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The organization of the projection of the centrifugal fibres to the retina in the pigeon

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INTRODUCTION

Although the centrifugal fibres to the avian retina run for a considerable part of their extent independently of the rest of the visual pathway and after joining the optic chiasma remain localized within a narrow part of the optic nerve, as they approach the optic disc they appear to spread out to reach most parts of the retina. At present there is no evidence for a topical organization in the projection of the nucleus of origin of these fibres upon the retina, but on *a priori* grounds it seems reasonable to predict that some degree of organization exists in this projection in view of the precision with which the tectum is known to be represented upon the isthmo-optic nucleus (McGill, Powell & Cowan, 1966). For an anatomical investigation of this problem two methods are available. First, localized lesions could be placed in the isthmo-optic nucleus and the distribution of the resulting fibre degeneration mapped out within the retina; because of the small size and complex folding of the isthmo-optic nucleus, the technical difficulty of placing such partial lesions makes it highly improbable that satisfactory results could be obtained by this method. On the other hand, as it is known that the cells of the isthmo-optic nucleus undergo a profound retrograde reaction following removal of the contralateral eye (Cowan, Adamson & Powell, 1961) it seemed more promising to place circumscribed lesions within the retina and to study the distribution of retrograde cell atrophy within the nucleus. Previous experiments with pigeons had shown that the placing of localized retinal lesions presents little or no difficulty, but for several reasons it became clear during the course of this study that it would only be possible to determine the general pattern of the centrifugal projection upon the retina.

MATERIAL AND METHODS

Thirty-five pigeons were used for this study. Under ether anaesthesia lesions of varying size were placed in the retina in the manner previously described (McGill *et al.* 1966). The animals were allowed to survive post-operatively for periods ranging from 40 to 200 days and were then killed by an overdose of ether. The brains were fixed in a mixture of alcohol and acetic acid and subsequently embedded in paraffin wax. They were all sectioned at 25 μ in the standard horizontal plane used for all parts of this investigation. A 1 in 12 series of sections throughout the brain was first mounted and stained with thionin, and following this a complete series of sections through the dorso-ventral extent of the isthmo-optic nucleus was mounted and stained. The eyes were orientated and processed as in the previous study, to which reference should be made for the details. The distribution of the retrograde

cell degeneration in the isthmo-optic nucleus was plotted first on a series of outlines of sections through the nucleus, and then transferred to a standard series of outlines and to a reconstruction of the whole nucleus.

RESULTS

Although it is known that following enucleation of an eye in the pigeon the cells of the isthmo-optic nucleus undergo a progressive atrophy leading to profound cell loss after 2 months, the interpretation of cellular changes in this nucleus at similar periods after partial lesions of the retina has proved to be unexpectedly difficult. To some extent this difficulty is simply due to the fact that the nucleus contains relatively few cells, but also, because it is in the form of a highly convoluted lamina, sections through the nucleus seldom show a regular arrangement of the cells. For these reasons it has been almost impossible to delimit, with any degree of confidence, small areas of cellular degeneration.

In several of the early experiments small lesions comparable to those used in the study of the retino-tectal projection (McGill *et al.* 1966) were produced, but in the great majority of these no cellular degeneration or gliosis could be detected with certainty. In view of this difficulty only experiments with relatively large lesions have proved useful. On the other hand, several experiments with such large lesions have also been of little value either because they have directly involved the optic disc or because they have interrupted fibres to all quadrants of the retina. As an example of the unsuitability of experiments in which the lesion has encroached upon the optic disc P68 will be described. In this case the lesion was placed in the inferior part of the retina, but the spread of current has involved the antero-inferior third of the optic disc and has penetrated into the medial part of the optic nerve (Fig. 1). Although the area of direct retinal damage does not amount to more than about 15% of the total surface, the isthmo-optic nucleus on the contralateral side has undergone profound cell loss throughout a large part of its extent. The nucleus as a whole is considerably smaller than that on the unaffected side, and an estimate of the number of surviving cells indicates that there has been a cell loss of the order of 60%. That the cellular atrophy should be so severe and extensive after this lesion is not really surprising in view of the damage to the optic nerve. However, that there is some organization in the arrangement of the centrifugal fibres is suggested even in a case such as this because the cellular degeneration in the isthmo-optic nucleus is largely confined to the ventro-lateral two thirds of the nucleus.

The absence of unequivocal retrograde cell degeneration in the isthmo-optic nucleus after small peripheral lesions in the retina on the one hand, and the unsuitability, for purposes of localization, of experiments with lesions in which the disc is involved on the other, has greatly limited the number of experiments which are useful for this study. Of these only six will be described in which the lesions were large enough to give clear and localized degeneration in the nucleus and in which the optic disc has escaped injury. Although in most of these experiments more than one quadrant of the retina has been damaged, it has been possible, by comparing the pattern of degeneration in the nucleus, to determine the projection of the nucleus upon the different quadrants of the retina with a reasonable degree of confidence.

