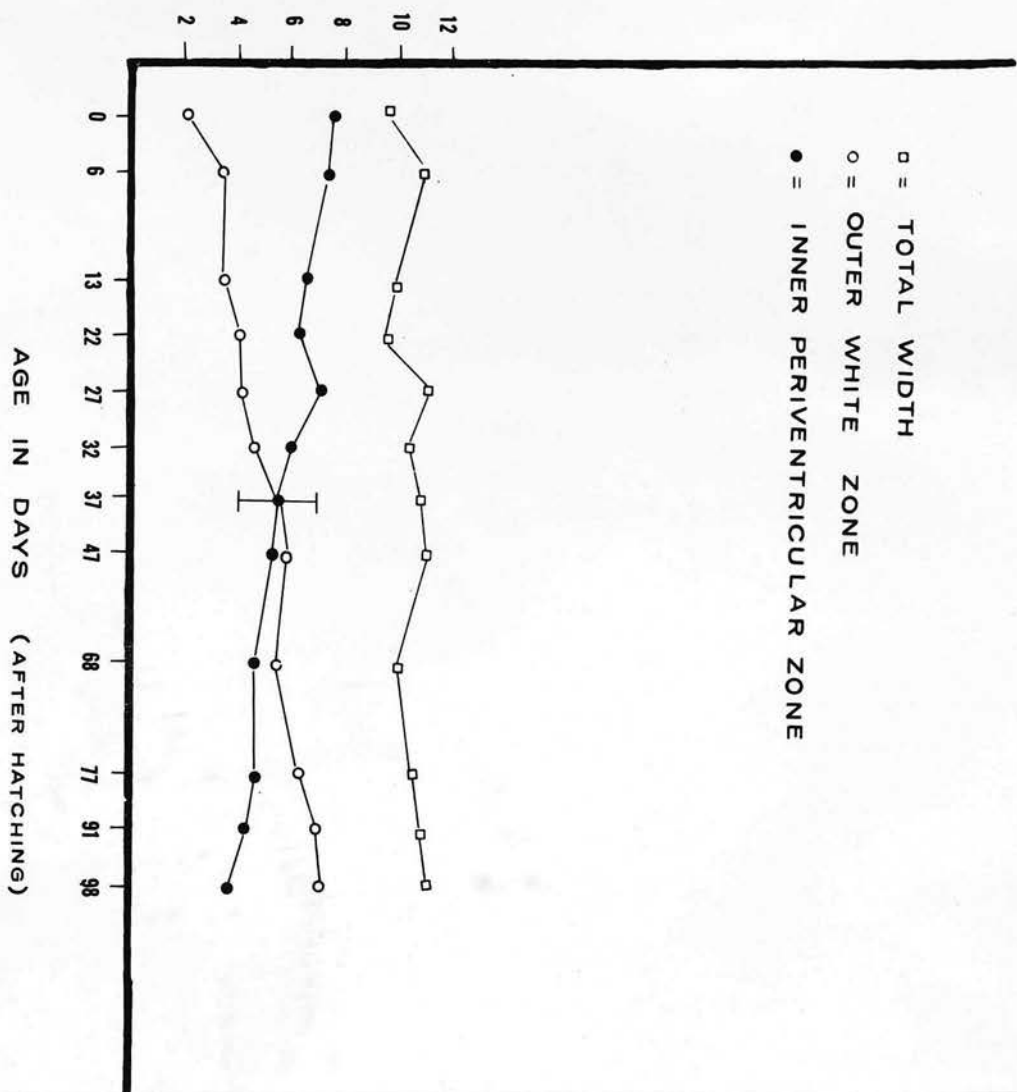
<u>FIG.</u> 19 and 20

<u>MIGRATION   OF   NEUROBLASTS   IN   THE   DEVELOPING   OPTIC   TECTUM</u>

Fig. 19 (upper) Fresh preparation of the optic tectum of a
trout (10 days after hatching) showing the early migration of
differentiating cell - note the two cell bodies attached
together.   Cells of the grey periventricular zone are in the
basal region of the Photomicrograph.   Phase contrast, scale
equals 0.03 mm.

Fig. 20 (lower) Fresh preparation of the optic tectum of Salmo-
fario (22 days after hatching) showing the dendritic formation
of the migratory neuroblast.   Note the attachment of parent
cell with the differentiating cell.   Phase contrast, scale
equals 0.02 mm.
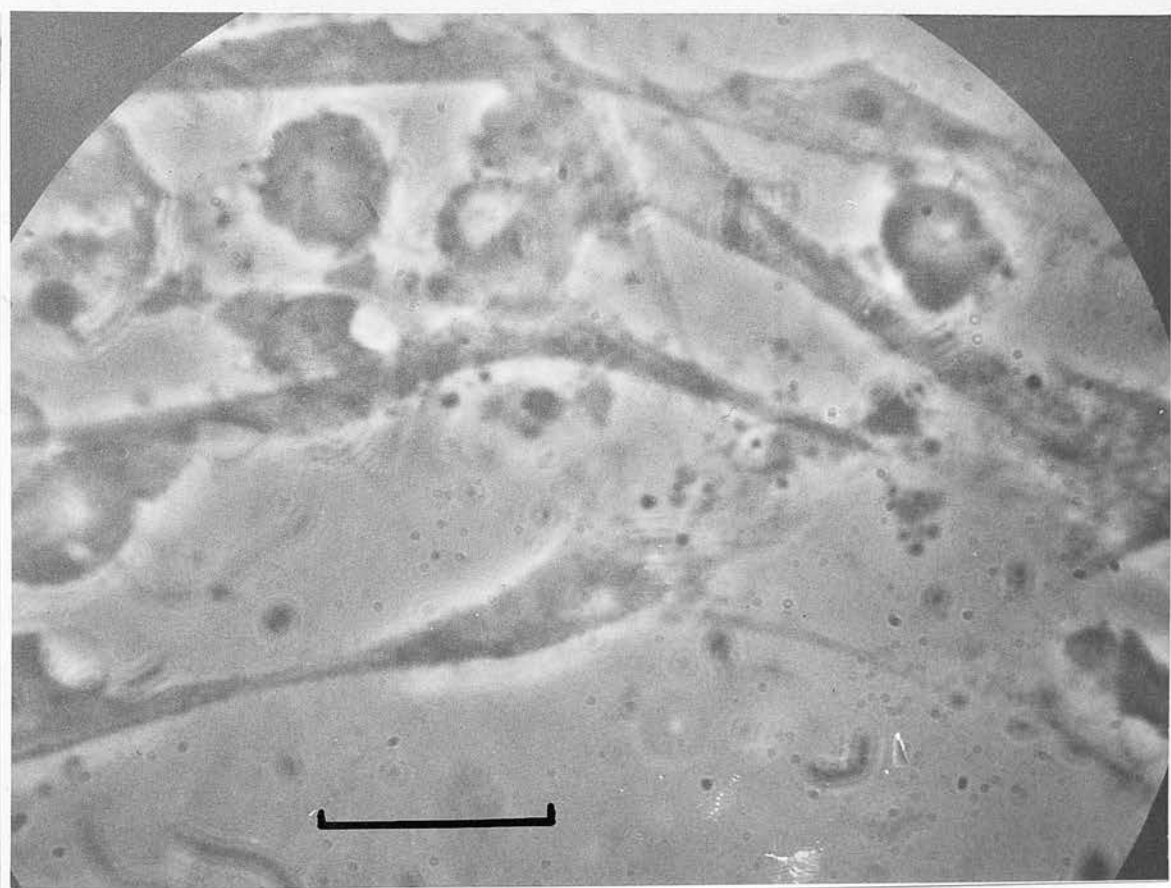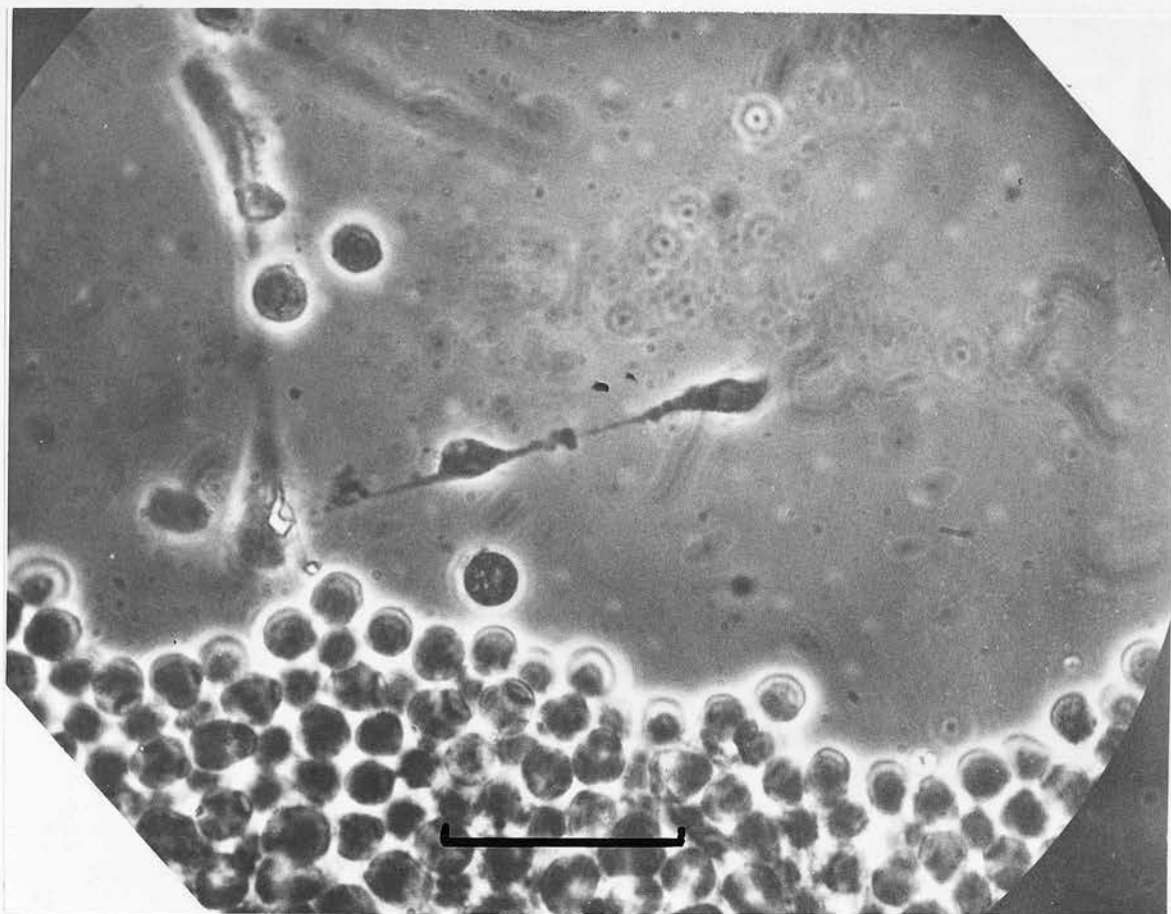
FIG. 21

MIGRATION OF NEUROBLASTS IN THE DEVELOPING OPTIC TECTUM

Fresh preparation of the optic tectum (18 days after

hatching) showing the parallel processes on the right

side of the Photomicrograph - note the oval mass in one

of the processes.   The ovoid cell from where these

processes originate is very clear.   Phase contrast,

scale equals 0.03 mm.
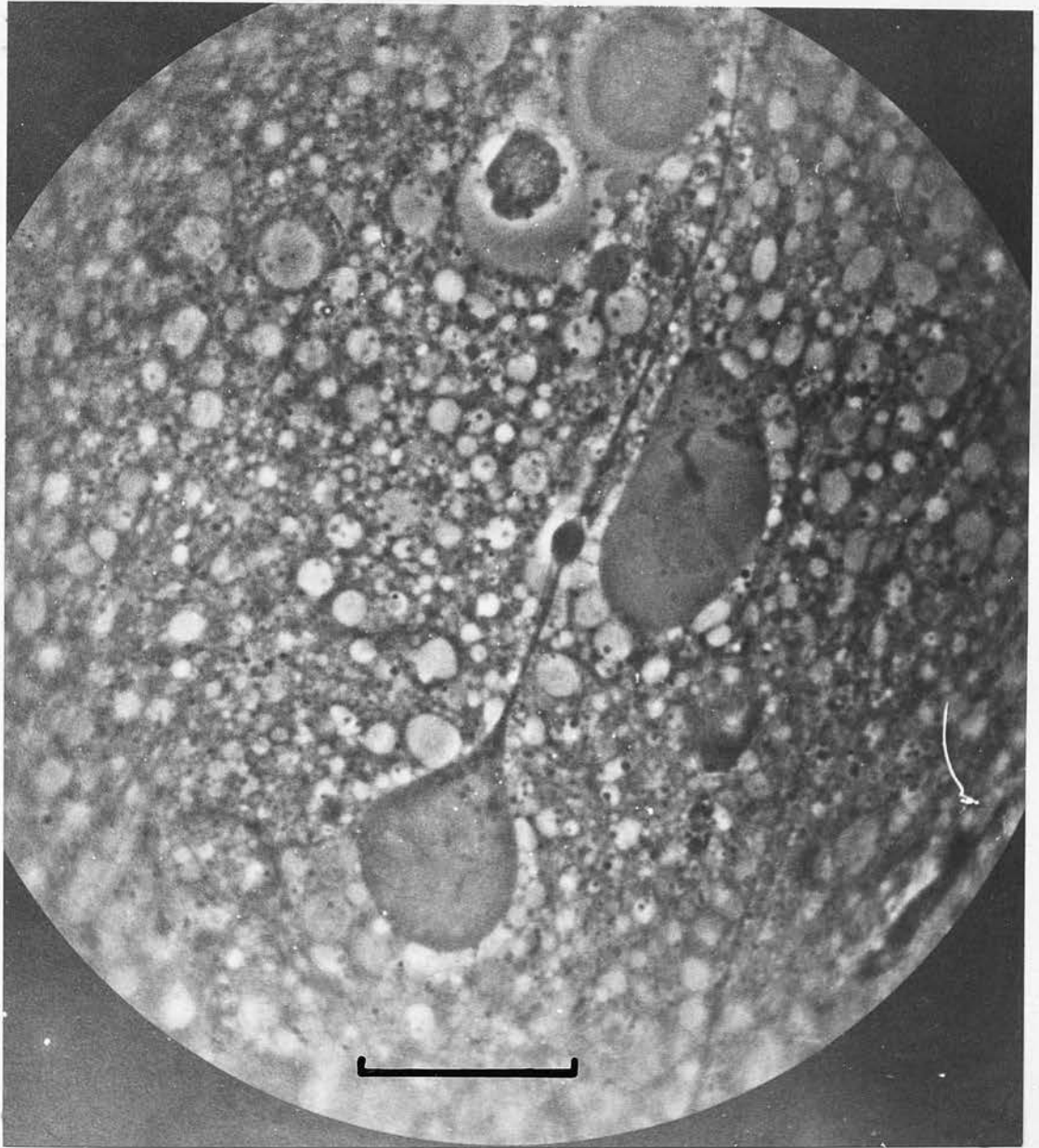
FIG. 22

The projection of the left visual field unto the right optic

tectum in the Goldfish (Figure has been taken from Jacobson

and Gaze, 1964). The map of the left visual field showing

stimulus positions is compared with normal 'Blind' project-

ion.
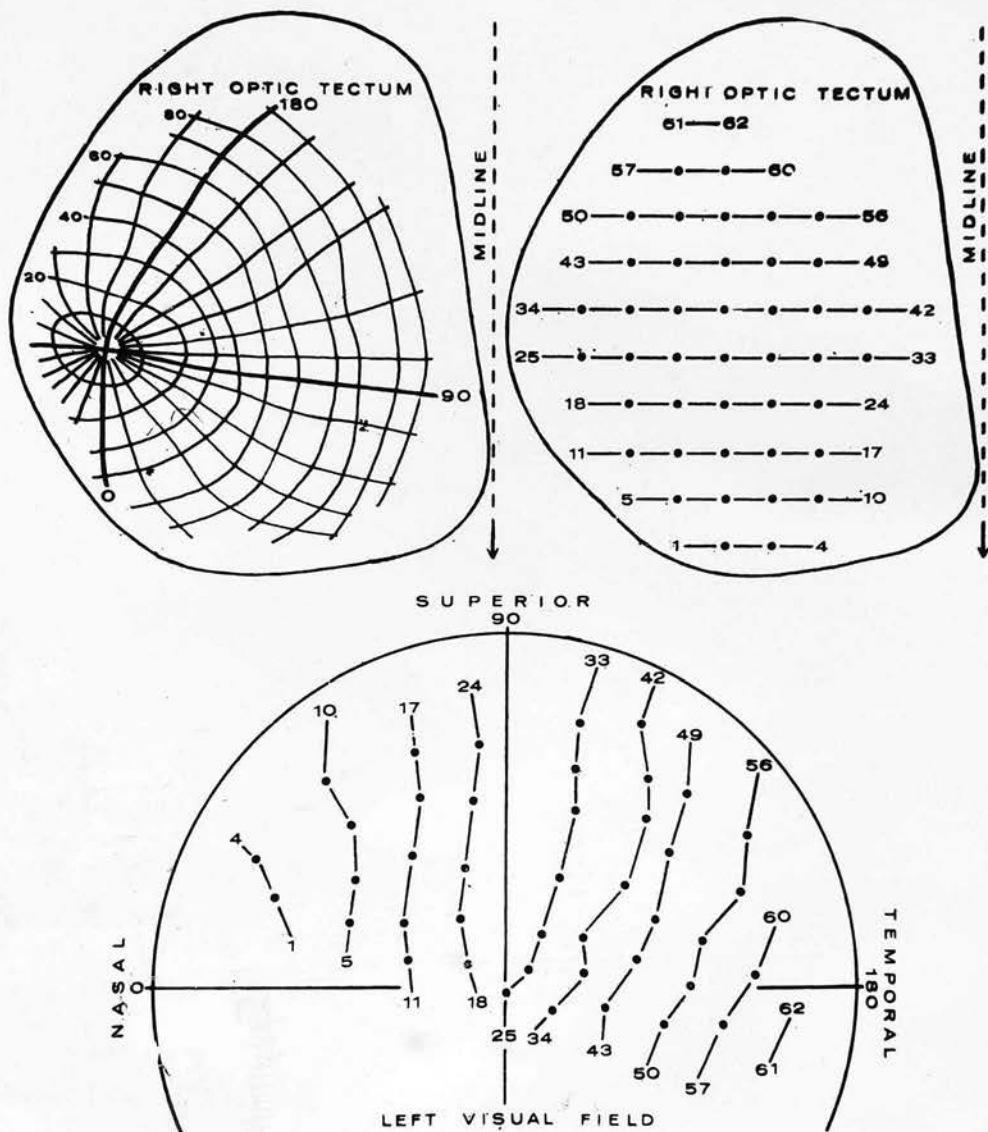
. . . —. The projection of the left visual field onto the right optic tectum in the goldfish.
Upper right.—Dorsal surface of tectum showing rows of numbered electrode positions. The arrow along the midline points rostrally.

Lower.—Chart of left visual field showing optimal stimulus positions, each corresponding to its appropriate electrode position. The chart covers 100° from the centre of the field.

Upper left.—Map of the meridians and parallels of the visual field on the tectum, prepared from the information in the other two diagrams.

The following visual maps (Figs. 23-40) are redrawn from the perimeter chart and the area of the tectum is reconstructed from the histological sections and then superimposed on the area seen at the time of recording (Details are given in the text).

The following abbreviations have been used:

C = Caudal

I = Inferior

L = Lateral

M = Medial

N = Nasal

R = Rostral

S = Superior

T = Temporal

The scale at which the area of the optic tectum is drawn, equals 200 microns. The scale has been kept constant in all experiments.

FIGS. 23 and 24

THE PROJECTION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM

Upper: (23) Upper left diagram of the outline of the right

optic tectum shown 34 electrode positions at which

action potentials were evoked in response to a light

stimulus in the left visual field at the positions

shown in the visual chart.   At each electrode

position on the tectum, a maximum response was

evoked by the stimulus at a position indicated by

the number in the upper half of the visual field.

The perimetric chart extends for 100° outwards from

the centre of the field.

