NEURAL DEVELOPMENT AND REGENERATION IN THE VISUAL SYSTEM OF TELEOSTS

by

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INTRODUCTION

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

After ablation of one eye in the larva of Drosophila, marked reduction in also occurred several days after metamorphosis, particularly in the optic epithelial layer, was shown by Stanišić (1906). However, the reduction in also was confined to the contralateral side of the optic lobe when Duxon (1911) and 1952 removed one eye in larvae of Drosophila.

Quantitative analysis of the degree of hypoplasia in the larvae of Hyalopoda, when unilateral excision of the eye was performed by Haupt (1922 and 1928). There was a marked reduction in the size of the embryonic optic lobe as compared with the other layers of the system, which showed a slight thinning. Moreover, there was an increase in the cell population on the contralateral lobe in the ipsilateral known. This analysis was done between the time of operation and metamorphosis. In this work the enlargement of the optic lobe 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 optic 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 iridens, 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. Saar (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. Sauer and Chittenden (1959); M. E. Sauer 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 Sauer's hypothesis.

Microspectrophotometric measurements of DNA in the nuclei of the chick neural tube were made by M. E. Sauer and Chittenden (1959) to test the hypothesis of the inter-mitotic migration of nuclei. Their results supported the hypothesis of F. C. Sauter (1935). M. E. Sauer 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 neuroblasts of the subependymal layer lost their histological characteristics (light nuclei, 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 densely packed ribosomes and several mitochondria. There was no endoplasmic reticulum or intercellular 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 neurofilaments, 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 granular lamina as seen in urodèles. 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 irradiation 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. autoradiography, 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 cytoplasm 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 μ 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 discernible 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 extracellular 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 underlie 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 goldfish, 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 source 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 sources 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. \text{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 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 Stickleback (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 traumatized 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 behaviour 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 Amblystoma (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 μm 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 undertaken. 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 understanding 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 termination of optic fibres in the tectum, there was a direct projection to the hypothalamus 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 (Frobst, 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 quadrant lesions resulted in specific localised degeneration in the contralateral tectum. They further reported that the nasal retinal quadrant projected to the caudal part of the contralateral optic tectum, the temporal retinal quadrant projected to the rostral part of the tectum; the dorsal retinal quadrant projected to the lateral part of the tectum and the ventral retinal quadrant projected to the medial part of the tectum. The above mentioned findings were further confirmed histologically by Legnissa (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 available 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 (1941a and b), using a silver nitrate technique was unable to find any evidence of retino-topic projection onto the tectum in Necturus and Catfish (Amphius nebulosus). The optic nerve fibres in Amphibia retain a fasciculation according to retinal quadrants 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 (1941a 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 Székely, 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 (Vejbasya, 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 quadrants 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 Székely, 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 electrophysiological techniques. They reported that the superior retinal quadrant is represented inferiorly in the tectum and the anterior quadrant 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 (1962a) in the frog, and Gaze, Jacobson and Székely, (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 (1958a and b) showed the point to point projection of the visual field on the optic tectum of the frog. The naso-superior, naso-inferior, tempo-inferior and tempo-superior quadrants 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); Koppanyi (1955); Sperry (1951 a,b, and c; 1955, 1965); Stone (1953 and 1964); Székely (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 regenerating 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 regenerating 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 quadrants of retina in adult Goldfish and cut the optic nerve. After regeneration of the optic nerve, he recorded electrophysiologically the retinotopic projection and discovered that the projection from the retinal quadrant 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). Székely (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 antero-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 (Székely, 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 gradients which are supposed to be active in the development of retinal and the tectal specificity. These results are compatible 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 temporaria 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 (Boellet. 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 stage 30 and the animals recovered normal vision. However, beyond stage thirty, it was found impossible to obtain a normal reimplantation 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 up to 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 dorsoventrally 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 combinations, 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 muscles they supply and were implanted into antagonistic muscles, neuromuscular transmission was re-established but with uncoordinated fin movements which remained so. It was shown that implantation of the crossed nerves 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 concept of myotypic respecification. It appears necessary to reinvestigate the old findings concerned with respecification 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 electrophysiologically 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?
EXPERIMENTAL METHODS