The experimental evidence for delimiting the parts of the isthmo-optic nucleus related to the anterior and posterior halves of the retina is unequivocal, and that for the origin of the fibres to each of the anterior quadrants is equally strong. Within the projection to the posterior half of the retina, however, we have no lesion which can be said to involve selectively the fibres to either the postero-superior or the postero-

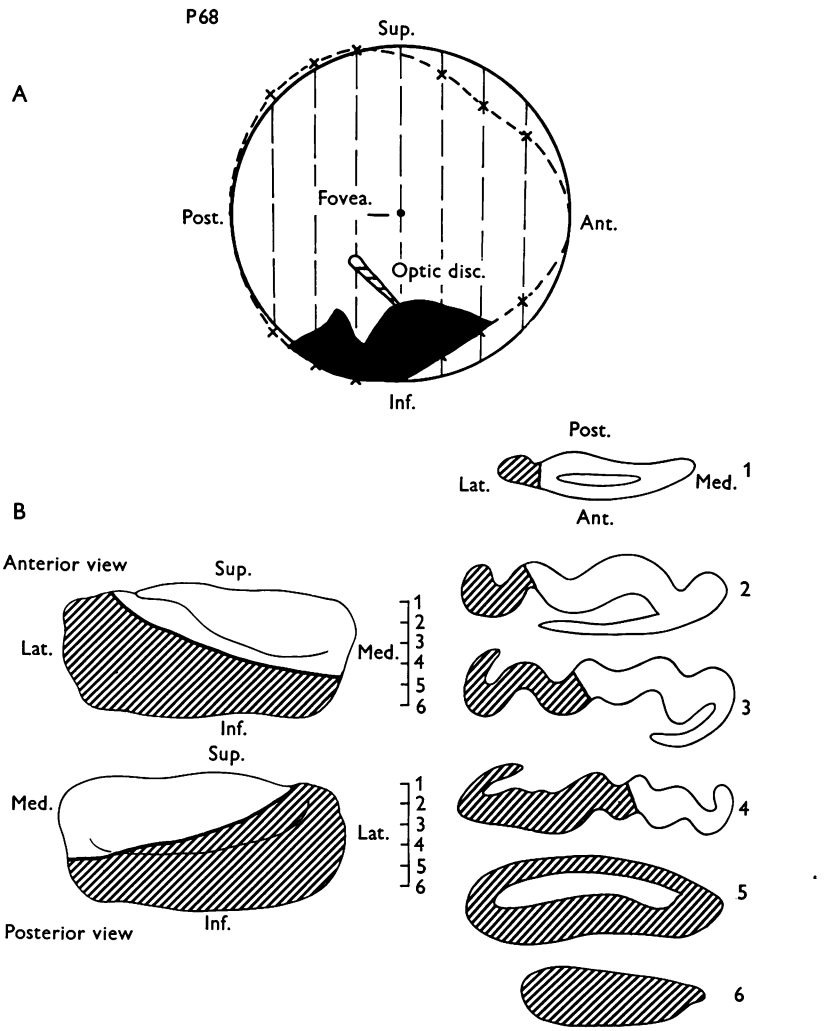

Fig. 1. The lesion and the distribution of the resulting retrograde cell degeneration in the isthmo-optic nucleus in Expt. P 68. In A, the site and extent of the lesion is shown in solid black on a standard outline of the *right* retina (continuous line) upon which the reconstructed outline (interrupted line) of the retina of this experiment has been superimposed. In B, the cellular degeneration is shown by hatching on a standard series of outlines of horizontal sections through the dorso-ventral extent of the *right* isthmo-optical nucleus and upon anterior and posterior views of a reconstruction of the nucleus. (To facilitate comparison of the results of different experiments *all* the lesions, in this and in subsequent figures, are shown on outlines of the *right* retina, and the cellular degeneration on sections and reconstructions of the *right* isthmo-optic nucleus.)

inferior quadrant, but there is indirect evidence to suggest that the organization of the fibres to the posterior half of the retina is comparable to that for the anterior quadrants.

The first experiment of this group to be described, P82, establishes the basic organization of the projection of the isthmo-optic nucleus to the anterior and posterior halves of the retina. The lesion in this case (Fig. 2) is roughly triangular in outline and is situated in the anterior half of the retina, immediately below and in front of the