Lower: (24) The outline of the right optic tectum shows 48

electrode positions.   The method of mapping is

similar to the one described above.   The projection

of the left visual field in both cases was made by

'Blind Mapping' technique described in the text.
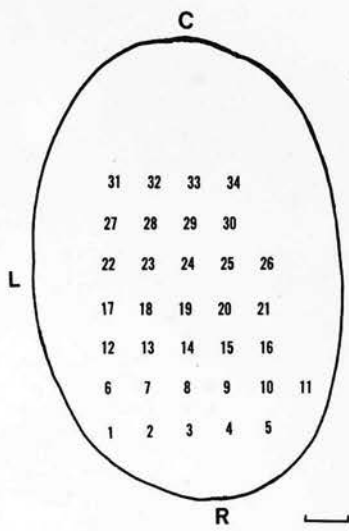
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
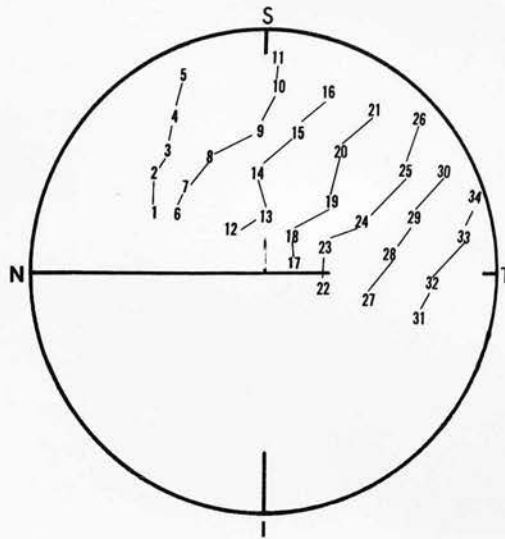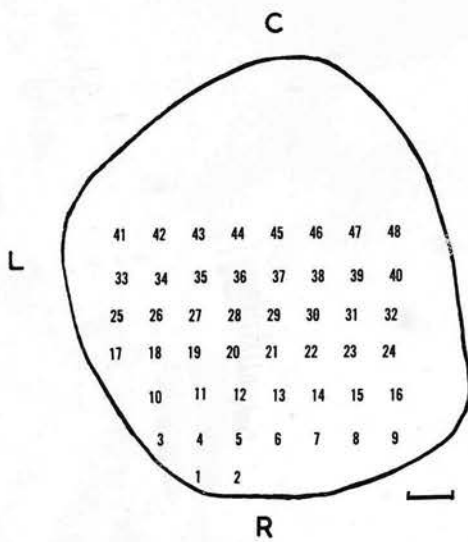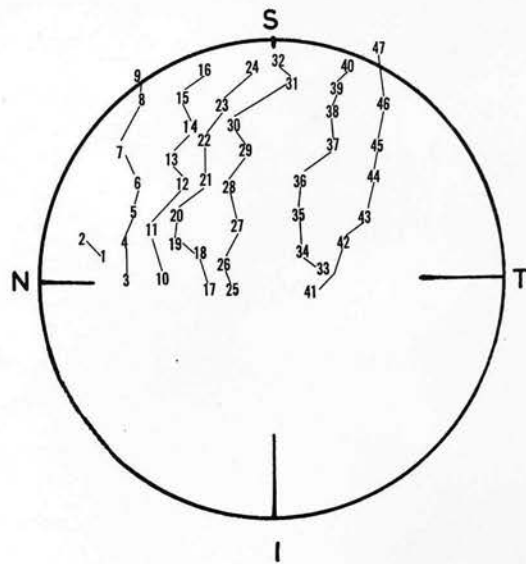
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

NORMAL "BLIND" PROJECTION

FIG. 25

(Experiment TT 9)

Upper left diagram illustrates the method of plotting the
histological sections in the experimental animals.   Lower
diagram shows the reconstruction of the tectum and the graft
(inner hatched area).   Upper right diagram represents the
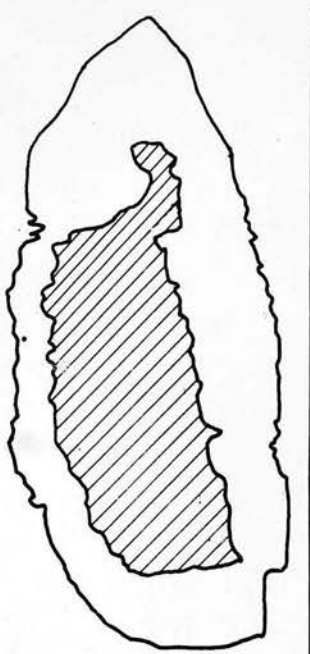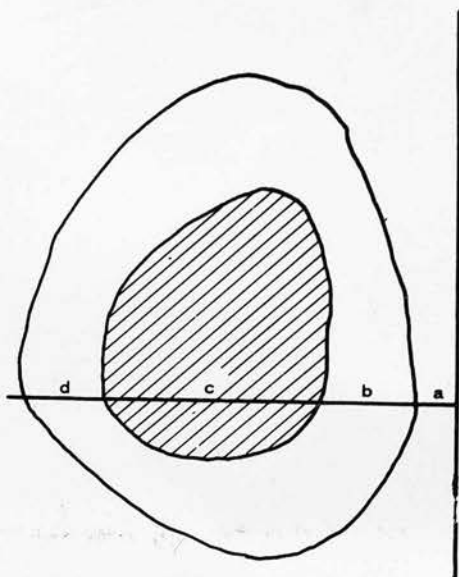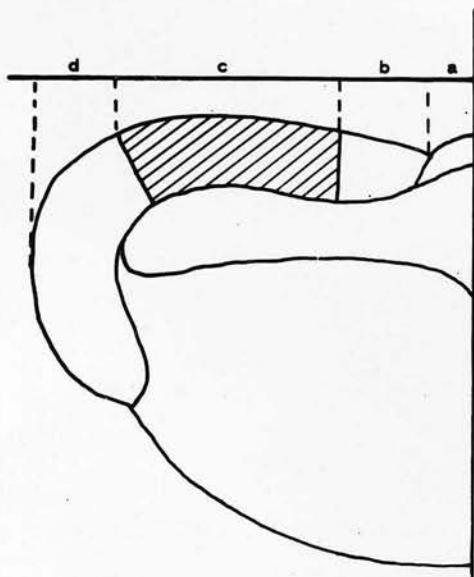final area of the graft in relation to the tectal boundary.

FIG. 26

(Experiment TT 9)

The representation of the left visual field on the right

optic tectum, mapped 107 days after tectal reimplantation

in normal orientation.   The conventions are the same as

in Fig. 24.

CAUDAL

MIDLINE

RIGHT TECTUM

ROSTRAL

S
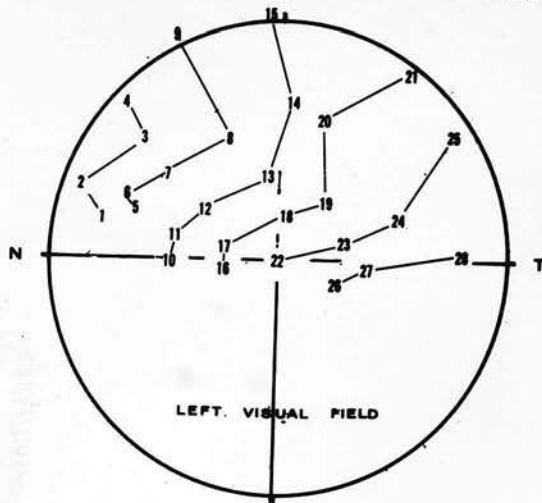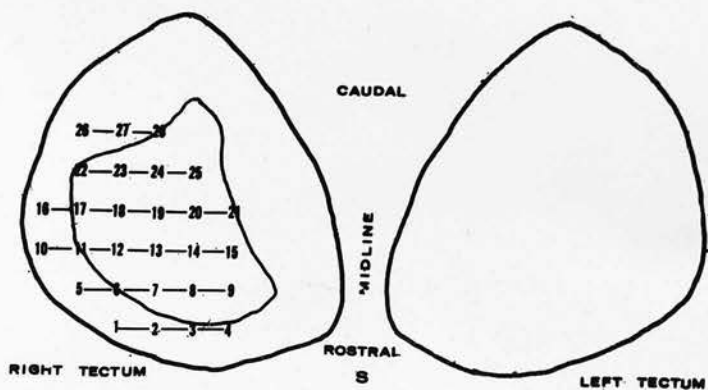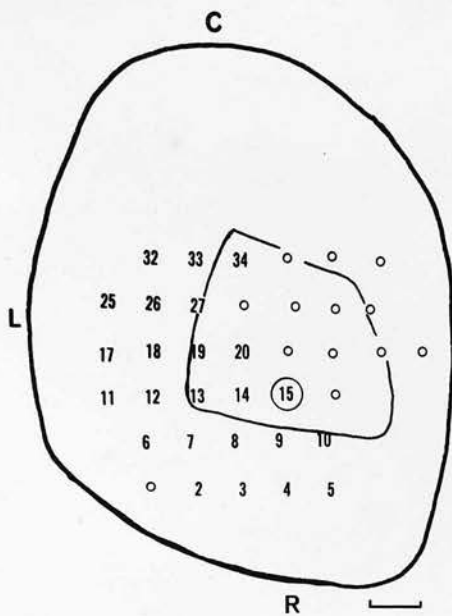
LEFT TECTUM
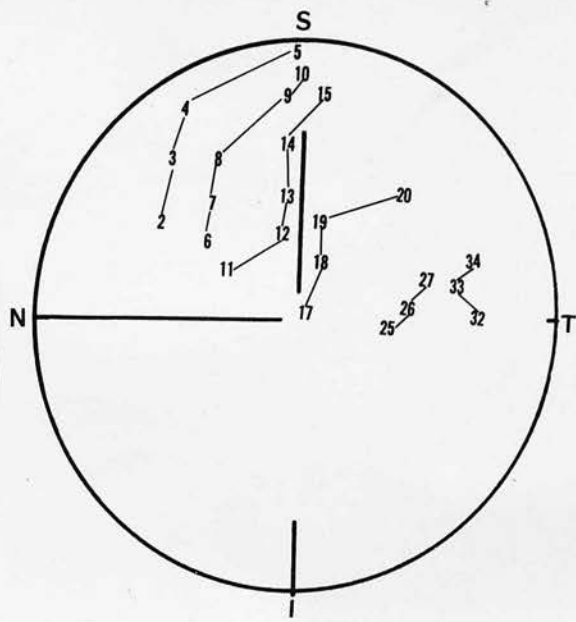
LEFT VISUAL FIELD

FIG. 27 A and B

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT OPTIC
TECTUM MAPPED 36 and 42 DAYS RESPECTIVELY AFTER TECTAL REIMPLANTATION

Upper: (27A, Experiment TT20); The numbers on the tectal
diagram represent electrode positions and the numbers
on the perimetric chart show the corresponding stimulus
positions in the contralateral visual field.   The
empty circles on the tectum represent electrode
positions from which no responses could be obtained.
The area within smaller square in the tectum is that
of the reimplant.   The perimetric chart extends for
100° outwards from the centre of the field.   Encircled
electrode position 15 represents the site of electro-
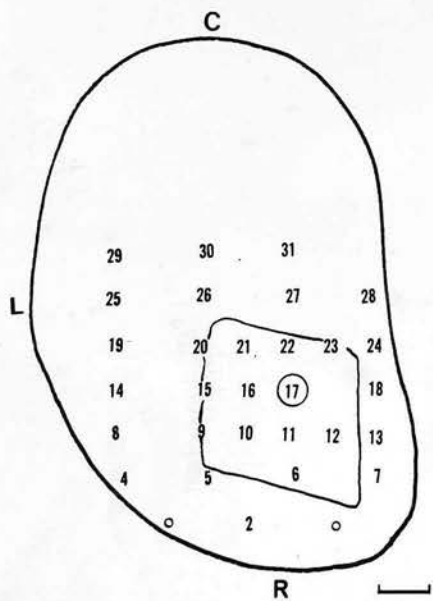lytic lesion.   The conventions otherwise are the
same as in Fig. 24.

Lower: (27B, Experiment TT19); The representation of the
retinotectal projection 42 days after tectal
reimplantation.   Electrolytic lesion at electrode
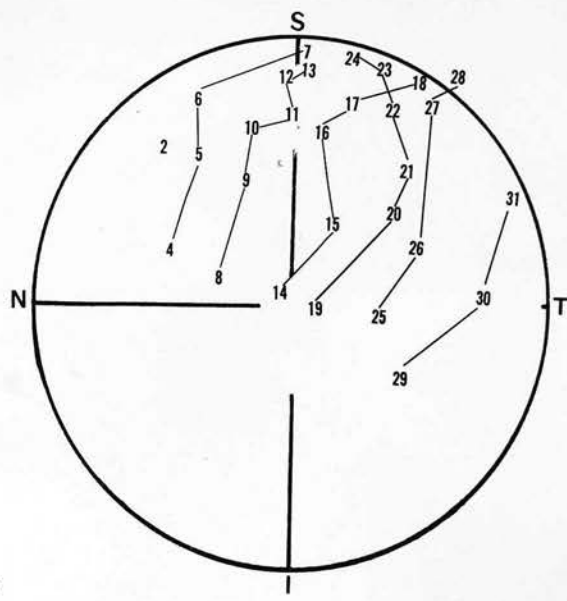position 17.   The conventions are the same as in
Fig. 27A.

RIGHT OPTIC TECTUM   **LEFT VISUAL FIELD**

RIGHT OPTIC TECTUM   **LEFT VISUAL FIELD**

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (28A, Experiment TT25); Retinotectal projection,
mapped 64 days after tectal reimplantation.
Encircled electrode position 15 represents the
electrolytic lesion. The conventions are the
same as in Fig. 27A.

Lower: (28B, Experiment TT22); Retinotectal projection,
mapped 66 days after the tectal reimplantation.
Encircled electrode position 7 indicates the
site of electrolytic lesion. The conventions
are the same as in Fig. 27A.

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
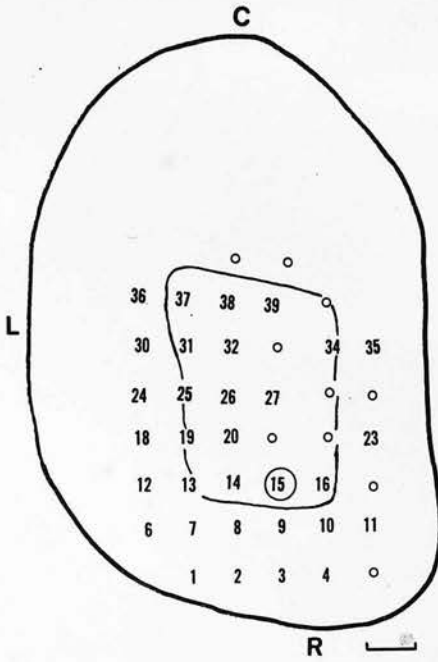
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (29A, Experiment TT17);   Retinotectal projection,

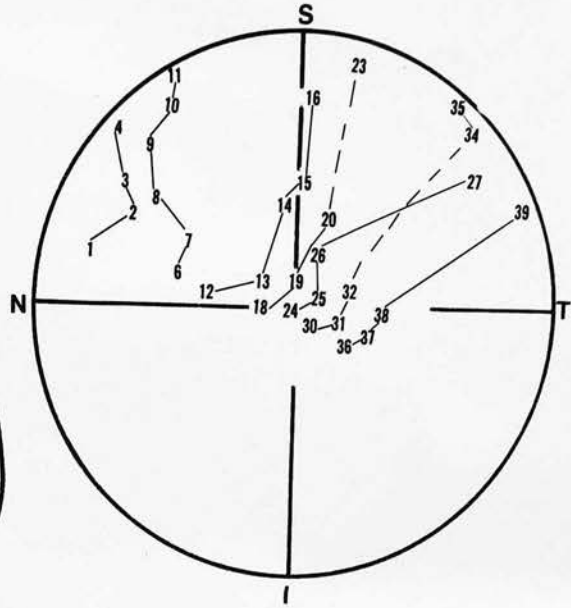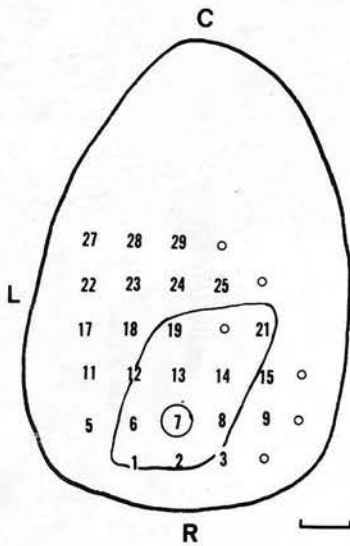mapped 76 days after tectal reimplantation.

Encircled electrode position 14 represents the

site of electrolytic lesion.   The conventions

are the same as in Fig. 27A.

Lower: (29B, Experiment TT26);   Retinotectal projection,

mapped 82 days after tectal reimplantation.

Encircled electrode position 5 indicates the

site of electrolytic lesion.   The conventions

are the same as in Fig. 27A.
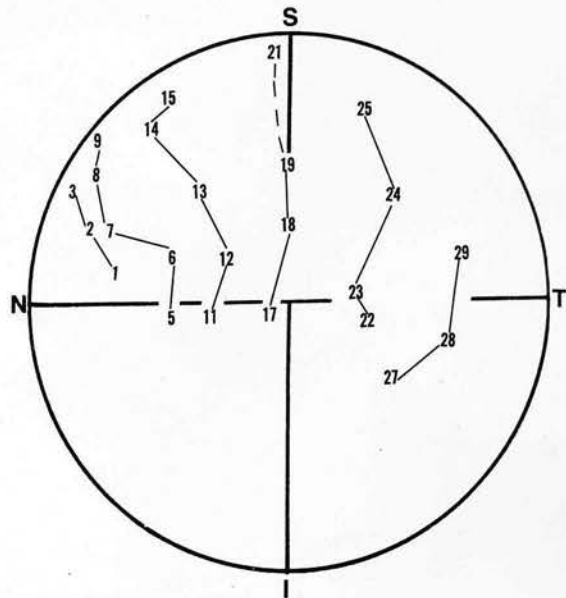
RIGHT OPTIC TECTUM   **LEFT VISUAL FIELD**

RIGHT OPTIC TECTUM   **LEFT VISUAL FIELD**

FIG. 30 A and B

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (30A, Experiment TT5); Retinotectal projection,

mapped 86 days after tectal reimplantation.

Responses were evoked only from the electrode

positions numbered.   No responses were recorded

from the electrode positions marked 0.   The

conventions are the same as in Fig. 27A.

Lower: (30B, Experiment TT28); Retinotectal projection,

mapped 87 days after tectal reimplantation.

The conventions are the same as in Fig. 27A.

RIGHT OPTIC TECTUM

LEFT VISUAL FIELD
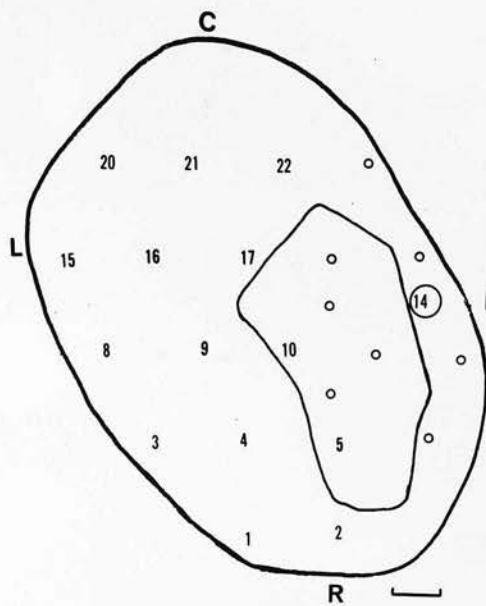
RIGHT OPTIC TECTUM

LEFT VISUAL FIELD

FIG. 31 A and B

THE PROJECTION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (31A, Experiment TT33); Retinotectal projection,
mapped 89 days after the tectal reimplantation.
Large circles (2 at electrode position 15 and 35
and other two empty) show the site of a electrolytic
lesion. These lesions were observed in histological
sections. The conventions are the same as in Fig.
27A.

Lower: (31B, Experiment TT35); Retinotectal projection,
mapped 94 days after the tectal reimplantation.
Encircled electrode position 9 shows the site of
electrolytic lesion. The conventions are the
same as in Fig. 27A.

RIGHT OPTIC TECTUM

LEFT VISUAL FIELD

RIGHT OPTIC TECTUM

LEFT VISUAL FIELD

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (32A, Experiment TT46); Retinotectal projection,
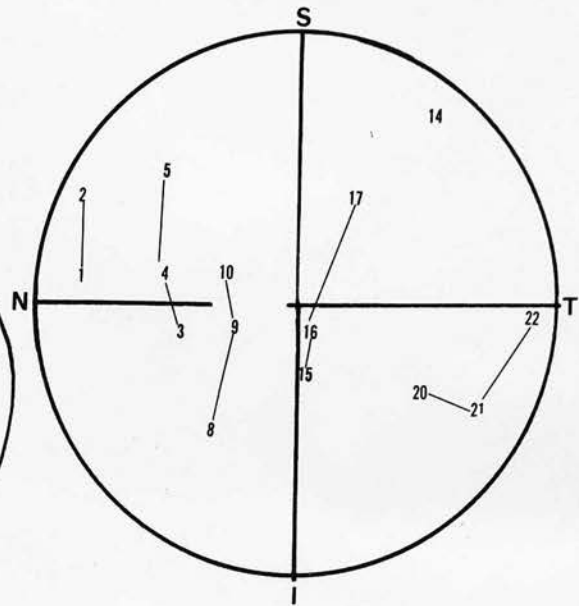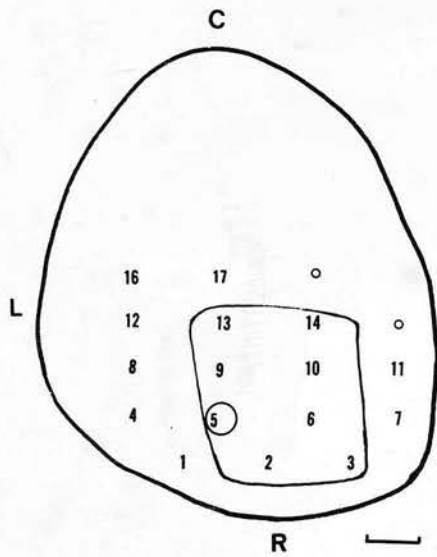
mapped 94 days after the tectal reimplantation.

Encircled electrode position 7 represents the

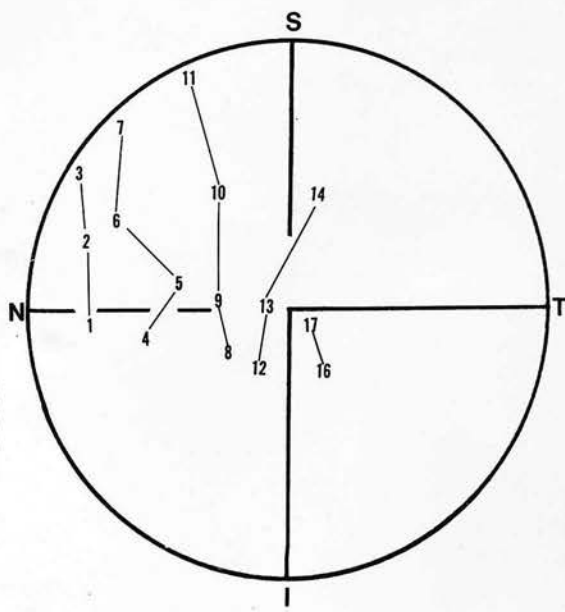site of electrolytic lesion.   The conventions

are the same as in Fig. 27A.