Goldfish (Carassius auratus) from 6 to 10 cm in length were used to map the visual field on the optic tectum. The animal was anesthetized by immersion in a five minutes in Lugol solution (1/4,000) of H2SO4 (Sulfuric acid) and then put up in a supporting dish of acrylic dental-repair material coated to the stage of its body. The Gill's were continuously perfused with fresh water in a continuous stream in the fish's mouth throughout the experiment. The animal was sealed 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 cranial cap made with a dental drill to expose the optic tectum and cerebral hemispheres. The skull flap was removed. The membranes covering the tectum were removed by suction. The optic lobes were kept stable in a pool of liquid paraffin.

After anesthesia, 0.01 cc. of bicuculline was injected intramuscularly. The optic lobes were drawn on an extensive graph paper with the help of a camera-lucida. The size of the field on the graph paper corresponded to that on the tegument.

The fish was positioned on the platform of a micro-manipulator with its left eye centered on an Alcian projection...
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-Sandoz) 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 through a tube inserted 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 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 200 μ 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 iridectomy 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 @ pH 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% aqueous 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-crine 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 animal's 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 optokinetic 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 \( 90^\circ \) clockwise rotation \( B \)
- Right tectum excised and reimplanted with \( 180^\circ \) clockwise rotation \( C \)
- Right tectum (caudal half) removed and Left Optic Nerve crushed \( D \)
- Right tectum excised on three sides only \( E \)

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<th>Experimental No.</th>
<th>Type of Operation</th>
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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 y 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 y 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 Lázár 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 periventricular 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 gréïsum Superficiare, 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 (l) 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 ventricles are small fusiform stained 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 technique. 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,l), 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. Table shows how the thickness of these various zones becomes modified during development.
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Thickness was measured in mmSL 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 respectively). 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.

<table>
<thead>
<tr>
<th>Photograph No.</th>
<th>Days before hatching.</th>
<th>No. of Cells per Unit Area, of the grey periventricular zone.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
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<tr>
<td>5</td>
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<td>6</td>
<td>2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Days after hatching.</th>
<th>No. of Cells per Unit Area, of the grey periventricular zone.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>95</td>
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<td>9</td>
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<tr>
<td>17</td>
<td>89</td>
</tr>
<tr>
<td>18</td>
<td>71</td>
</tr>
</tbody>
</table>

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 composed 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 occurrence 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 by 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 reimplemented 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 reconstruction. 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 temporo-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 (TT4) 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 $180^\circ$ 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 $25^\circ$ 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 $180^\circ$ 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 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 quadrant of the left visual field, whereas, the responses in the posterior most row (points 18-23) were evoked from naso-inferior to superio-temporal quadrant 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 projections 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 corresponding 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 quadrants 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 $90^\circ$ latitude for the accuracy of this progression, then in the naso-superior quadrant the row should run

while in the temporo-superior quadrant 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 \( \chi^2 \) 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.

\[
\begin{align*}
\text{Observed correct (O)} & = 8; \\
\text{Expected correct (E)} & = \frac{8}{4}; \\
E \frac{(O-E)^2}{E} & = 6; \quad \text{and} \quad \frac{(O-E)^2}{E} = 18.00
\end{align*}
\]

Thus here, \( \chi^2 = 18.00 \) 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 \( \chi^2 \) for the series of rows.
The $\chi^2$ 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 $\chi^2$ 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 TT52, Fig. 32B.

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<th>Expected Corrected (E)</th>
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### Experiment TT56, Fig. 33B.

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<th>Observed Correct (O)</th>
<th>Expected Corrected (E)</th>
<th>((O - E)^2 / E)</th>
<th>Probability on the basis of chance</th>
</tr>
</thead>
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<td>(4)</td>
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### Experiment TT55, Fig. 30A.

<table>
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<th>Expected Corrected (E)</th>
<th>((O - E)^2 / E)</th>
<th>Probability on the basis of chance</th>
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<td>(3)</td>
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<tr>
<td>(5)</td>
<td>1</td>
<td>1/4</td>
<td>2.25</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

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 commissural 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. TT149), 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 U 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 telophase, 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 migrating cells.