Lower: (32B, Experiment TT52); Retinotectal projection,

mapped 111 days after the tectal reimplantation.

The conventions are the same as in Fig. 27A.

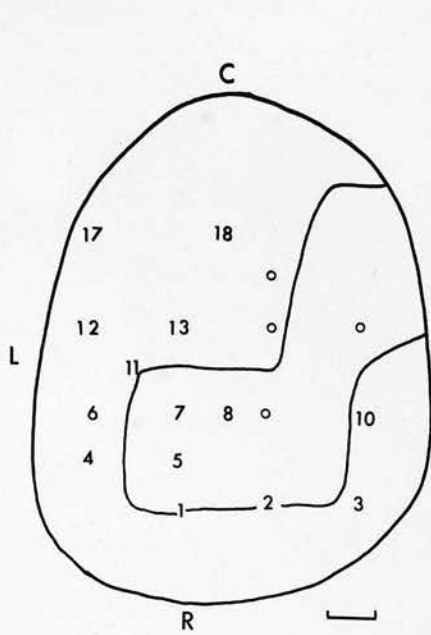RIGHT OPTIC TECTUM LEFT VISUAL FIELD
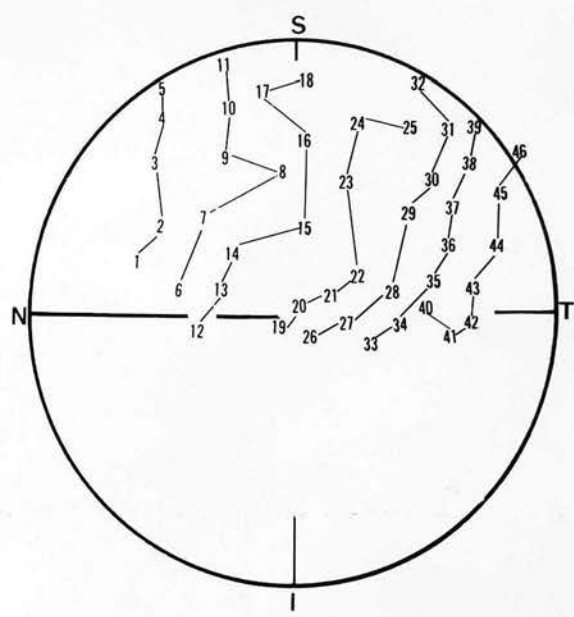
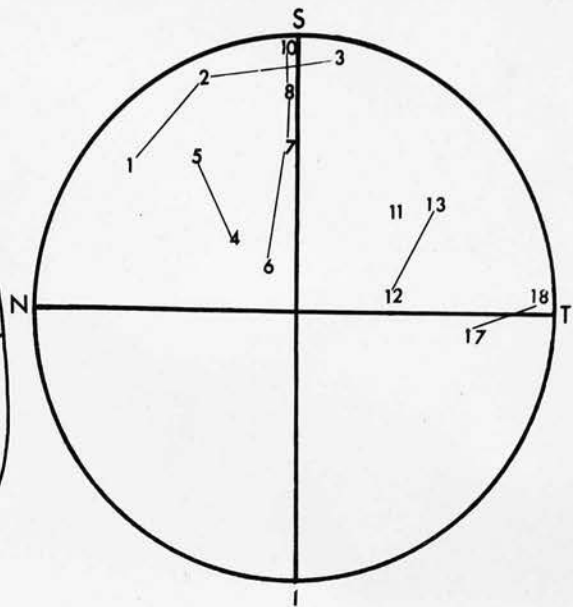RIGHT OPTIC TECTUM LEFT VISUAL FIELD

FIG. 33 A and B

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT
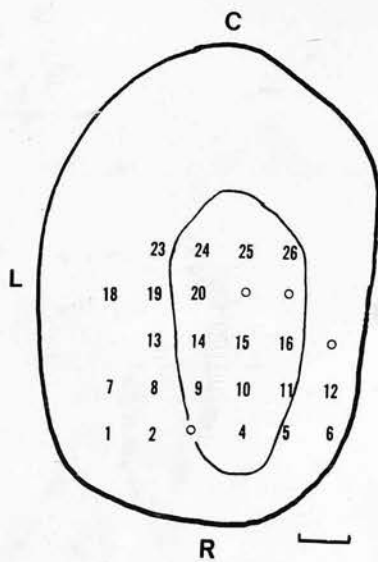
OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (33A, Experiment TT50); Retinotectal projection,

mapped 144 days after the tectal reimplantation.

The conventions are the same as in Fig. 27A.

Lower: (33B, Experiment TT36); Retinotectal projection,

mapped 148 days after the tectal reimplantation.

No responses were evoked at electrode positions

marked 0.   The conventions are the same as in

Fig. 27A.

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT
OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (34A, Experiment TT29); Retinotectal projection,
mapped 167 days after the tectal reimplantation.
No visual responses were evoked at electrode
positions marked O.   The conventions are the
same as in Fig. 27A.

Lower: (34B, Experiment TT44); Retinotectal projection,
mapped 194 days after the tectal reimplantation.
Encircled electrode position 5 represents the site
of electrolytic lesion.   The conventions are the
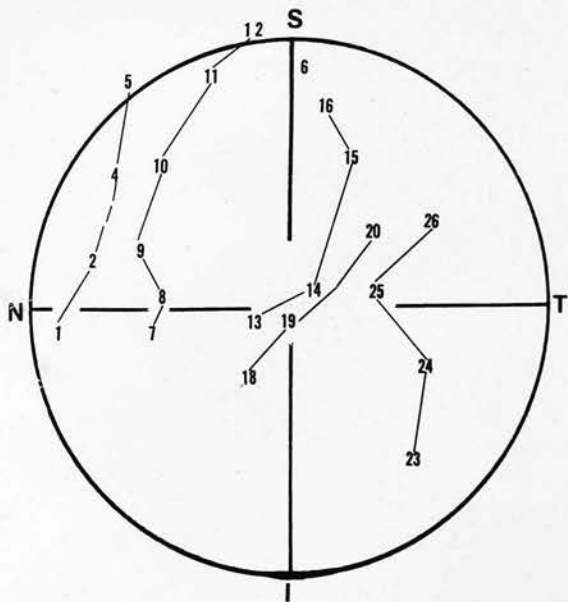same as in Fig. 27A.

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
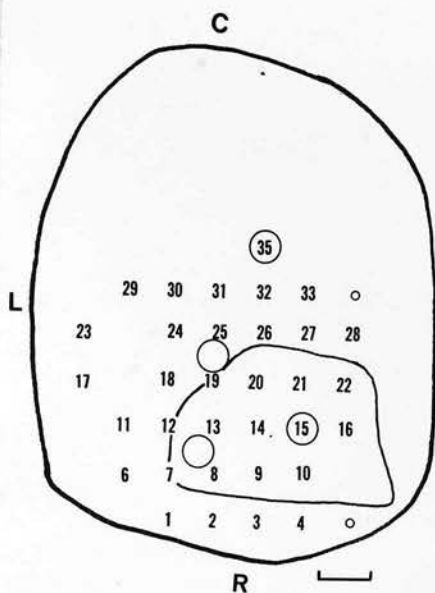
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

FIG. 35 A and B
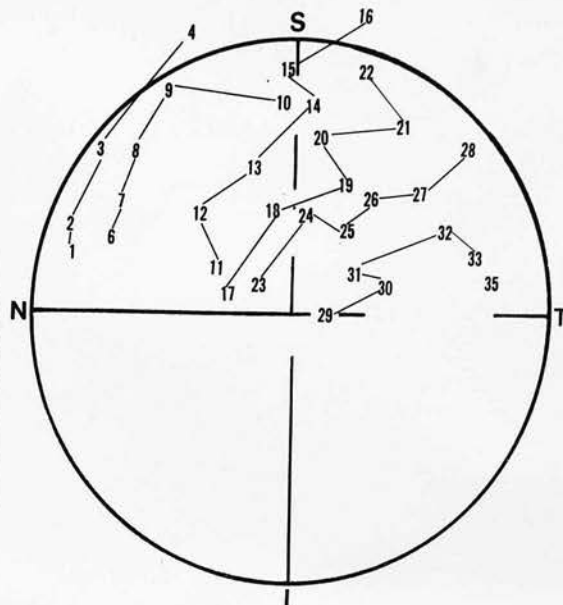
THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (35A, Experiment TT45); Retinotectal projection,
mapped 239 days after the tectal reimplantation.
No visual responses were recorded at electrode
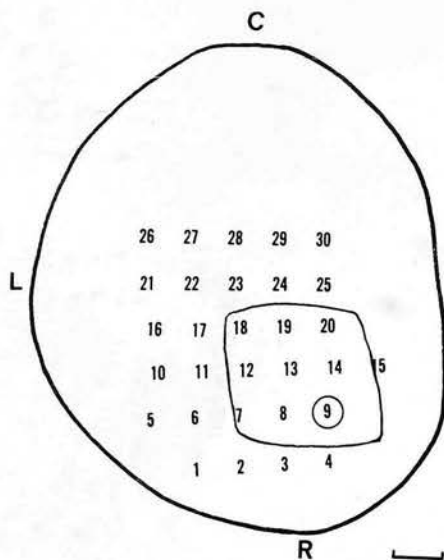positions marked O. The conventions are the same
as in Fig. 27A.

Lower: (35B, Experiment TT48); Retinotectal projection,
mapped 366 days after the tectal reimplantation.
Encircled electrode position 17 indicates the site
of electrolytic lesion. The conventions are the
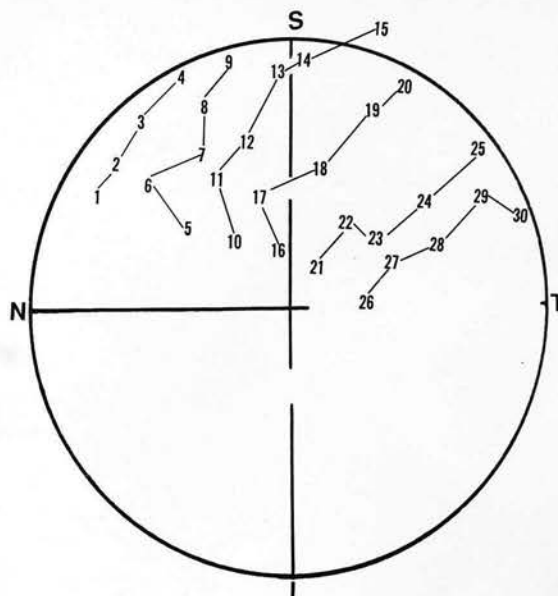same as in Fig. 27A.

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

FIG. 34A and B

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (34A, Experiment TT29); Retinotectal projection,

mapped 167 days after the tectal reimplantation.

No visual responses were evoked at electrode

positions marked O.   The conventions are the

same as in Fig. 27A.

Lower: (34B, Experiment TT44); Retinotectal projection,

mapped 194 days after the tectal reimplantation.

Encircled electrode position 5 represents the site

of electrolytic lesion.   The conventions are the

same as in Fig. 27A.

RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
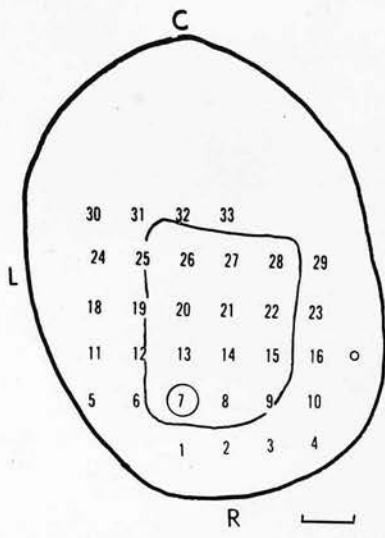
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
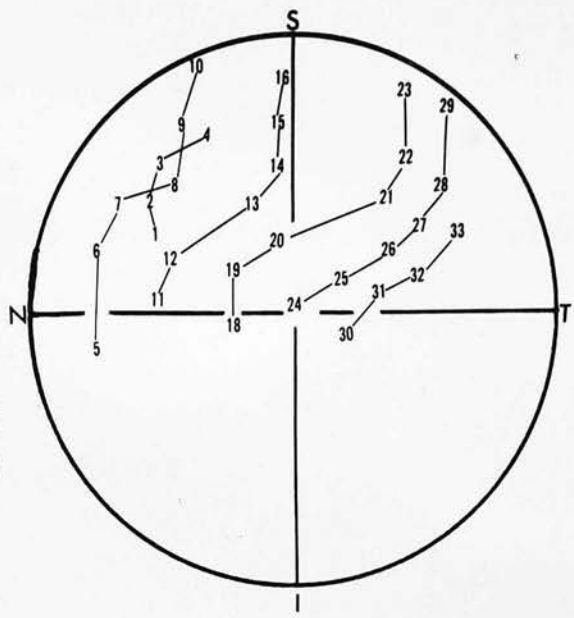
FIG. 35 A and B

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER TECTAL REIMPLANTATION

Upper: (35A, Experiment TT45); Retinotectal projection,

mapped 239 days after the tectal reimplantation.

No visual responses were recorded at electrode

positions marked O.   The conventions are the same

as in Fig. 27A.

Lower: (35B, Experiment TT48); Retinotectal projection,

mapped 366 days after the tectal reimplantation.

Encircled electrode position 17 indicates the site

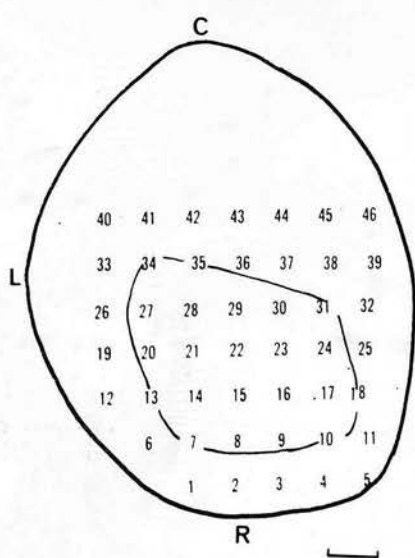of electrolytic lesion.   The conventions are the

same as in Fig. 27A.

RIGHT OPTIC TECTUM **LEFT VISUAL FIELD**

RIGHT OPTIC TECTUM **LEFT VISUAL FIELD**

FIG. 37A

Upper: (37A): Left diagram shows 9 electrode positions,
within small square in the tectum, which were changed
from the chronological order so as to represent 90°
clockwise rotation. Right diagram shows the
predicted visual map which would occur due to 90°
rotation of the tectum. This is a hypothetical
case. This reconstruction is based on Fig. 22.

FIG. 37B

THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM WITH 90° ROTATED TECTAL REIMPLANTATION

Lower: (37B, Experiment TT21); Retinotectal projection,
mapped 61 days after 90° rotated tectum. Encircled
electrode position 29 indicates the site of electro-
lytic lesion. The conventions are the same as in
Fig. 27A.
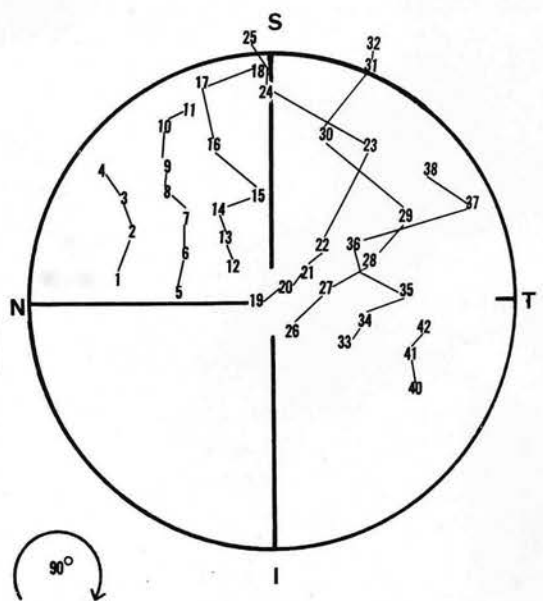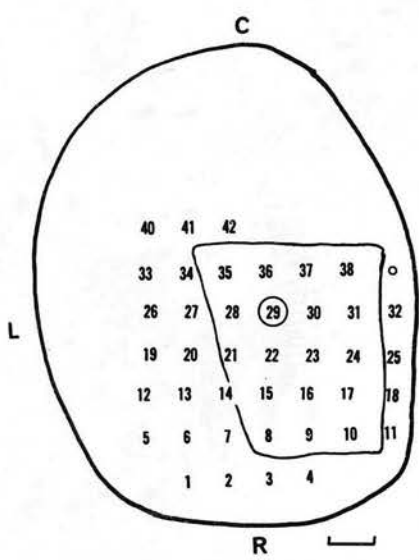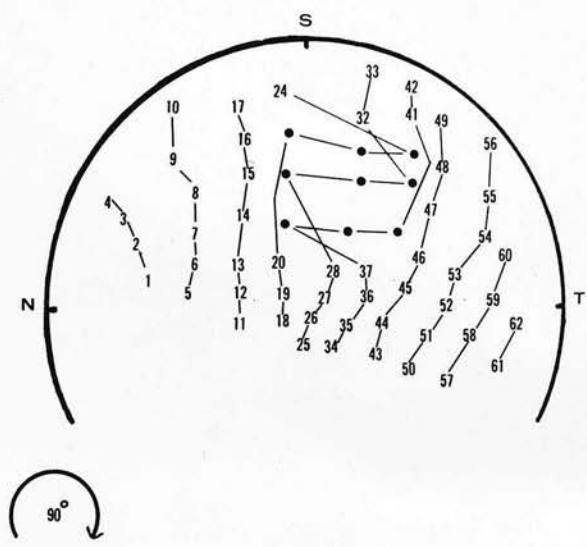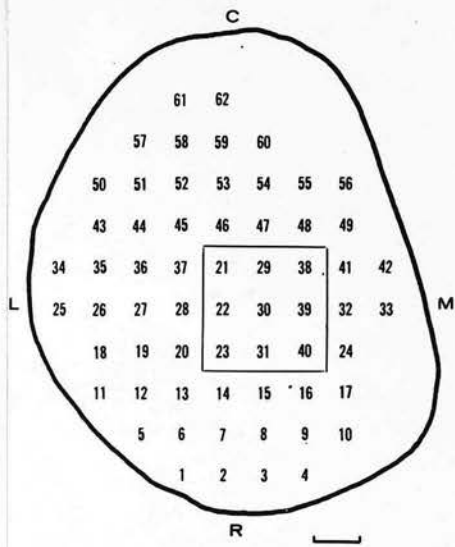
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
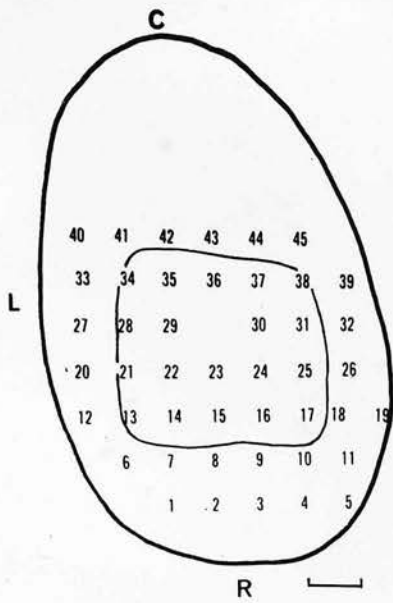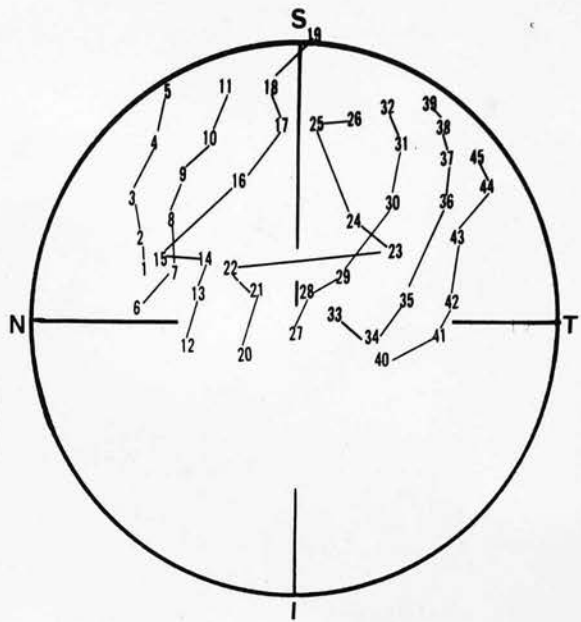
THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT

OPTIC TECTUM AFTER 90° ROTATED TECTAL REIMPLANTATION

Upper: (38A, Experiment TT53); Retinotectal projection,

mapped 171 days after 90° rotated tectal

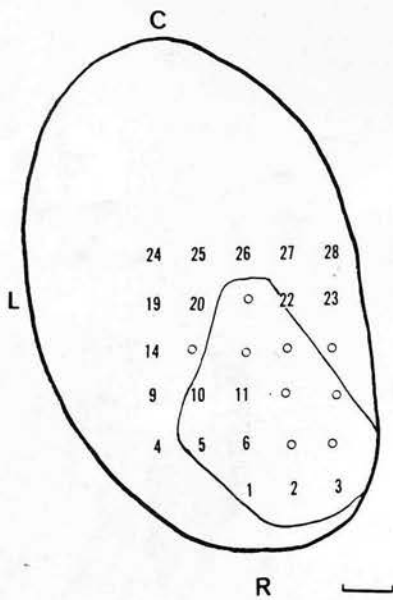reimplantation.   The conventions are the same as

in Fig. 27A.

Lower: (38B, Experiment TT54); Retinotectal projection,

mapped 172 days after 90° rotated tectal

reimplantation. No visual responses could be

obtained from the electrode positions marked O.

The conventions are the same as in Fig. 27B.
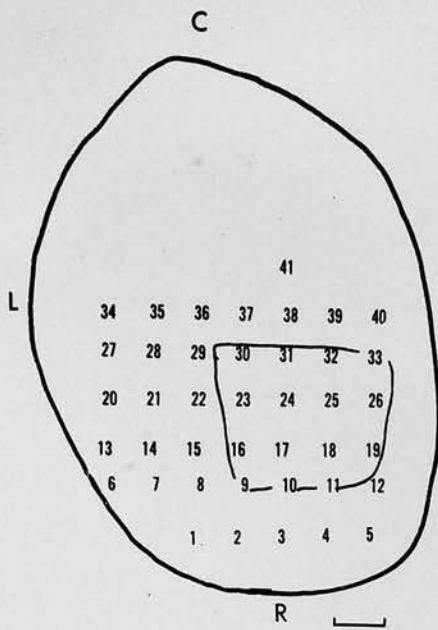
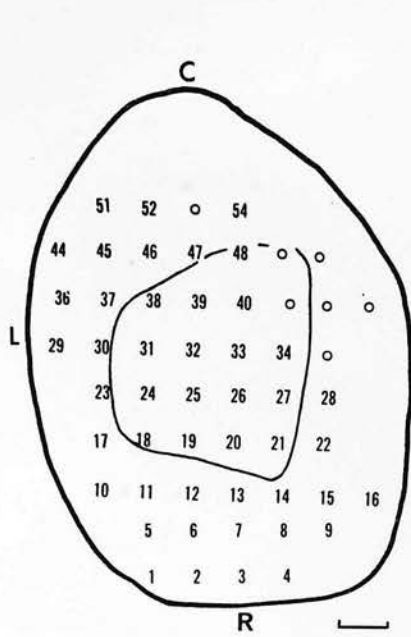RIGHT OPTIC TECTUM   LEFT VISUAL FIELD
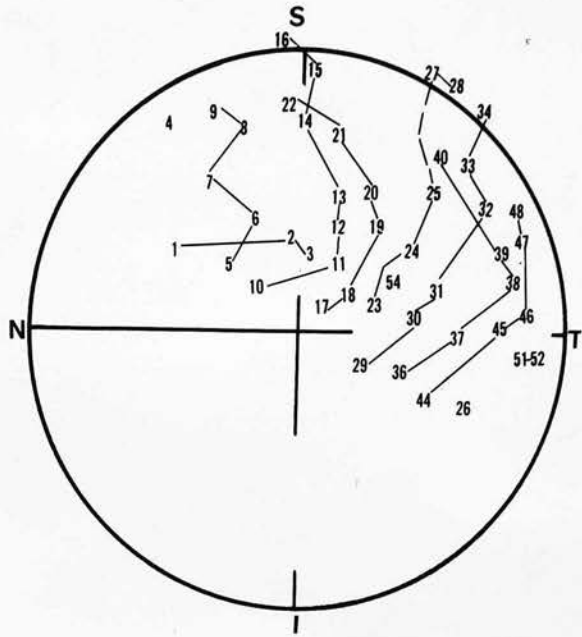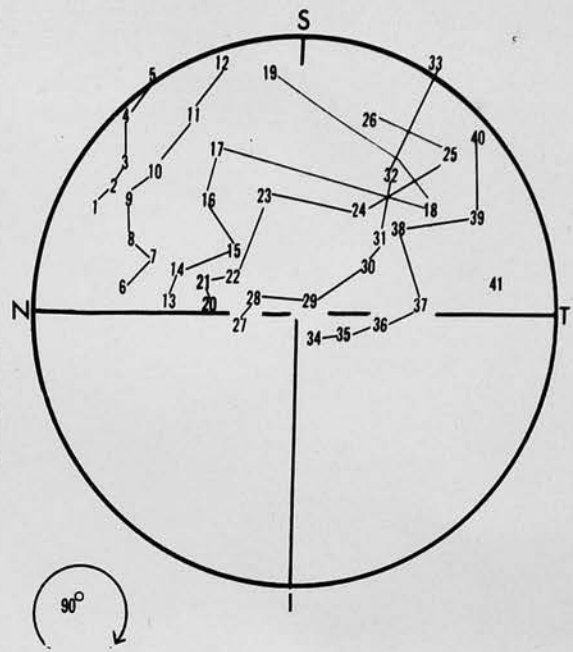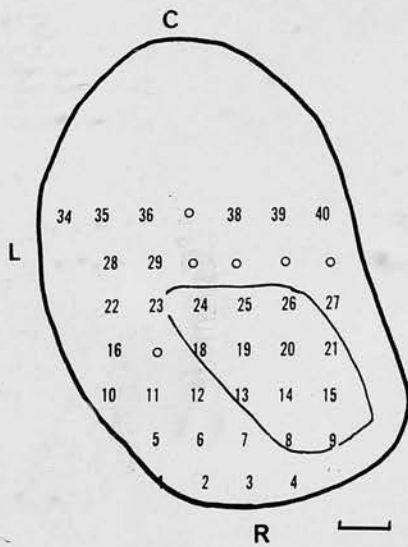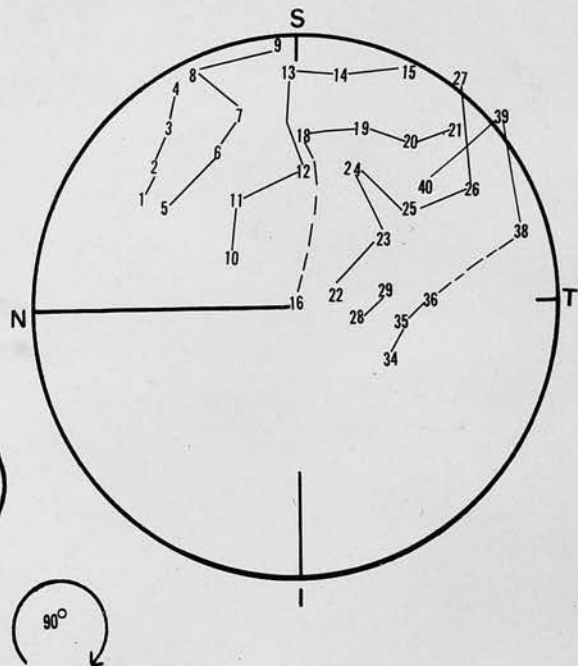
RIGHT OPTIC TECTUM   LEFT VISUAL FIELD

FIG. 38C

Photomicrograph of the Goldfish optic tectum taken before
the mapping experiment.   Left side shows the right optic
tectum and the area of the graft.   A few conspicuous fibre
bundles are seen near the edges of the graft.   The fibre
pattern on the left tectum (right side) is very clear.
Rounded lobe (on top of the picture) is the cerebellum.   The
scale is equal to 600 microns.
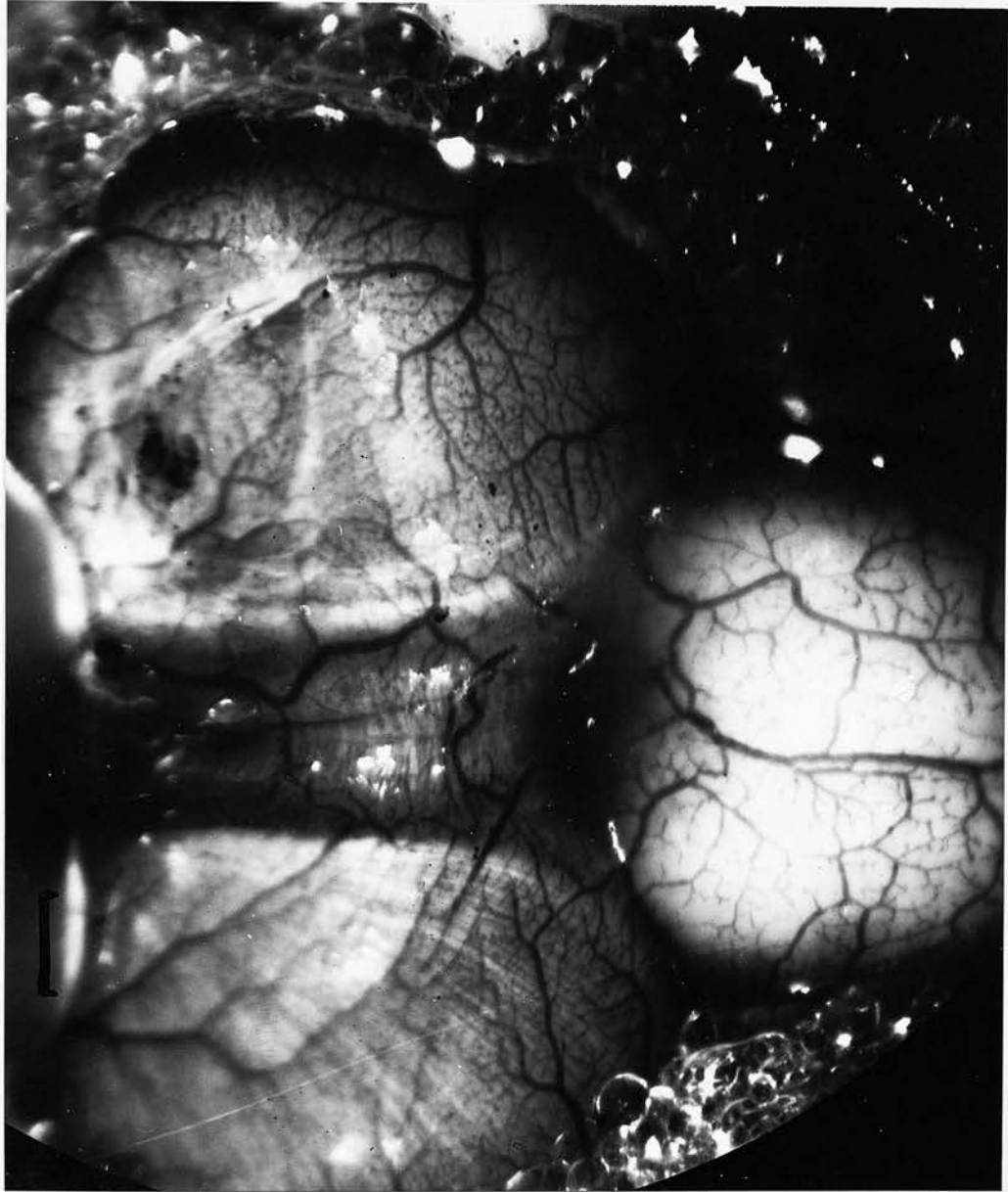
THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE RIGHT
OPTIC TECTUM AFTER 180° ROTATED TECTAL REIMPLANTATION

Upper: (39A, Experiment TT57); Retinotectal projection,
mapped 121 days after 180° rotated tectal
reimplantation. No visual responses could be
obtained from the electrode position marked O.
The conventions are the same as in Fig. 27A.

Lower: (39B, Experiment TT66); Retinotectal projection,
mapped 91 days after 180° rotated tectal
reimplantation. No visual responses were
obtained from the electrode positions marked O.
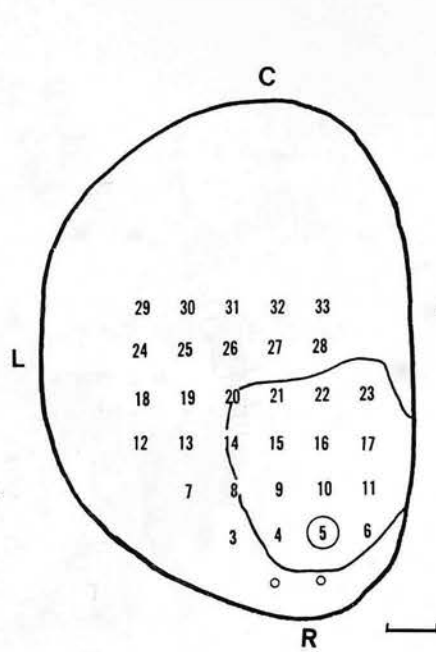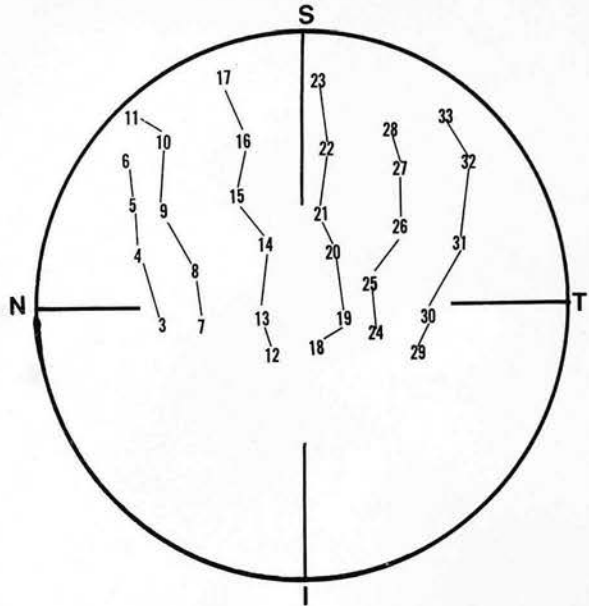The conventions are the same as in Fig. 27A.

C

S

RIGHT OPTIC TECTUM

LEFT VISUAL FIELD

180°

C

S

RIGHT OPTIC TECTUM

LEFT VISUAL FIELD

180°
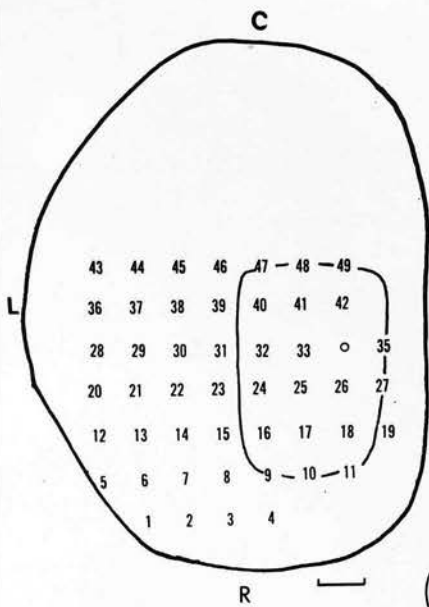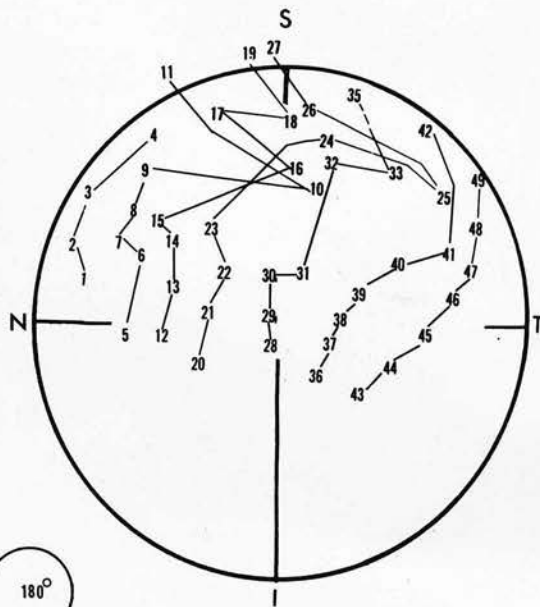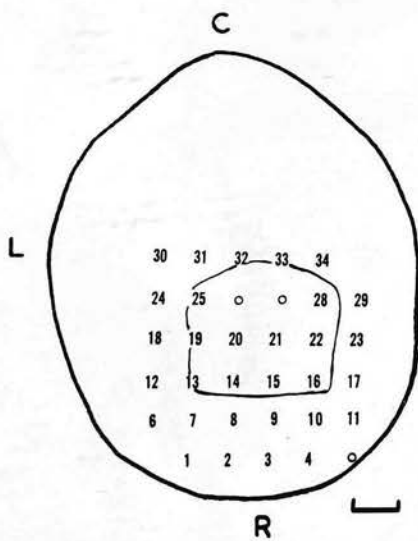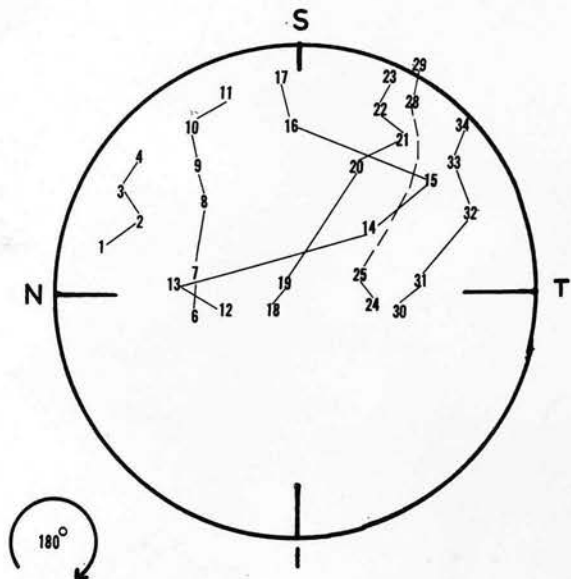
THE REPRESENTATION OF THE LEFT VISUAL FIELD ON THE

RIGHT OPTIC TECTUM

(Experiment TT55)

Retinotectal projection, mapped 114 days after the removal

of posterior tectum.   Dotted-hatched area represents the

extent of the tectal removed.   No visual response could be

obtained from the electrode positions marked O (within the

hatched area).   The conventions are the same as in Fig. 27A.
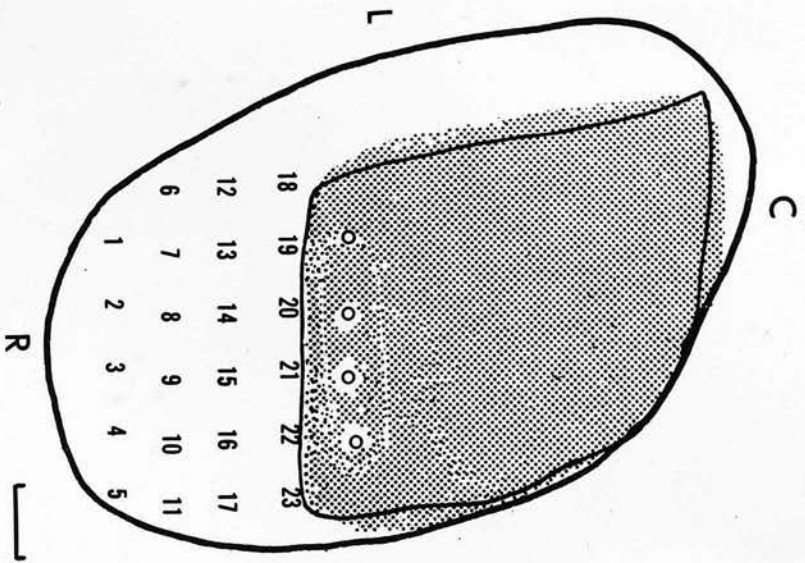
RIGHT OPTIC TECTUM
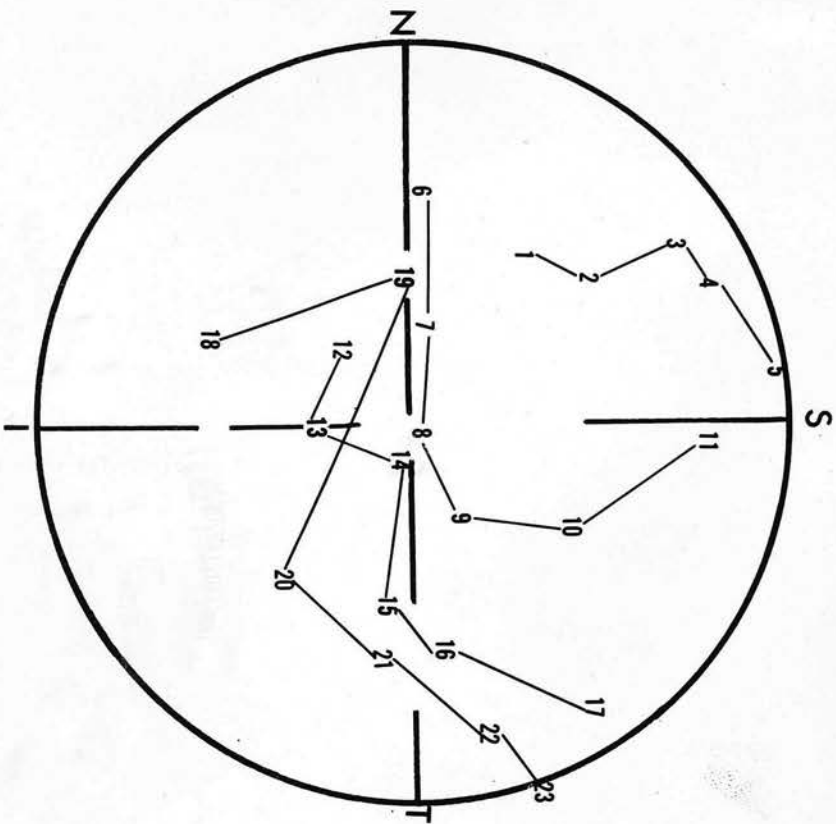
LEFT VISUAL FIELD

THE REPRESENTATION OF THE VISUAL FIELD (RIGHT AND LEFT)

ON THE OPTIC TECTUM

(Experiment TT62)

Retinotectal projection, mapped 97 days after the removal of

posterior tectum (Dotted-hatched area; upper left). The

representation of the right visual field on the left optic

tectum (unoperated) of the same animal is also shown. The

conventions are the same as in Fig. 27A.