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 Glee’s 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, Glee’s 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
DEVELOPMENT OF TECTAL NEURONS OF SALMO-FARIO

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.
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.
PLATE 4.

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.
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 stratification of the optic tectum.
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.
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.
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.
AGE IN DAYS (BEFORE HATCHING)
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

AGE IN DAYS (AFTER HATCHING)

TOTAL WIDTH

OUTER WHITE ZONE

INNER PERIVENTRICULAR ZONE
FIG. 19 and 20

MIGRATION OF NEUROBLASTS IN THE DEVELOPING OPTIC TECTUM

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.
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' projection.
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.
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.
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 electrolytic 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.
FIG. 28A and B

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.
FIG. 29 A and B

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.
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 O. 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.
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 an 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.
FIG. 32 A and B

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

Lower: (34B, Experiment TT4.4); 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.
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 0. 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.
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 0. 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 0. 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.
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 electrolytic lesion. The conventions are the same as in Fig. 27A.
FIG. 38 A and B

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 0. The conventions are the same as in Fig. 27B.
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 picture) is the cerebellum. The scale is equal to 600 microns.
FIG. 39 A and B

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 0. 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 0. The conventions are the same as in Fig. 27A.
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 0 (within the hatched area). The conventions are the same as in Fig. 27A.
FIG. 40B

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.
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.
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.
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.
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.
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.
Photomicrograph reconstruction of the transverse section of the tectum from the middle of the 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.
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.
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 periventricular 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.
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.
FIG. 48

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.
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.
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.
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.
Fresh preparation of the 3 days old tectal graft showing cytoplasmic bridge between 2 separate lobes. Phase contrast, the bar represents 0.01 mm.
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 \( \mu \) Holmes' Silver impregnated sections. The bar represents 0.01 mm.
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.
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 μm Glees' reduced Silver stained sections. The bar represents 0.1 mm.
DISCUSSION

The neuro-anatomical picture of a given nucleus (e.g. nucleus) is compounded from the results of applying several different experimental techniques. In the case of the optic tectum the idea of different measures in the number of strata present are misleading. Sholl (1954) demonstrated that the ratio of the number of Golgi-dispersed cells to the number of Nissl-stained cells remained roughly constant (3:17) through the layers of n. tecti. Sholl (1954) concluded that the number of cells along the Golgi-stained cell representation of the total present, and that the method was by inference. In the present studies on the optic tectum of rhesus studied by Golgi techniques (P. Maco, 1950; Maco y Cajal, 1911; Miller and Crosby, 1936 and Leighton, 1955) discrepancies in the results are evident. By using extensively variations of the rapid Golgi-technique, the present findings indicate that the extent and completeness of interpretation is governed by the length of exposure to the fixation; the duration of the degeneration, by the exposure to silver tincture. It is quite apparent in the present study that partial degeneration of neurons and their processes has occurred. These observations are in conflict with the idea that Golgi degeneration, when it occurs, affects
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) demonstrated 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) discrepancies 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. Discrepancies 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 Székely (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 Székely 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 thalamo-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 Székely (1967) in frog has also been observed in these studies. It is suggested that these cells form the supporting 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 neuroblast migration and the formation of the outer white zone and does not include a study of earlier stages, in which neuroblasts 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.

Sauß 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 up to 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; Spädel, 1933; Weiss, 1934 etc.)

Detwiler (1947) showed the regeneration of gross morphological structures after unilateral extirpation 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 Coulombre, 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 ectodermal cells but the final pattern of arrangement of the cells is determined by those regions of the ectoderm with which the most stable pseudopodial 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 pseudopods 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 contrasted 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 modulating 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 optokinetic 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 histological 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). Electrophysiological 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 submammalian 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/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 retinotopic 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 (1951a, 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 retinotectal 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 up to 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 commissural 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 Rijssel, 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 pO2 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 undifferentiated cells of the cylindrical ependyma also start differentiation 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 ependymoglial 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 projection. 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 phenomena 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 cannot 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 organization 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.
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