OPTIC TECTUM

RIGHT   LEFT

RIGHT VISUAL FIELD   LEFT VISUAL FIELD
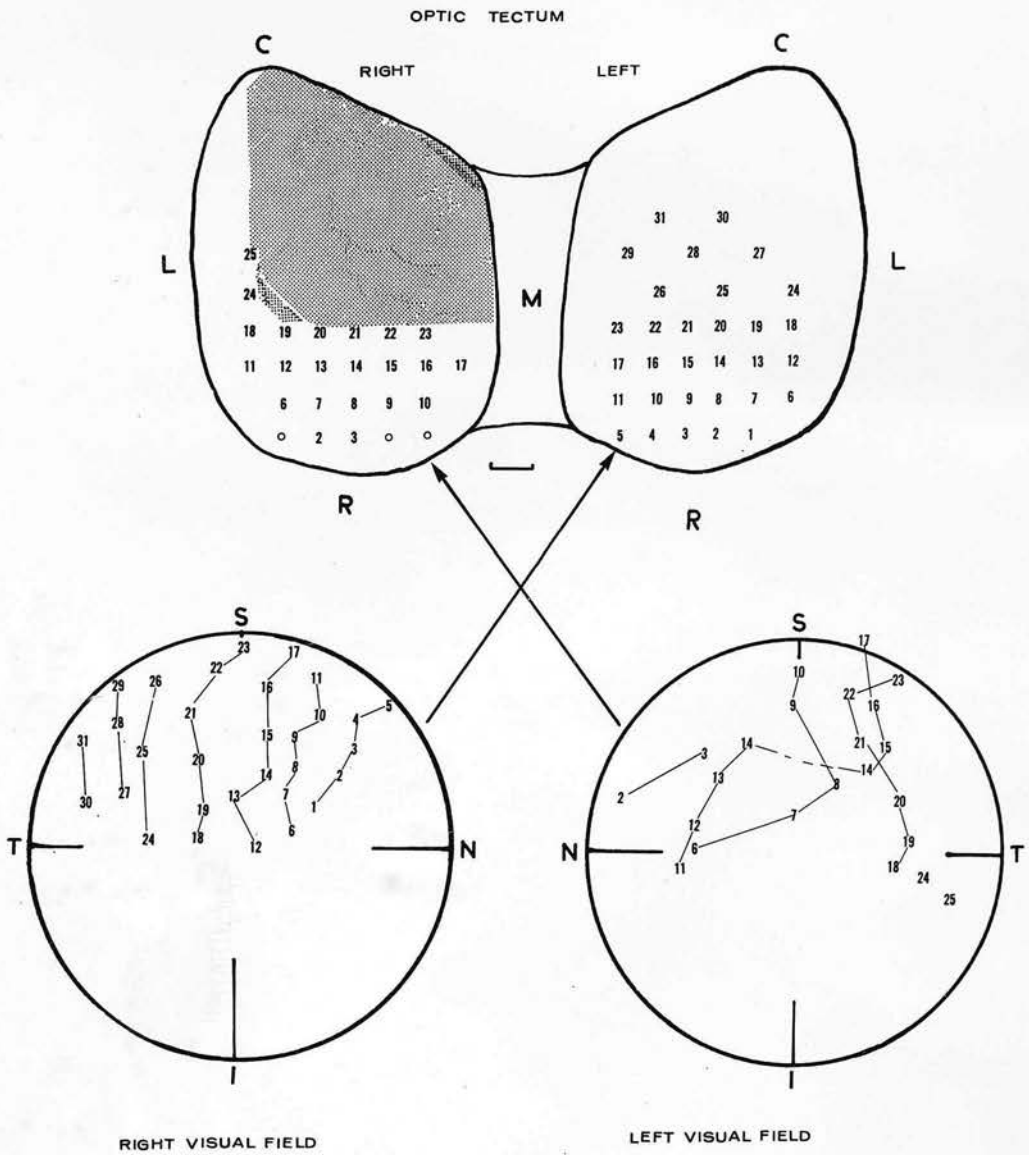
FIG. 41A

Photomicrograph of a transverse section through the mid-brain of Goldfish, (normal animal) caudal to the exit of the oculomotor nerve.   Scale equals 0.3 mm.
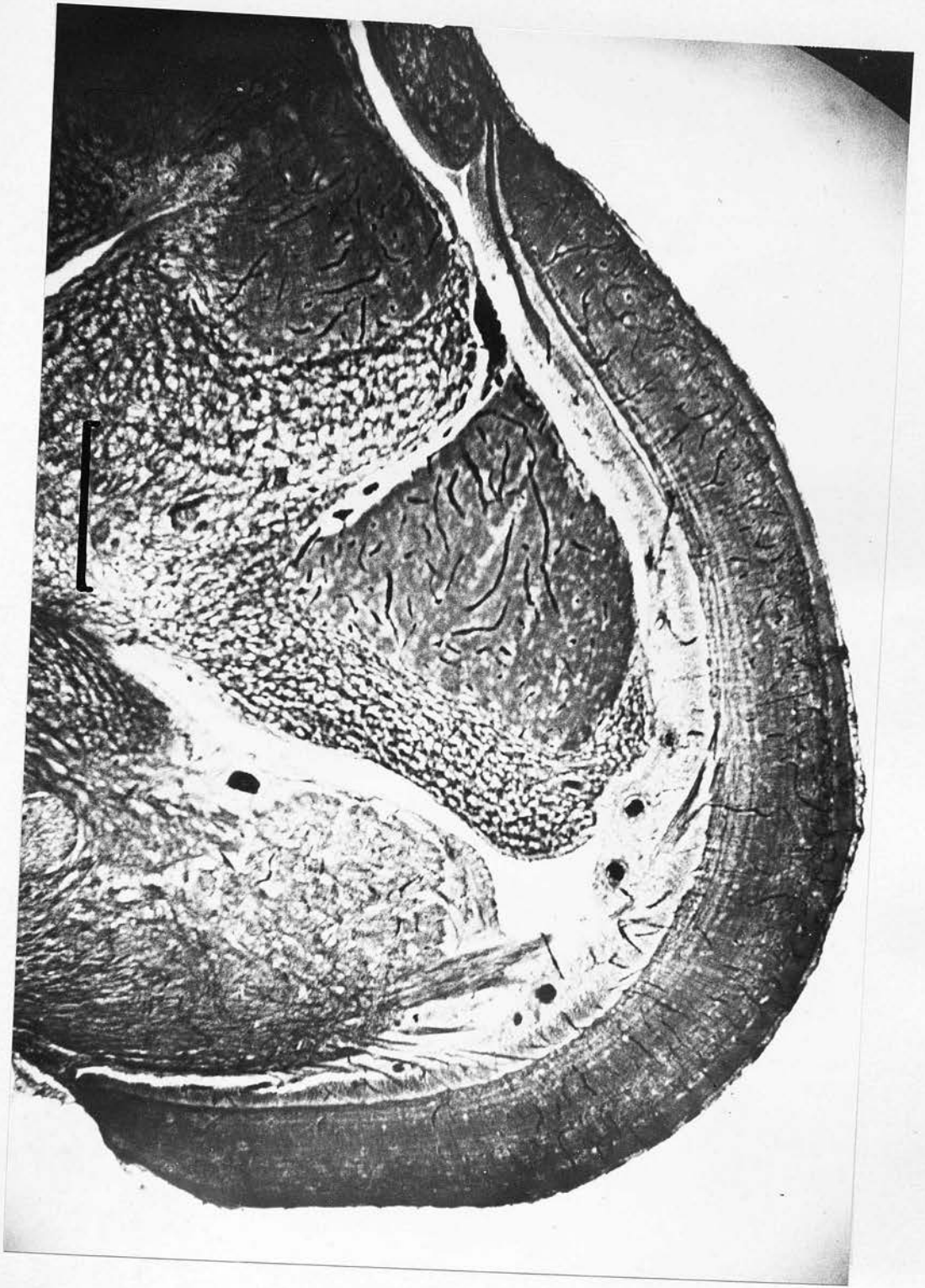
FIG. 41B

Photomicrograph of a transverse section through the mid-brain of Goldfish (normal animal) showing various layers (Holmes' Silver preparation).   The bar represents 0.1 mm.

FIG. 42

Photomicrograph of a transverse section through the mid-brain
of Goldfish.   This fish was the one giving the result shown
in Fig. 26.   The extent of the graft is between the two
lightly stained areas.

15 μ Holmes' Silver stained sections.   The bar represents
0.03 mm.

<u>FIG. 43</u>

Photomicrograph of a transverse section of the tectum at
the site of incision (u shaped, lightly stained area).
This fish was the one giving the result shown in Fig. 26.
Right side of the picture shows the lack of layered
structure.   Bar represents 0.1 mm.
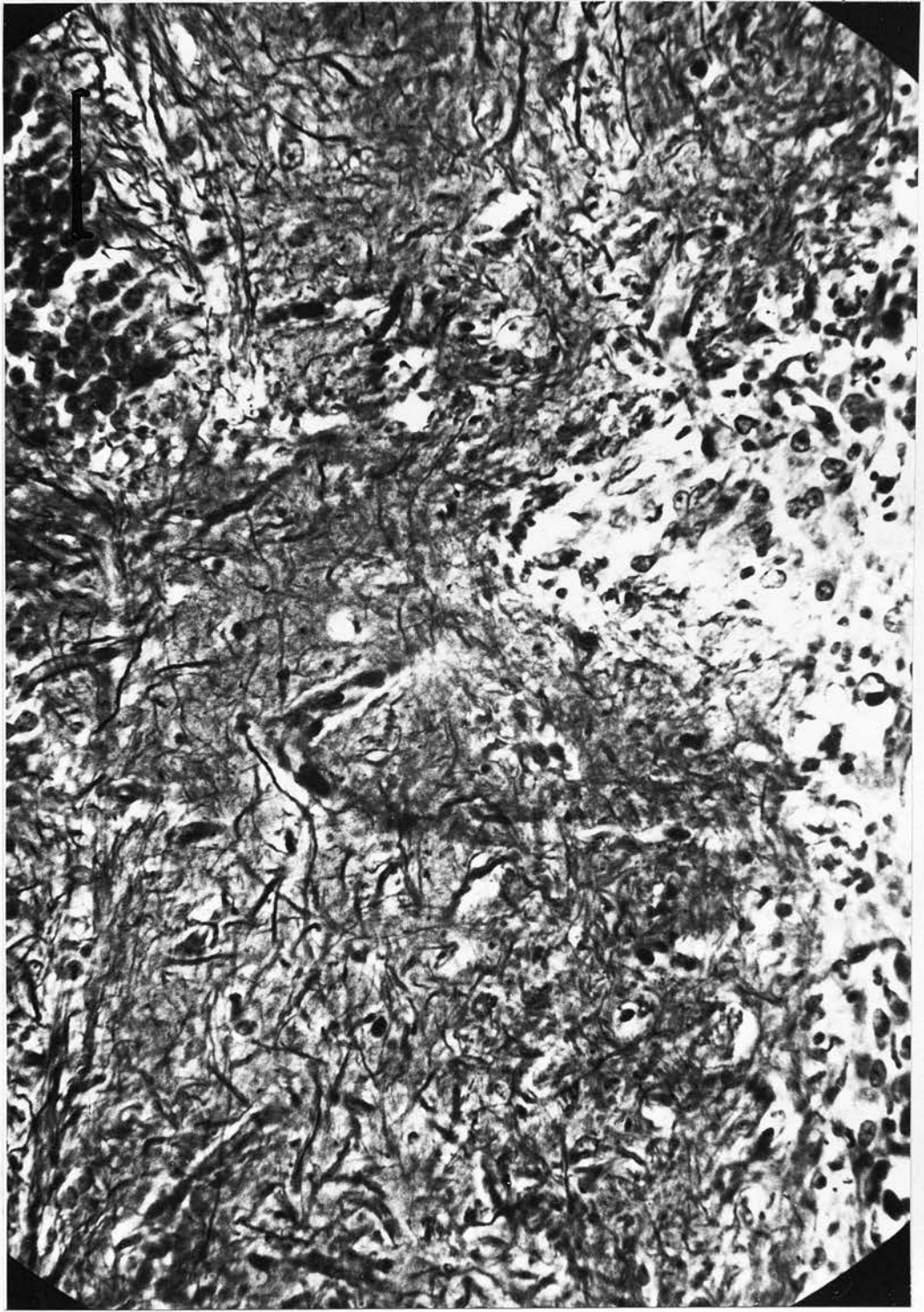
FIG. 44

Photomicrograph of a transverse section of the tectum in
the middle of the graft showing the absence of normal
layered structure.   This fish was the one giving the
result shown in Fig. 26.   15 μ Holmes' Silver stained
section.   The bar represents 0.1 mm.

FIG. 45

Photomicrograph reconstruction of the transverse section of
the tectum from the middle of graft showing absence of normal
layered structure.   U shaped fibre bundle is evident.   This
fish was the one giving the result shown in Fig. 34A.
15 μ Holmes' Silver stained section.
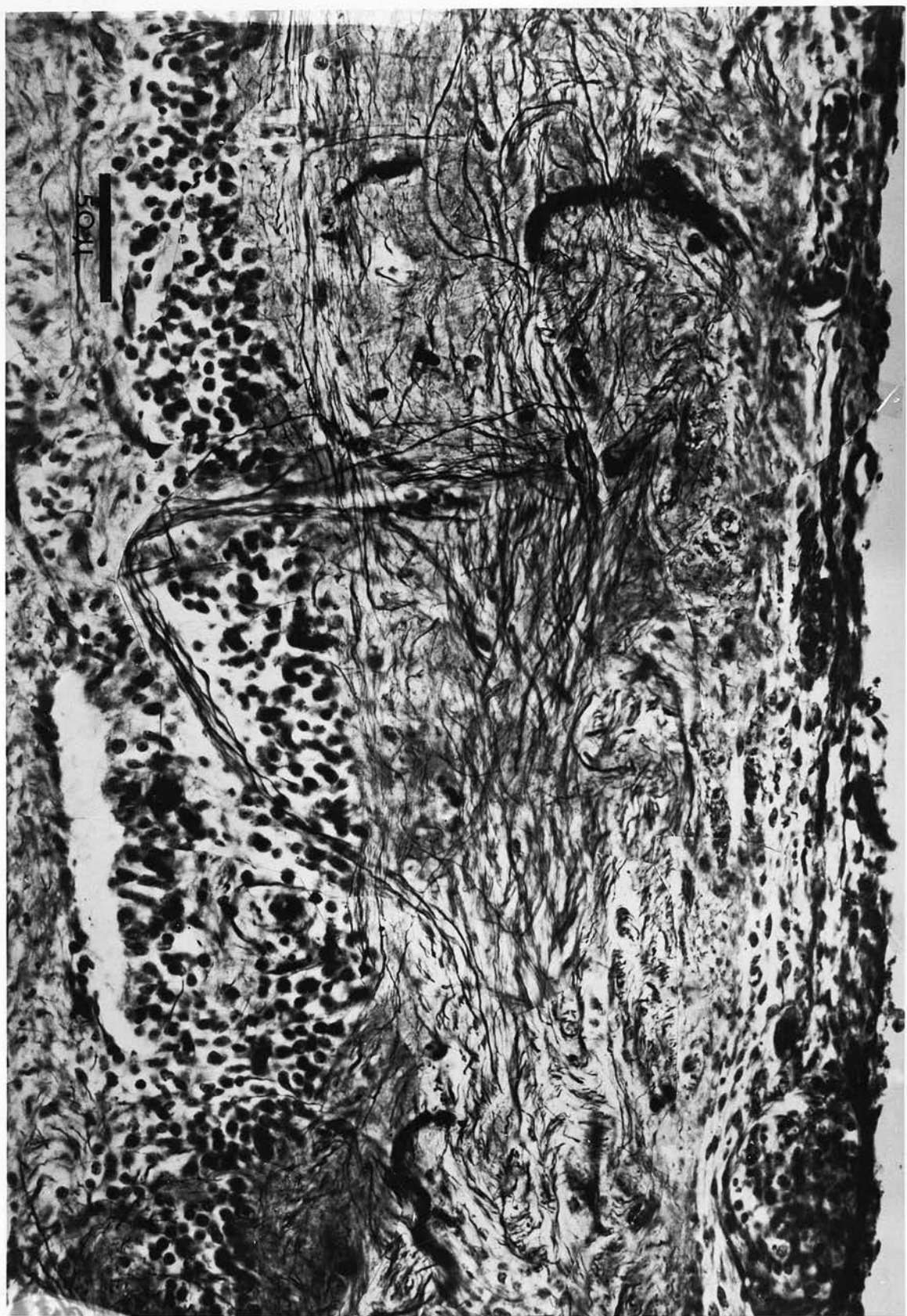
FIG. 46A

Photomicrograph of a transverse section through the mid-brain
of goldfish showing the histological details within the graft.
There is a lack of layered structure.   This section passed
through the middle of the graft.   This fish was the one
giving the results shown in Fig. 38A.   The bar represents
0.1 mm.   15 μ Holmes' Silver stained section.
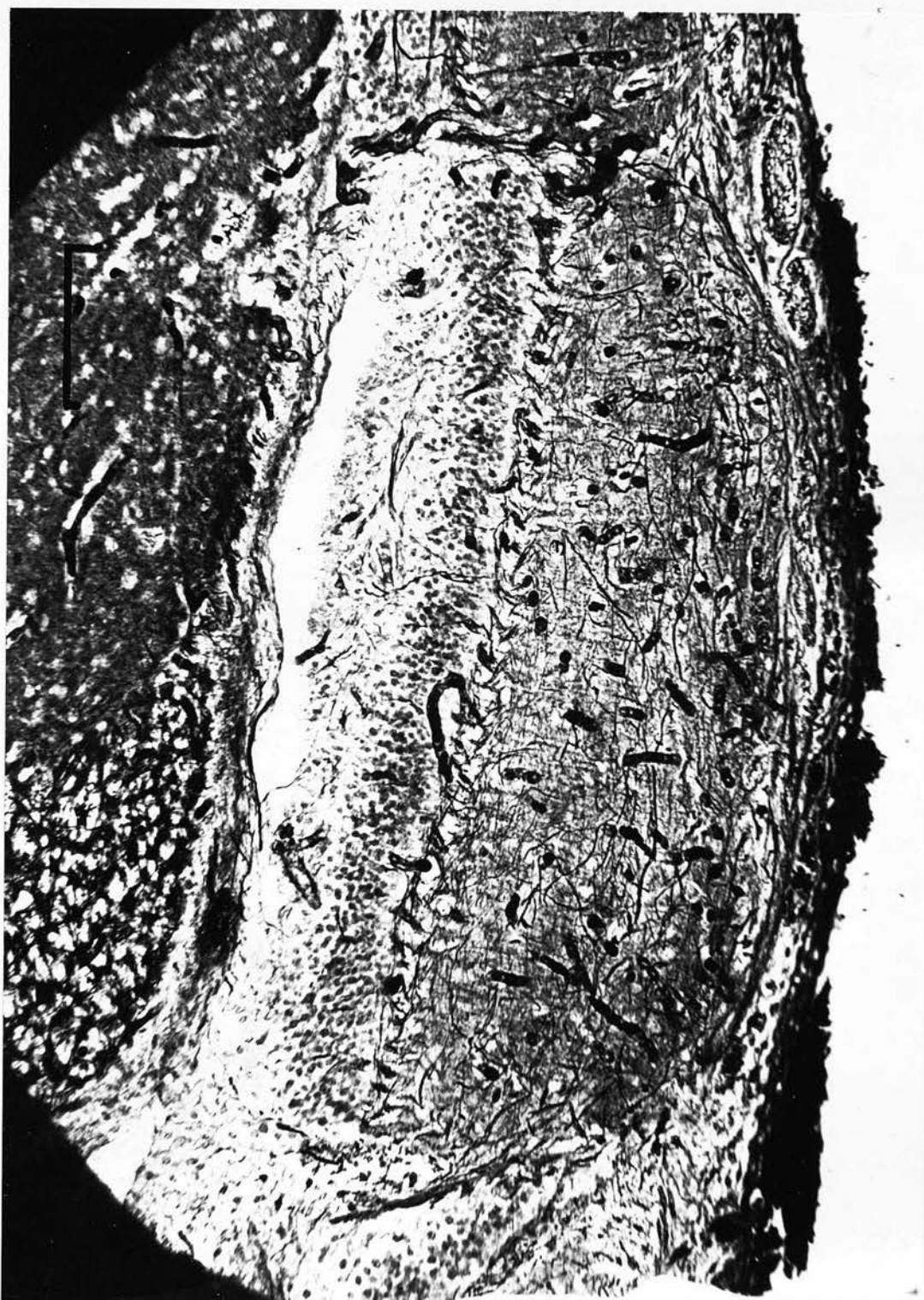
Photomicrograph of a transverse section through the mid-brain
of the goldfish showing the histological details in the
posterior region of the graft.   Lack of layered structure
is evident.   This fish was the one giving the results
shown in Fig. 38A.   The bar represents 0.1 mm.   15 μ
Holmes' Silver stained sections.
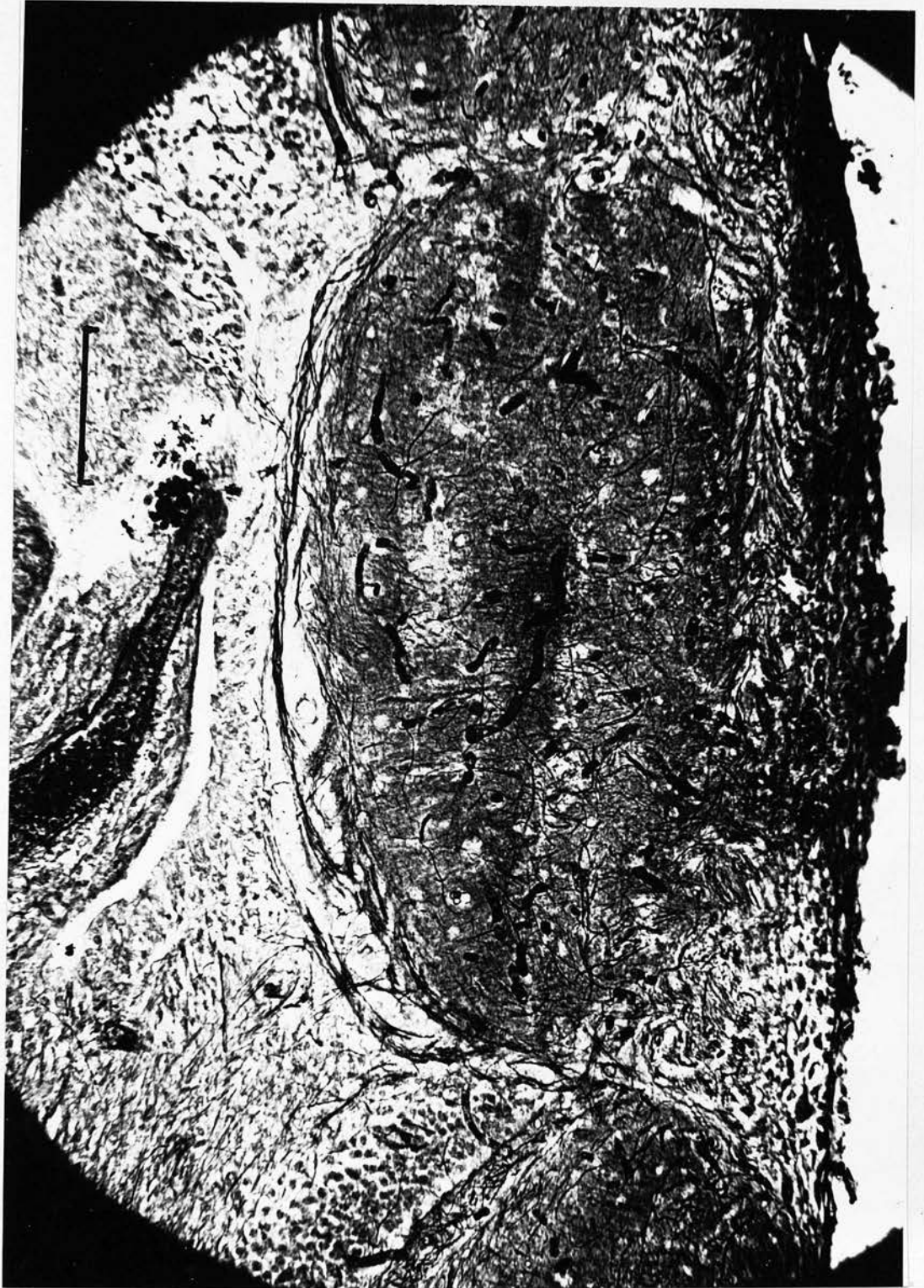
FIG. 46C

Photomicrograph of a transverse section through the mid-brain
of the goldfish showing the histological details on the
anterior region of the graft.   U shaped fibrous bundles which
hang down from the graft are shown.   Total lack of layered
structure of the tectum and the absence of grey periven-
tricular cellular zone is shown.   15 μ Holmes' Silver
stained sections.   The bar represents 0.2 mm.   This fish
was the one giving the results shown in Fig. 38 A.

FIG. 47

EXPERIMENT TT52

Goldfish brain was cut into transverse serial sections 15

microns thick, and a camera lucida drawing of every tenth

section in the region of the lesion is shown in the

figure.   The optic tectum within the graft is abnormal

in appearance but the optic tectum surrounding the graft

is normal.   This fish was the one giving the results shown

in Fig. 32B.
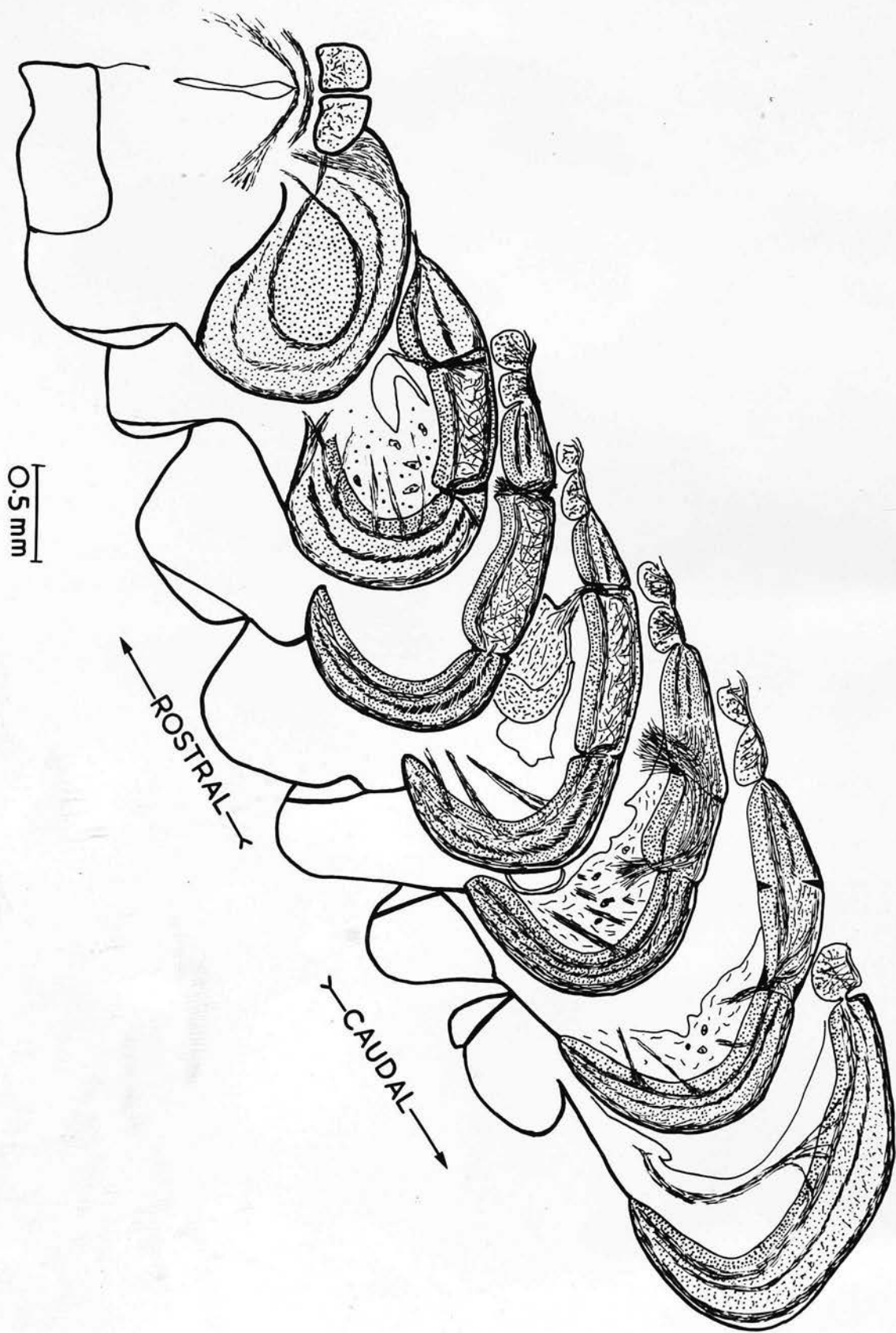
0.5 mm

ROSTRAL

CAUDAL

Photomicrograph of a transverse section through the mid-brain of goldfish showing the histological details of 2 days old graft.   Vacuolization and the formation of incision canal at the site of lesion is shown.   10 μ thick Iron Haematoxylin stained sections.   The bar represents 0.1 mm.

<u>FIG. 49</u>

Photomicrograph from the lower layer of 2 days old tectal
graft (Fig. 48).   Hyaline area surrounding the nucleus
is shown.   10 μ thick Iron Haematoxylin stained sections.
The bar represents 0.03 mm.
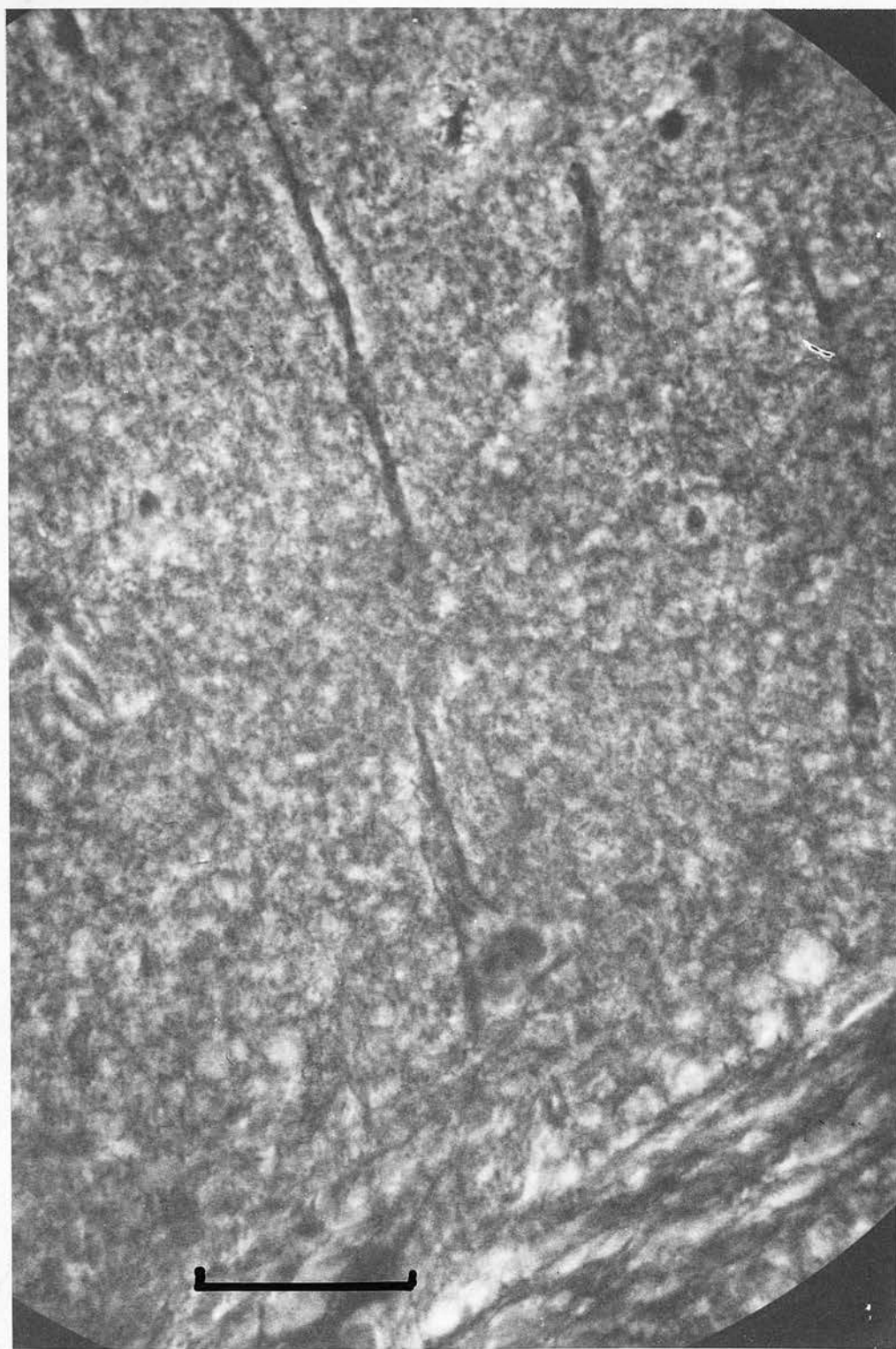
FIG. 50

Photomicrograph of a transverse section through the mid-brain of goldfish showing the extent of the graft and the site of lesion filled with meningeal tissue and the vacuolization in the outer zones of the graft.   The graft is 3 days old. The bar represents 0.1 mm.

<u>FIG. 50A</u>

Photomicrograph from the grey periventricular zone within the graft showing mitotic activity.   In the centre of the picture 2 daughter cells represent the late telophase. The bar represents 0.01 mm., a 3 days graft.
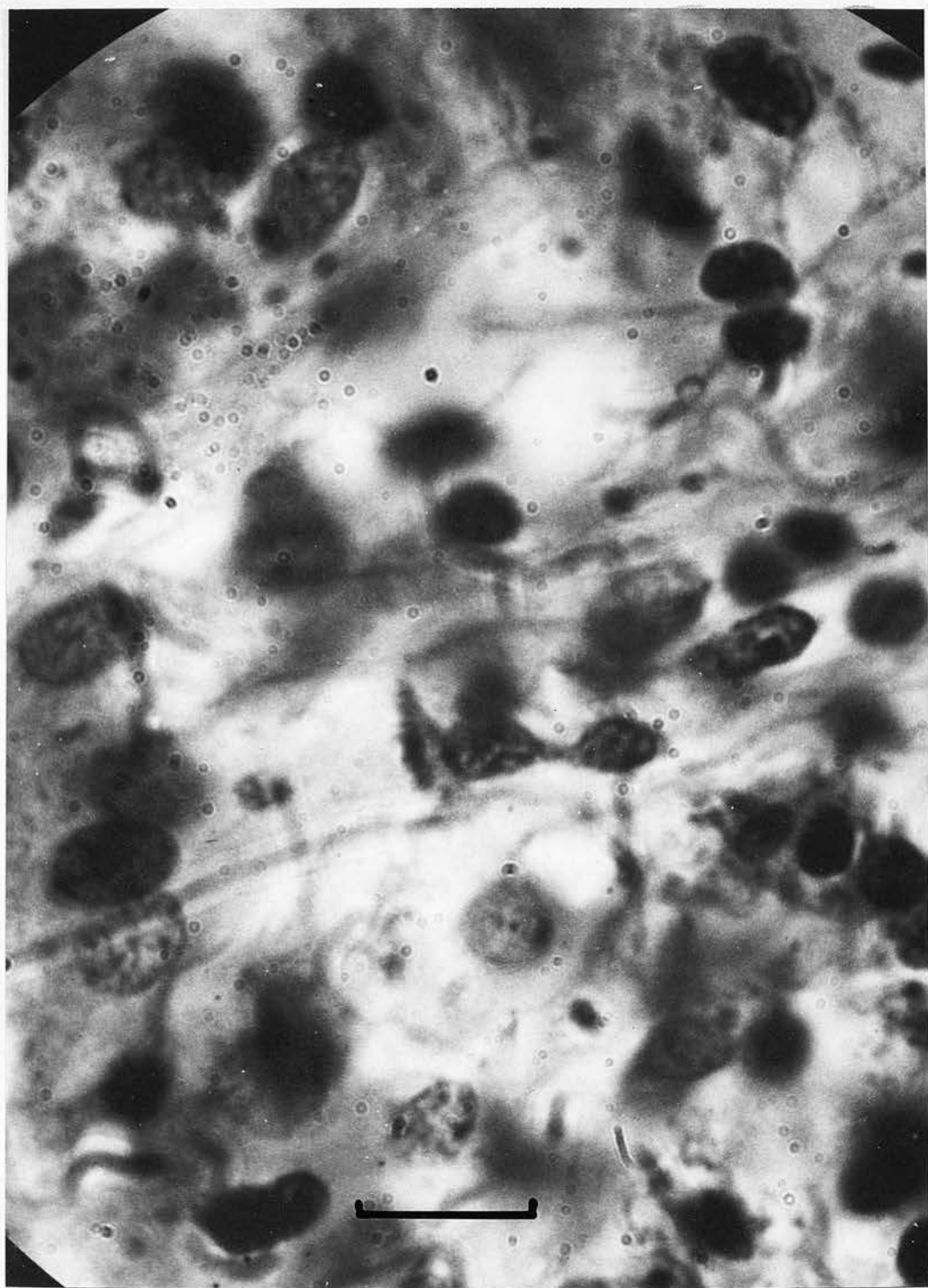
FIG. 50B

Fresh preparation of the 3 days old tectal graft showing
cytoplasmic bridge between 2 separate lobes.   Phase
contrast, the bar represents 0.01 mm.
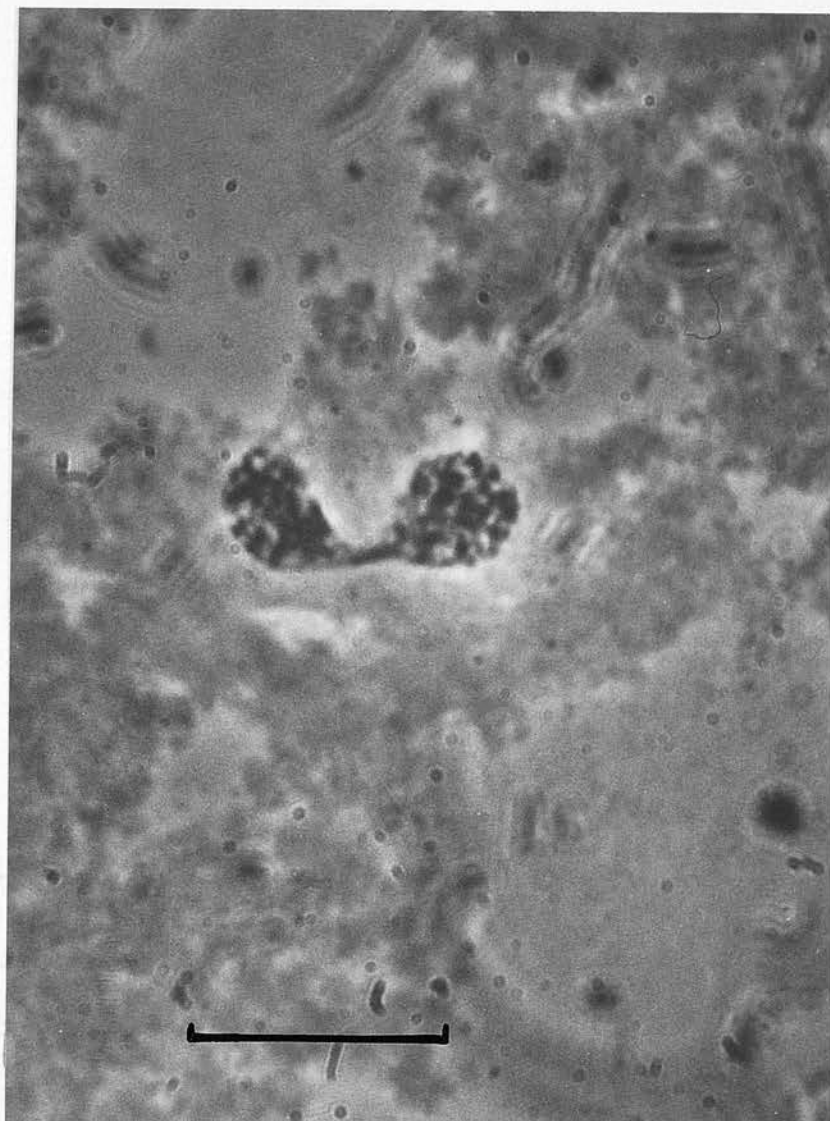
FIG. 51A

Photomicrograph of the transverse section of the optic tectum
of goldfish showing 12 day graft with lack of layered
structure and absence of most of the fibrous layer in the
outer zones.   15 μ Holmes' Silver impregnated sections.
The bar represents 0.01 mm.

FIG. 51B

Photomicrograph of the transverse section of the optic tectum

of goldfish showing 12 days graft with lack of layered

structure.   On the left hand side, picture shows the

formation of fibrous bridge.   Outer zones of the tectum

are chaotic.   10 μ Glees' reduced silver stained sections.

The bar represents 0.1 mm.

FIG. 52

Photomicrograph of the transverse section of the optic tectum
at the site of lesion. This animal was with 20 days graft.
Picture shows the emergence of fibrous bundles from the host
tissue (left side). Lack of layered tectal structure is
evident. 15 μ Glees' reduced Silver stained sections. The
bar represents 0.1 mm.

DISCUSSION
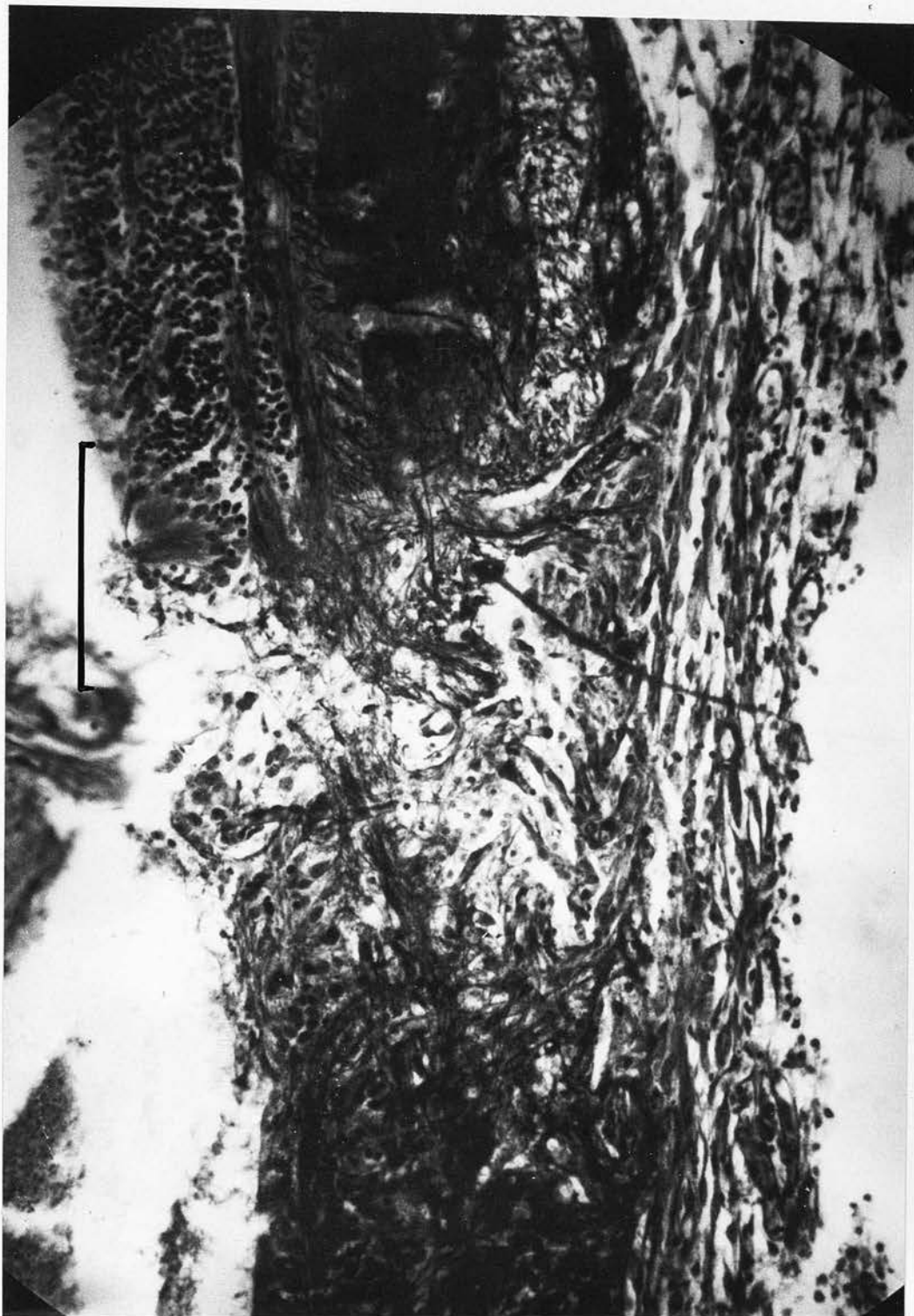
The neuro-anatomical picture of a given nucleus (e.g.
tectum) is compounded from the results of applying several
different experimental techniques.   In the case of the
optic tectum the ideas of different workers on the number
of strata present are conflicting.   Sholl (1956) demon-
strated that the ratio of the number of Golgi-impregnated
cells to the number of Nissl-stained cells remained roughly
constant (1/17) through the layers of cerebral cortex. From
his extensive study of cerebral cortex, Sholl (1956) concluded
that the number of cells revealed by the Golgi method was
representative of the total present, and that the next step
was by inference.   In the previous studies on the optic
tectum of fish studied by Golgi-techniques (P. Ramon, 1890;
Ramon y Cajal, 1911; Huber and Crosby, 1934 and Leghissa,
1955) discrepencies in the results are evident.   By using
extensive variations of the rapid Golgi-technique, the
present findings indicate that the extent and completeness
of impregnation is governed by the length of exposure to
the fixative; the darkness of the impregnation, by the
exposure to silver ions.   It is quite apparent in the
present study that partial impregnation of neurons and their
processes has occurred.   These observations are in conflict
with the idea that Golgi impregnation, when it occurs, affects

the entire neuron with all its processes. Most of the
partial impregnation is encountered in the axons. However,
with Golgi-Cox techniques, the axon is rarely seen.
Dendrites are generally seen with apparently complete
impregnation. Discrapencies in the results of previous
workers can be explained if one takes into account partial
or complete impregnation of the tectum. In the last few
years, Golgi technique has attracted the attention of numerous
workers and new refined Golgi techniques with known reaction
chemistry are available. The picture of the optic tectum
given here shows the presence of a few cell types which have
not been observed so far. Some of these cells could be seen
only with single impregnation and others with double or triple
impregnations. Moreover, double or triple impregnation
obscures certain cells due to the heavy deposition of silver
ions in the inter-cellular spaces.

Looking at the pattern of optic nerve fibres spreading
over the tectum it becomes clear that most of the neurons in
the optic tectum may receive information directly from the
optic nerve fibres.

Lazar and Székély (1967) have found in the frog that the
number of optic nerve fibres and the total number of tectal
neurons is approximately the same. They suggested that each
tectal cell may receive visual information directly from optic

fibres.   In order for this to be true, cells in the lower periventricular zone would have to have processes extending up to the sixth layer, this being the deepest part to which optic fibres penetrate.   Although Lazar and Székély could see such centrifugal fibres in their frog Golgi preparations, few periventricular cells in the fish have such processes (Kirsche, 1961 and Kirsche and Kirsche, 1964 have seen cells without any processes in the periventricular zones in the fore brain and mid brain of the fish and frog). Whether or not similar cells exist in the lower zones in the frog and fish tectum can only be judged in electron microscopic preparations.

The presence of horizontally orientated pyramidal cells and inverted pear-shaped cells in the external plexiform layer is a new finding.   Both cells have a branching axon.   Their axons and horizontally orientated dendrites run in the vicinity of optic terminals.   It is quite possible that these cells or their dendrites form synapses with optic fibres and pass their message to the spino-tectal and bulbo-tectal tracts, and thalmo-tectal portion of the brachium tecti, which are known to originate from this zone.   In double and triple impregnations, these cells become obscure.

The presence of ependymo-glial cells mentioned earlier by P. Ramon (1890); Wlassak (1893); Gaupp (1899) and Lazar

and Székély (1967) in frog has also been observed in these
studies.   It is suggested that these cells form the supp-
orting elements of the optic tectum.

Apart from a few horizontally orientated cells in the
tectum, most of the cells and their processes are vertically
organised as are the optic fibres and their terminals.
These factors suggest the possibility that optic tectum
may be organised on a similar pattern to cat's visual
cortex into vertically orientated columnar integrative units
of cell assemblies (Hubel and Wiesel, 1963).

The present study is concerned primarily with neuro-
blast migration and the formation of the outer white zone and
does not include a study of earlier stages, in which neuro-
blasts are differentiated from early neural tube cells. This
would be necessary for a more extensive discussion of the
identity of primary neuroblasts and fibre groups in terms of
adult neurons and fibres.

The following generalizations can be made about the
lamination of the optic tectum from the present observations
on its development and growth.

1.   The first migratory neuroblasts appear on the outer
      boundary of the grey periventricular zone, 22 days
      before hatching.

2.   The migration of neuroblasts, which starts from the

anterior-lateral margin of the tectum, proceeds mediocaudally.

This observation is in accordance with the initial neuroblast differentiation in the mesencephalon of the chick (Lyser, 1966). The organisation of the tectum may result from the very early differentiation of the medullary plate and neural tube. Corner (1964) demonstrated the developmental capacities of neural plate under experimental conditions (e.g. experimental regional induction or development of isolated regions). He reported the development of characteristic patterns of function in the neural plate of Xenopus laevis. The pattern of early migration of neuroblasts invading the outer white zone implies a specific regional differentiation within the neural tube at the time of initial differentiation of the neuroblasts. Although Lyser (1966) showed in the initial neuroblast differentiation of the mesencephalon of the chick, the presence of a characteristic specific pattern in location and sequence of differentiation of groups of neuroblasts; no evidence for a specific pattern in the case of individual cells has been found.

EVIDENCE FOR THE MIGRATION OF NEUROBLASTS

Contrary to Tilney's (1933) migratory lamination theory, no evidence for the primary, secondary and tertiary migratory laminations was found in Nissl stained preparations in the

present experiments; although a band, one cell thick, was found next to the most superficial layer of the grey periventricular zone. This band remained stationary and of the same thickness even when most of the outer layers were formed.

Agnevine and Sidman (1961) and Berry and Rogers (1965) studied the histogenesis of the cerebral cortex autoradiographically in mouse and albino rat respectively. The pattern of migration was essentially the same in both cases. Berry and Rogers (1965) asserted that the hypothesis that the significance of these migrations may be the establishment of intra-cortical synaptic patterns (Agnevine and Sidman, 1961), must be rejected in favour of their suggestion that the processes of cells do not grow until migration is completed. The present observations strongly suggest that at least during the histogenesis of the optic tectum, the migratory cells which were attached with the parent cells start extending their processes and establish themselves in the area with the help of dendrites (22 days after hatching). At this stage most of the migration is nearly over. Berry and Roger's suggestion that differentiation of the migratory neuroblast only occurs after migration is complete must be rejected in the light of the present observations.

Differentiation starts immediately when the migratory cell, along with its parent cell, reaches a certain level in the outer white zone of the tectum. It is further suggested that the migratory cell detaches itself from its parent cell only after it has formed connections with the surroundings.

Sauer and Walker (1959); Sidman, Miale and Feder (1959); and Fujita (1962 and 1963) asserted that cells of the germinal or ependymal layers were morphologically alike and mitotically active. Fujita (1963) considered these primitive germinal cells to be a morphologically homogenous cell population and called them matrix cells. It is generally agreed now that the entire cellular population of the optic tectum is formed from the matrix cells.

The present results agree with the suggestion of Berry and Rogers (1965) regarding the histogenesis of the cortex upto a point when the parent cell as well as the migratory cell (which is attached by cytoplasmic extension from the parent cell) have one nucleus each. Berry and Rogers suggestion that a nucleus with scanty cytoplasm becomes independent and rounded and develops its processes separately, has no supporting evidence . However, it has been shown in the present study that the story during histogenesis is slightly different from that predicted by Berry and Rogers (1965). The peripheral migratory cell, after attaching

itself to the basal parts of the zonal layer, starts

extending its processes which presumably are the dendrites.

It is suggested that the cell, after dendrite formation,

detaches itself from its parent cell and follows the growth

processes afterwards.   The radial orientation of both axon

and apical dendrites may result from growth by contact (Weiss,

1955) with the radially arranged ependymal processes.

THE PROBLEM OF SPECIFICATION IN NEUROGENESIS

The primary cells which emerge from the neural

epithelium give definite signs of specificity (Corner, 1964).

Each cell follows a particular course during migration to

reach its location and it aggregates with its own type which

suggest a specificity in regard to its own direction.   There

are small variations, especially the question of radially

orientated cell bodies, which are probably due to a number

of other factors influencing the pathways at the same time.

Probably mechanical factors exert some pressure on the out-

growing embryonic fibres as has been shown in the adult

animal where these factors determine the pathways of nerve

fibres (Harrison, 1914; Spéedel, 1933; Weiss, 1934 etc.)

Detwiler (1947) showed the regeneration of gross

morphological structures after unilateral extripation of

parts of the brain or spinal cord in amphibian embryos.

These regenerates have been shown to possess a smaller

number of cell types (Stefanelli, 1951). In Mauthner cells, the time of determination and specification was traced back to the early medullary plate stage (Stefanelli, 1951). These results suggested that specification of early neuroblasts must be fixed in the neural epithelial stage and presumably is genetically controlled. In chick embryo, specification of the tectal neurons has been shown to occur independently before the ingrowth of the optic nerve (De Long and Coḷombre, 1965). The present studies provide no information about tectal specification. In amphibia. there is, as yet, no evidence concerning tectal specification. No experiment has been devised, as yet, to investigate the time of specification of tectal neurons during development in lower vertebrates.

In an experimental analysis of dual origion of the trigeminal ganglion in the chick embryo, Hamburger (1961) showed that the peripheral trigeminal pathways, equally accessible to all fibres, are actually followed only by one type. These discriminating specifications are considered to be biochemical differentials in the substrate. Hamburger (1962) suggested that preneural pathways have to be regarded as sites of biochemical interactions between axon and substrate and not merely as a mechanical track system.

The roll of cell exudates in cell interaction in the

selective adhesion of cells and in directional cell migration, is being studied by many workers (Weiss, 1945; Moscona, 1960 etc.).   Moscona reported evidence for type specificity of exudate from retinal cell.   On the basis of this evidence it is possible to postulate that different axon types produce specific exudates which match the matrix on which they grow during development as well as during regeneration.

The immigration of primary mesenchyme cells in developing sea urchin eggs was analysed by Gustafson and Wolpert (1961). The mesenchyme cells carry out pseudopodial movements and random contacts are formed with ectoderm cells but the final pattern of arrangement of the cells is determined by those regions of the ectoderm with which the most stable pseudo- podial contacts are established.   One has thus the impression that the affinity of the primary mesenchyme cell for specific regions of the ectoderm is greater than that of other regions of the ectoderm.   Wolpert and Gustafson (1967) reported that the random manner in which pseudo- pods are formed in the sea urchin embryo and the highly variable pathways taken by cells seem to preclude the possibility that the mechanism of pattern formation is based on preformed pathways or chemotaxis.   It is easy to presume that cells will move up a gradient of adhesiveness. Wolpert and Gustafson further suggested that all that is

required to specify the pattern taken up by cell is an appropriate pattern of adhesiveness in the wall and random pseudopodial activity. Even when the additional pattern is formed the cells continue to send out and withdraw pseudopods. This leaves the cell sensitive to changes in the adhesiveness of the wall, and the latter rearrangement of the cells can be accounted for on this basis.

EVIDENCE OF GRADIENT:

A gradient of metallic deposition was made to offer a surface with progressively increasing adhesiveness for cells (Carter, 1965). This gradient was constructed by spraying palladium onto cellulose acetate, to which the cells would not attach. Mouse fibroblast cells moved up this gradient in the direction of increasing adhesion to the substrate and their uniformity of movements constrasted with the apparently random movement of cells on evenly metallized cellulose acetate or on glass. As the cells crowded together on the upper end of the gradient, progressively increasing cell to cell contacts interfered with the movement of individual cells. Nevertheless, cell migration continued in this direction. Carter (1965) suggested that movement towards surfaces offering greater adhesion is a general phenomenon applicable to all cells which are dependent on contact with a surface for their mobility. He further suggested that the movement

of a cell is controlled by the relative strengths of its
peripheral adhesion, and that the movements directed in
this way, together with the influence of patterns of
adhesion on cell shape, are responsible for the arrangement
of cells into complex and ordered tissues. Cellular
mobility and the adhesive properties of cells could thus
provide the link between gene action and morphology.

Leghissa (1951) concluded, from his observation of the
development of the trout optic tectum, that retinal as well
as other centripetal fibres have a definite action in modulat-
ing the functional structure of the optic lobe. He did not
mention the migration of neuroblasts from the inner grey zone
to the outer white zone. The different stages of development
mentioned by Leghissa were only considered from the point of
view of the total thickness of the tectum and the layers formed
by various neurons. Behavioural development (especially
visual) was completely ignored by Leghissa. It is important
to know that at what stage visual behaviour starts. The

present study shows that the histological appearance of the
tectum before migration starts is one of total lack of
differentiation. A great deal of mitosis is observed in
these cells, 22 days before hatching. Up to nine days
before hatching most of the cells which are migratory in
the outer white zones and the lower cellular zone are still
undifferentiated or early differentiating neuroblasts.
Active multiplication in the lower areas is evident. The
early white substance when studied under high magnification
shows the presence of numerous thin fibres which are mostly
the extensions of newly differentiating underlying cells.

Optokinetic responses start at the stage of development
when the outer white zone of the tectum is equal in thickness
to the inner grey zone. Most of the outer layers are formed
at this time and no mitotic activity is noticed in the grey
periventricular zone. It is deduced from these observations
that optic nerve fibres which started invading the tectum
nine days before hatching have formed synaptic connections
with the tectal neurons and started relaying the visual
information to the tectum. Presumably the onset of opto-
kinetic behaviour starts with the completion of connection of
optic nerve fibres with tectal neurons. At the time when the
first optokinetic responses were observed in the trout,
electrophysiological recording from the tectum gave no results.

The methods used in the present study for recording the visual responses by means of a large electrode gave no information about the formation of functional connections. In a few cases complex spontaneous potentials from the tectum were recorded but never in response to a visual stimulation. Visual responses from the tectum were first recorded 75-80 days after hatching in the trout. This is at the time when the outer white zone has increased greatly and the inner grey zone has decreased to a considerable degree. Most of the outer layers are formed and the optic lobe in general has increased in size.

The second part of this project is concerned mainly with a limited aspect of the function of the visual system concerned with the localization of a visual stimulus in space. The present experiments provide evidence for a precisely organized topographical projection of visual space onto the optic tectum of the Goldfish. In these experiments only two kinds of stimuli were employed, - a spot of light ($\frac{1}{2}$-2° in diameter) and black cardboard discs of various sizes, used on an illuminated background. The distance between the various electrode positions on the tectum relates to the distance between the positions in the visual field and is fairly consistent over all the tectum explored. These findings are in good agreement with the results of Jacobson and Gaze (1964)

and Schwassmann and Kruger (1965). Ioannides (1963)
observed in horizontal and vertical sections of the eye of
goldfish that the distribution of ganglion cells appears to
be fairly uniform. Jacobson and Gaze (1964) studied the
magnification factor from the periphery to the centre of the
visual field and concluded that there was no area centralis
in the superior half of the visual field. An enlarged
representation of the nasal retina on the tectum of the
rainbow trout was observed by Akert (1949 b). He concluded
that temporal visual field or nasal retina possesses low
resolving power. These results were based on the histol-
ogical degeneration traced after removing one quadrant of
retina. It is possible that this crude technique of retinal
lesions did not reveal the proper spatial extent of these
lesions. However, the present electrophysiological study
is not in accordance with the above observations of Akert
(1949 b). The present results do agree with the earlier
histological observations on the projection of the retina
onto the tectum in various fish (Lubsen, 1921; Akert, 1949;
Leghissa, 1955 and Attardi and Sperry, 1963). Electro-
physiological techniques make it possible to map the entire
visual field in a single animal as compared with the
histological technique in which only one quadrant of the
retinal projection at a time can be studied in a single animal.

A severe criticism of these experiments is that the origin of the response in the tectum cannot be accurately assigned in view of the structural complexity of the region. It is generally thought that optic potentials evoked from the tectum are presynaptic in nature. The reasons for this belief are as follows:

The majority of investigators using electrical stimulation of the optic nerve considered the fast component in the tectal response to be presynaptic i.e. generated by the optic nerve terminals (Buser, 1955). Recently, Karamian et. al. (1966) studied the characteristics of the visual system in sub-mammalian vertebrates. They considered the fast wave which was produced only after electrical stimulation of the optic nerve (not produced by light stimulation) to be presynaptic in nature. The waves with 6 m. sec. latency/in Lampreys and 4 m. sec. latency in frogs (producing the big slow negative wave) were considered to be fast components in the tectal response. These authors showed that (a) it was possible to record these waves from the optic tracts after removal of the brain (b) they could be revealed after the local administration of Nembutal to the brain (c) these waves persisted at relatively high frequencies of stimulation. However, analysis of the responses to optic nerve stimulation tells us little about the nature of the responses to light

stimulation of the eye. The intra-retinal delay is so
great, and the latencies of the responses so variable,
that it has so far not proved possible to assign pre or
post-synaptic origin to the unit responses recorded here.

The arguments relating to the origin of the tectal unit
potentials originate from the work of Maturana et. al. (1960)
and may be summarized as follows:-

1.   The tectal responses can not come from "fibres of passage"
     on the tectum, else a retinootopic map could not be found
     as observed.

2.   Therefore the responses either come from post-synaptic
     elements (somatic, dendrites) or from specific places
     on the pre-synaptic axons.

3.   The responses are pre-synaptic, since receptive fields
     recorded   in the tectal neuropil are identical to
     these in the optic nerve.

4.   The most likely point on the pre-synaptic axon for a
     specialized pick-up region is the terminal bush of the
     axon.

While all these arguments suggest that the tectal unit
recordings come from pre-synaptic endings, this has not yet
been proved. There remains an element of doubt about the
origin of these potentials. This difficulty becomes serious
when the retina is mapped after regeneration of optic nerve

fibres into the grafted region of the tectum; this imposes a limitation on the interpretation of the experimental results.

In spite of these defects, the present methods have given some insight into the mode of projection of the retina onto the grafted region of the tectum. It has been possible to confirm that the grafted tectum gives a more or less similar projection to the normal one despite the fact that the anatomical structure of the graft is grossly abnormal. These studies have also given some information on the mechanisms of optic nerve regeneration and suggested certain similarities between regeneration and development.

RESTITUTION OF RETINO-TECTAL PROJECTION IN THE ANIMALS WITH
TECTAL REIMPLANTATION

The evidence obtained by Gaze (1959); Maturana, Lettvin, McCulloch and Pitts (1959); Gaze and Jacobson (1963); and Jacobson and Gaze (1965) that the normal projection from retina to optic tectum is restored following regeneration of the optic nerve, conforms with Sperry's hypothesis, accounting for the selectivity with which optic nerve fibres, either during normal development or during regeneration, form their central connections. However, all these observations do not throw any more light on the process

of regeneration and the reformation of connections.

Sperry (1951 a.b.&c.,1955, 1956, 1963, 1965) has summarized a great variety of experiments that support his hypothesis that the restoration of spatial relations in the visual system is achieved as a result of growth processes which cannot be modified by function and experience. The precise nature of these growth processes has not been worked out as yet, although numerous hypothetical suggestions are present in the literature.

The experiments in this series show that the regeneration of optic nerve fibres may result in the restoration of incomplete and normal retino-tectal projections, in spite of the fact that the histological structure of the graft was chaotic in almost all animals studied. In the early stages of regeneration (Fig. 27 a and b) a few localizable points within the graft were recorded. These positions were situated mostly on the lateral edge of the tectum. In a subsequent experiment (Fig. 30A) only rostral and lateral regions of the graft responded to visual stimulation. Later stages (Fig. 31 and 32) gave sometimes virtually normal projections. It appears that the regenerating optic nerve fibres do not form connections in the tectum simultaneously. Visual restoration starts, in most cases, from the rostro-lateral margin towards medio-caudal extension of the graft. These results are comparable

with the mode of optic nerve expansion during development
(Kollros, 1953). In two animals recorded, 239 and 366
days post operatively (Fig. 35 A and B),many points within
the graft did not respond to visual stimulation. In these
cases most of the dorsal tectum was removed but at the time
of mapping the size of the graft had become considerably
reduced. Early responses recorded from the graft, generally
had wider multi-unit receptive fields. These responses were
fairly localized and the responses evoked were from multi-
unit receptive fields of 5-10° diameter (Fig. 34B). These
results suggest that the regenerating optic axons have
widely spread terminal arborizations in the initial stage of
regeneration which in the later stages shrink to a more
correct and localizable region (Cronly-Dillon, 1967). The
hypothesis that fibres from the central part of the retina
possess a greater degree of specificity than the peripheral
region of the retina, as suggested by Cronly-Dillon (1967)
for the regenerating newt retina, in unnecessary. Gaze and
Watson (1967) studied retinal regeneration in newt, using
tritiated Thymidine to follow cell division and migration.
They found that most of the fully regenerated retina had
come from the retinal ciliary margin, where active cell
division takes place even in the normal adult eye. Thus
the central retina is "older" than the peripheral retina
and may be expected to form adequate connections first.

The retino-tectal projection in normal and experimental animals has been mapped. More or less complete restoration of the RT projection has been observed, in some experimental animals. At the scar tissue, certain optic nerve fibres may fail to reach their destination directly or may reverse their direction of growth on the way. Some regenerating fibres may never reach the grafted tectum to form specific connections. In a regenerating peripheral nerve trunk more small nerve fibres can be counted than in the original stump (Mark, et. al., 1966), because each axon sends out a group of fine fibres, only part of which will establish functional connections. This multiple sprouting of regenerating nerve fibres, if it also occurs in regenerating optic nerves, might conceivably account for the relatively large visual fields which were found when recording from postero-medial region of the graft. In later stages of regeneration most of the receptive fields were smaller than those in early regeneration. Further, as Sperry has inferred, nerve specificities must somehow operate to ensure that the connections which are formed and functional are those compatible with previous patterns of optic projection and existing patterns of central nervous organisation. In the present experiments it is the central nervous organisation pattern which has been interfered with. The efficiency with which such processes of fibre specificity

might operate is not known.

The number of connections finally successfully formed should be reflected in the degree of restoration of the retino-tectal projection.   78.4% restoration of visual activity (behaviourally) has been reported in fish after optic nerve section (Weiler, 1966).   Restoration of visual function in the present experiments could be almost 100% although behavioural tests have not been applied to these animals. From the retino-tectal projection results, however, one could infer the restoration of visual function in some cases to be fairly good.

We may consider the following mechanisms which might operate to restore appropriately central nervous connection in the grafted tectum.

(a)   The optic axons might, on entering the grafted region, form synaptic connections randomly with whichever central neurons happen to lie in the path of their growth.   Only those connections which by chance happen to correspond to the previous terminations, however, would be functional. Thus the number of successfully restored functional connections would be determined purely by the number of regenerating fibres which by chance made the appropriate central connections. The animal might then still give a normal retino-tectal projection as a few fibres only for one particular retinal

locus may be required to give a visual response.

(b) The optic axons might enter the tectum as above, but in the course of growth, each axon might give rise to a large number of exploratory processes. Many of these growing fibre tips would encounter an unsuitable chemical milieu; while other would push into more favourable regions and thus direct the growth of the fibres. If a fibre happened to contact a nerve dendrite or cell with an appropriate specific chemical constitution, a functional synaptic ending would be formed. Only then would the fibre cease exploratory growth. If each fibre which entered the graft searched a relatively large area throughout the course of its growth, if growth continued until synaptic connections were formed, and if synaptic connections were formed only when the specific chemical conditions were favourable, a larger fraction of the fibres would be expected to form the appropriate functional connections than would be the case with random regeneration. In this case a fairly high order of a restitution of the RT projection would be expected.

Under the present experimental conditions, the results indicate that in favourable cases almost all the optic nerve fibres which have entered the graft presumably made connections specific enough to restore the RT projection map. We are compelled to assume that the second mechanism provides greater

efficiency in the establishment of functional connections.

We have to enquire here whether the present series of experiments allowed sufficient time for the second mechanism (mentioned above) to achieve its maximum degree of restoration. Growth processes may continue for a considerable time before the nerve fibres make contacts with appropriate central neurons.

The distance from the back of the retina to the caudal tip of the optic tectum (in a 9 cm. fish) is about 10 mm. It is known that peripheral regenerating nerves may have a rate of growth of 0.2 - 1.4 mm. per day. Early regeneration in these grafts occurred from 36 days in the anterior and lateral region of the graft (Fig. 27A). Complete restitution time in these experiments was about 94 days post-operatively. In 9 cm. fish at 0.2 mm. per day this time should be about 50 days. Most of the present experiments were done on 6-8 cm. long fish. This time would allow for a slow advance of the growing nerve fibres through tectal tissue. One has to presume that after the lesion in the tectum optic nerve fibres first degenerate towards the retina followed by regeneration. In the present experimental series, reimplantation of the tectum was done without cutting or crushing the optic nerve near the eye.

A certain variability in the results may be attributed to individual factors e.g. variable extent of the lesion,

variation in the general condition of the animal and the
severity of the operation.

The main conclusion which is to be drawn from these
experiments is that there exists a pattern of nerve
specificities which is operative in the formation of
connections between retina and the central associative area.
This mechanism would provide the high degree of specificity
in synaptic arrangements found to conform to the neuronal
patterns already laid down in the course of individual
embryonic development; and it could provide a high degree
of restoration of detailed vision.

If retina and tectum are specified separately (De Long
and Coulombre, 1965) and the optic nerve has formed
connections in such a way that each tectal cell forms its
appropriate specific nerve connection with optic nerve fibres
(Gaze et. al. 1963) and this pattern of point to point
projection is restored after optic nerve section, one would
expect that this relationship between tectum and retina
would remain unchanged even if we interfered with the tectum.
The observations of Attardi and Sperry (1963) and Jacobson and
Gaze (1965) confirm that if half of the retina of an adult
goldfish is prevented from regenerating, the fibres from the
other half retina regenerate only to their correct places in
the tectum. Now, if there is a rigid specificity, the

ingrowing optic nerve fibres, if they arborize widely enough,
should be able to search out appropriate loci in the tectum
where the tectum is rotated 90° or 180° in one or other
direction. Figs. 37 and 38 show the results of 90° clockwise
rotation (Fig. 37A). It is evident from these experiments
that point to point specificity is maintained upto a great
degree in these rotated grafts. The striking diversion of
the retino-tectal projection in the central region which
corresponds to the rotated tectum suggests that the appropriate
rotation of the maps may be occurring. Deviation from the
normal fan-like spread of the optic nerve fibres over the
roof of the tectum, notably a diversion at the site of lesion
in the 90° rotation tectum (Fig. 38C), suggests that
exploratory fibres in the tectum have diverted their route
towards more suitable loci. The results of recording from
the animals with 180° rotation cannot be interpreted, largely
because there are too few experiments in this series. In
the section dealing with experimental result there has already
been some discussion of the validity of the visual responses
which originate from the grafted tectum. However, it is
necessary to see what structures in the grafted tectum form
synaptic connections with the regenerating optic axons.

Following excision of the tectum it has been seen that
cells in the outer white zone generally degenerate followed
by vacuolisation of the outer white zone. Surprisingly, only

the cellular layers of the grey periventricular zone and efferent or commisural fibres generally remain intact. How can this happen when the blood supply has been totally cut off?.

The following possibilities may account for this phenomenon:

(a) These cells are very small in size as compared with the more superficial cells. Comparatively few of these cells extend their processes into the outer zones. We have to presume that cells with long dendritic processes degenerate in the absence of terminal connections on the dendrites. Outer cells which are rounded and with very small dendritic extensions do not degenerate as they do not form synaptic connections with the outer layers. These cells may have a low metabolic rate and they survive for a far longer time.

(b) Presuming that every cell in the graft is affected by degeneration, the ependymal cells from the host tissue creep into the graft and form a cellular bridge over the site of the lesion. Ependymal cells may divide in response to injury in the host tissue and within two days they penetrate into the graft. These new cells may differentiate in the graft and extend their processes in the outer white zone. The technique employed in the present study did not allow us to see the early movement of cells if there is any. Autoradiographic techniques are required for this. Cellular movement in other parts of the brain in response to injury have been

observed, particularly the formation of a cellular bridge between valvula cerebelli and the tectum (Fig. 47). A few mitoses have been observed in the underlying cellular zones in the graft as well as in the surrounding host tissue. It is probable that new cells are formed from the deep zones of the graft and the adjacent host regions. These newly formed cells would then extend their processes in the outer degenerated areas and form synaptic connections with regenerating optic axons.

(c) Blood vessels in the brain of Carassius gibelio are accompanied by periventricular spaces communicating with the meningeal space and with the ventricular space all of which contain cerebrospinal fluid. This fluid is moved through the perivascular spaces by the pulse wave in the same direction as the blood stream and also through the thin roof of the third ventricle (Van Rijsel, 1946). It is presumed here that ependymal cells and the joining tissue take up oxygen by diffusion from the cerebro-spinal fluid which is continuously bathing these cells. We do not know the diffusion rate neither do we know anything about pO_2 value of cerebro-spinal fluid of the fish. Cells in the grey periventricular zone may keep alive for sometime due to oxygen uptake from cerebro-spinal fluid till vascularization extends into the graft.

It is relevant here to mention the recent findings of
Gaze and Watson (1967) who reported mitosis in the ependymal
cells of newts, 15 days following the section of the optic
nerve. These authors studied autoradiographically the
labelling of ependymal cells with Tritiated Thymidine at
different stages of degeneration and regeneration of the
optic nerve fibres. No labelling in the ependymal cells
was reported between 2-10 days after nerve section. A few
adjacent cells in the lower cellular grey zone, as well as
ependymal cells, were reported to incorporate ^{3}H Thymidine
in 15 days post-operative animals. The significance of
this labelling of ependymal cells in the newt was not
discussed. It is possible that newly formed ependymoglial
cells replace the degenerating cells and form synaptic
connections with the regenerating optic axons.

Kirsche (1960) and Richter (1965) have reported
regeneration of optic tectum following its excision in
fish. Kirsche described the presence of undifferentiated
cells, or matrix tissue, in three different zones of the
tectum, and laid emphasis on the importance of this tissue
for regeneration. He labelled different matrix zones as
dorsal, caudal and basal zones. These zones lie next to the
ependymal cell layer. He further reported the presence of
matrix tissue in the normal fish, from which neuron and glial
cells originate continuously, increasing the size of the

tectum. In older fish, this tissue was completely spent. Kirsche reported that matrix cells start differentiation in response to a stimulus exerted by the lesion. He further reported that not only the matrix zones but many undifferent- iated cells of the cylindrical ependyma also start differ- entiation following lesions in the tectum. Although extensive regeneration was reported by Kirsche in Goldfish tectum, especially when the matrix zones were left intact, restoration to normal morphological structure has never been shown even 300 days after the operation. It has been found difficult to identify any matrix zones (Kirsche and Kirsche 1961) in the tectum of Goldfish. The presence of this undifferentiated cell mass could only be confirmed by electron microscopic study. On the other hand, Richter (1965) showed complete restoration of the tectum following a small lesion. In the present experiments, normal morphological restoration following either excision or reimplantation has never occurred. Moreover, in the outer zones of the graft very few cells have been seen. The numerous fibre bundles in this zone make the structure of the graft look chaotic.

The ependymal cells in fish (Clupea sardina) have been studied by Mitro (1895). Most of the ependymal processes have subpial end-feet. They have unusually broad processes

of specialised cytoplasm extending across the width of the neural tube (Kruger and Maxwell, 1966). Distinct ependymal processes with vascular end-feet have been demonstrated in fishes (Horstmann, 1954). Kruger and Maxwell (1966) suggested, on the basis of electron microscopic study of the ependymal cells of different teleosts (especially Paralabrax nebulifer), that these cells are not simply primitive supportive elements. A similar type of ependymal or ependymoglial cells has been reported here in the Golgi study of the optic tectum of Goldfish. It is highly probable in the light of Gaze and Watson's (1967) observation, that ependymo-glial cell may divide in response to injury to the tectum.

In some long term experiments in which the fish were allowed to survive for almost a year there was very poor restoration of the retino-tectal projection. In these cases, most of the dorsal part of the right optic tectum was removed and then reimplanted (Fig. 35A and B). At the time of recording, the area of the graft had become reduced but within the available graft many points were not localizable. One may assume, on the basis of the hypothesis already mentioned, and in accordance with the normal growth of the tectum, that first regeneration would have occurred in the rostro-lateral region of the graft. There are two possibilities concerning what is happening in the graft in these unsuccessful

cases; (a) either the graft has degenerated altogether or the optic nerve fibres which were supposed to innervate that area have degenerated as they could not find suitable loci in the tectum, (b) one year is not enough for regeneration over so large an area of grafted tectum.

In TT48 (Fig. 35B) although most of the points in the remaining graft were localizable, the orientation of the map was slightly abnormal. The crossing over of the projection lines in this case can be compared to the early regeneration stages in the animals with smaller grafts, (Fig. 27B).

THE "ELASTIC" PROPERTIES OF THE VISUAL SYSTEM

In regeneration of optic nerve in the frog (Gaze and Jacobson, 1963) the recovery of a projection which is retino-topically organized in the naso-temporal but not in the dorso-ventral axis, suggests that the organization of the projection is determined separately in these two axes of the retina. Experiments on "compound eyes" (Gaze et. al. 1963 and 1965) suggested that the organization of the connections between retina and tectum may be determined by two gradients acting at right angles in the naso-temporal and dorso-ventral axes of the retina and similarly in the rostro-caudal and medio-lateral axes of the tectum.

The projection from each half of the "compound eye" appears
to have spread out to occupy the whole tectum and does not
seem to have connected only with half the tectum as might
have occurred, if the specification of each cell were
absolute. Moreover, their hypothesis is in accordance with
the idea that independent parallel specification of the
retina and tectum occurs so that each cell in the retina
and tectum acquires a specific identification in a biaxial
gradient system. Gaze et. al. (1963) gave excellent diagrams
of the gradient system which was postulated as specifying the
retina and tectum in normal and "compound eye" experiments.
Specification of a cell in the gradient only depends on
whether the cell is above or below its neighbours in the
gradient system. These authors showed that after bisecting
the retina in Xenopus embryos, when the naso-temporal retinal
gradient had become determined but not the dorso-ventral one,
the remaining half retina connected to the whole tectum.
The authors argued that if the number of cells in the retina
or presumably in the tectum is halved surgically, the
gradient will still be complete but its slope will be
steepened. They postulated that once the gradients have
become established, halving the retina or tectum would not
produce the results comparable to "compound eye" experiments,
since each cell in the retina or tectum had already acquired

its unique values.   To test this hypothesis, Jacobson and

Gaze (1965) performed experiments on young adult goldfish.

They reported that either reduction in the number of optic

nerve fibres or in the size of the tectum receiving the

regenerated fibres may alter the normal retino-tectal pro-

jection.   These experiments have already been mentioned

elsewhere.   On the basis of these experiments they argued

that once the gradients have become established, halving

the retina or tectum does not produce the results seen in

"compound eye" experiments.   Unfortunately their results

were based on removing either the lateral or medial half of

the tectum along with optic nerve crush.   In both cases,

they showed that optic nerve fibres which were destined for

the ablated half of the tectum failed to make tectal

connections, whereas, an appropriate projection to the

remaining half of the tectum was restored.   These authors

did not try the removal of rostral or caudal parts of the

tectum.   The present experiments give results different from

those of Jacobson and Gaze (1965).   The fish in which the

anterior part of the tectum was removed along with optic

nerve crush did not survive at all.   In other series where

the caudal part of the tectum was removed along with optic

nerve crush, the regenerating optic axons spread in the

remaining half tectum in such a way as to suggest the

spreading of the whole visual field in the remaining half tectum. Moreover, histological examination of these brains showed that the caudal half of the tectum was absent and despite spreading of optic axons in the remaining anterior part of the tectum, normal stratification was evident. The only explanation of these experiments is that gradients which were supposed to have become established in adult animals were functional again when the optic nerve was crushed and the caudal tectum was removed. New regenerating optic nerve fibres appear to have established their connections with the half tectum, treating it as a full tectum. Since the retinal gradients are normal, the optic nerve fibres would be expected to form connections with the remaining half tectum. It may be inferred from these experiments that gradients in adult goldfish tectum work in rostro-caudal direction but not in lateromedial one. It appears that the 'elastic' properties of the system (Xenopus larvae "compound eye" experiments) are still at work but presumably in one direction only.

The time allowed for regeneration in the present series as well as in the experiments of Jacobson and Gaze (1965) is not sufficient to allow us to assess the true nature of these properties. In the face of the present observations it is difficult to agree with Jacobson and Gaze (1965) that the "elastic property of the gradient system does not operate in

the adult goldfish, but that a fixed system of place
specificities determines the positions at which regenerating
optic axons terminate in the tectum".

IS REGENERATION A 'MIRROR-IMAGE' OF DEVELOPMENT?

In embryogenesis the differentiation of the tectal layers
parallel the arrival of optic nerve fibres. When the nerve
regenerates however, the lobe is already developed. Once
the optic nerve connections have been made, section of the
nerve does not lead to complete regression of the lobe to
its previous undifferentiated state; involution occurs but
the layered structure persists (Gaze, 1960). Thus optic
nerve regeneration is not a parallel of organogenesis.
It is therefore invalid to propose that regeneration and
embryogenesis are identical processes.

In embryogenesis, since the environment of the growing
fibres is so much simpler than in regeneration, Gaze (1960)
has suggested that orderliness of the growth process results
in the normal spatial retino-tectal relationship. The axons
arriving at a particular part of the lobe could then induce
specificity in the central tissue. The optic lobe
differentiates from the anterior towards the posterior
pole (Kollros, 1953) which parallels the development of
cholinesterase activity in the lobe (Boell, et. al. 1955).

The optic axons enter the lobe from the anterior end.
Perhaps the order of arrival of the fibres determines their
systematic distribution over the lobe surface.   Under these
circumstances one would expect a projection from anterior to
posterior tectum.   This is directly supported by the
observations of Attardi and Sperry (1963).   At the onset
of regeneration, both the retinal and tectal cells are
specified, in the sense that there exists in the centres two
corresponding entities whose equally distinctive properties
match these groups.   The process of embryogenesis differs from
this.

During embryogenesis the optic tectum is an undifferentiated
mass of cells until the arrival of the optic afferents.   The
eye is connected to the brain by the optic stalk.   During
development the optic fibres are guided along this preformed
pathway.   There is no evidence to suggest that the point of
termination of any one fibre is determined in this way.   In
both processes there is no evidence that mechanical factors
play any part in fibre specificity which result in specific
tectal connections, other than the conduction of the fibres
towards the CNS as a whole.

Degeneration of the optic nerve may cause tectal atrophy
but its layered differentiated structured remains.   Thus
regeneration is an adaptive homeostatic reaction of the
organism to tissue damage;   not a primary establishment of

intercellular relations; and will involve phenomema different
to those involved in development.

Moreover, it can be inferred from the present findings
that restoration of the retino-tectal map in the graft tectum
cancnot be attributed to the tectal regeneration. Morphological
regeneration of the graft has not been observed in any case.

It is suggested that degeneration is not the reverse of
morphogenesis and that regeneration does not parallel
organogenesis. It is believed here that the substantial
gap between the processes of regeneration and development will
widen, following the further development of analytical
procedures to such an extent as to differentiate these
processes as separate aspects of physiology. Thus the
study of orderly regeneration in the CNS throws light on
normal mechanisms of development only in so far as both
processes represent orderly growth but with presumably
different mechanisms.

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SUMMARY

The structure of the developing optic tectum of the trout has been investigated using histological preparations and fresh material. The neuroblasts reached the pial surface and started to differentiate before separating from the ependymal processes. A new finding was the presence of inverted pear-shaped neurones in the plexiform external layer in Golgi sections of adult tectum. The organisation of the tectum was found to be similar to that described by Leghissa (1955).

The first optokinetic responses occurred in trout embryos at a stage of development at which the thickness of the outer white zone was equal to that of the inner grey zone.

The topographical representation of the visual field on the optic tectum of ten normal goldfish was mapped electrophysiologically using metal-filled micropipette electrodes. Responses were evoked by visual stimulation with spots of light and with black discs of various sizes.

Restoration of the visual projection over the right tectum following excision and reimplantation of the tectum in its normal orientation was studied in a series of goldfish at different times after the operation. There was a wide variation in the time taken for the restoration of the retinotectal projection map. From the third postoperative month some normal projections in the graft area were seen. It was concluded that optic nerve fibres can regenerate into the

graft, and that the graft may retain its original specificities, viz. its specialization to represent a particular part of the retina.

Cases where recovery occurred later than three months were those with larger areas of grafted tissue.

When the graft was reimplanted 90° rotated it was predicted that the recovered projection would show a corresponding 90° rotation over the grafted area. Results tended to show the predicted distortion in the projection though not at the edges of the grafts.

It was difficult to draw any useful conclusions from the retinotectal projection of 180° rotated tectum.

In another series when the posterior half of the tectum was removed and the entire optic nerve allowed to regenerate, the resulting projection was compressed into the remaining half tectum.

There was a gross abnormality in the histological structure of the grafted tectum even in those cases where the restoration of the visual map was more or less normal.

An attempt has been made using various microscopic techniques to compare regeneration within tectal grafts with normal tectal development.