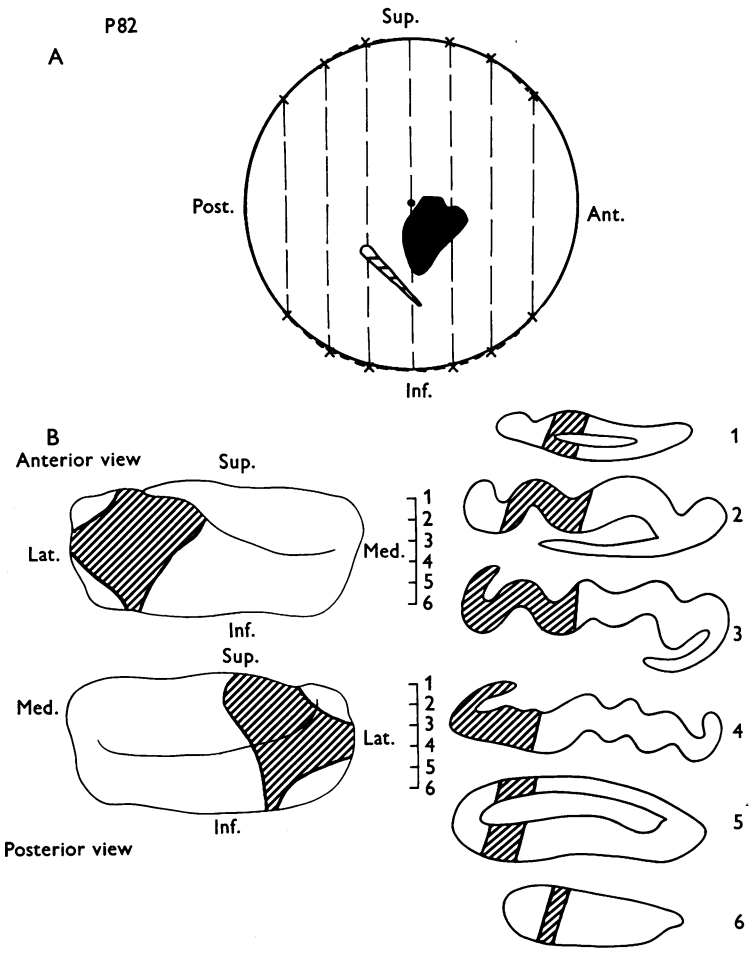

Fig. 2. The lesion in the retina and the extent of the retrograde cell degeneration in the isthmo-optic nucleus in Expt. P82.

fovea. The posterior margin of the lesion passes more or less vertically from just below the fovea to just above the optic disc, and so coincides with the vertical axis through the fovea. Similarly, its upper border is very close to, and approximately parallel with, the horizontal axis of the retina. If it is assumed that the centrifugal fibres radiate outwards from the optic disc in a way comparable to the course of the

optic nerve fibres it would seem reasonable to infer that this lesion has interrupted only those centrifugal fibres going to the anterior half of the retina, and of these mainly fibres directed towards the antero-superior quadrant. It may be pointed out here that in a previous paper in which an account was given of the distribution of the degenerating centrifugal fibres after lesions of the isthmo-optic nucleus, the arrangement of the fibres at the level of the optic disc was described and correctly illustrated (see Fig. 2, Cowan & Powell, 1963), but in the text it was stated, inadvertently that the optic nerve layer was thicker on the lateral (posterior) than on the medial (anterior) side of the optic disc. Re-examination of this material and of other retinae has shown that the optic nerve layer is, in fact, thicker on the medial side of the optic disc, on the same side as that on which the centrifugal fibres approach the retina as shown in the text-figure of the previous paper. That is to say, the majority of the centrifugal fibres enter the retina along the medial (or anterior) border of the optic disc, which is perhaps not surprising as approximately two-thirds of the retina lies antero-superior to the disc.

In the isthmo-optic nucleus of the contra-lateral side degeneration is confined to the lateral half of the nucleus and is more severe dorsally (Fig. 2). In the most dorsal sections there is a narrow zone of cell loss just lateral to the middle of the nucleus, and lateral to this in turn, the cells are appreciably shrunken. When traced ventrally the area of cell loss is found to increase in its medio-lateral extent, so that at the level of the middle of the nucleus it comes to occupy most of the lateral half of the nucleus. In the sections of the ventral half of the nucleus the degeneration is definitely less severe, the area of cell loss being limited to a narrow band at the junction of the third and lateral quarters of the cross-sectional areas, and lateral to this, including the postero-lateral limb, there is shrinkage and pallor of the cells. From this experiment it may be concluded that the anterior half of the retina receives centrifugal fibres from the lateral half of the isthmo-optic nucleus, and, in view of the fact that the lesion has interrupted considerably more fibres passing to the superior quadrant than to the inferior, the more severe degeneration in the dorsal half of the nucleus is probably explicable on this basis.

In the next experiment, P60, the lesion is more clearly limited to the antero-superior quadrant, and the distribution of the degeneration in the isthmo-optic nucleus supports the conclusion that the centrifugal fibres to this quadrant are derived from the supero-lateral quarter of the nucleus. The site of entry of the electrode through the posterior surface of the eye is marked by a small area of retinal destruction along its extreme periphery just above the horizontal axis. The definitive lesion is in the form of an irregular area of retinal destruction extending from the fovea in an anterior and superior direction to the anterior edge of the retina (Fig. 3). Apart from a small area of damage just below and in front of the fovea, there is a broad, approximately triangular area of damage immediately above the horizontal axis which must have interrupted all the centrifugal fibres to the antero-superior quadrant of the retina.

The area of retrograde cell degeneration in the isthmo-optic nucleus of the contra-lateral side is very well-defined in this experiment, and is limited to the supero-lateral portion. Here there is very severe cell loss, the outline of the affected area being only just detectable by the occasional shrunken cell which persists

(Fig. 4). In the most dorsal sections this atrophy occupies almost exactly the lateral half of the nucleus. The affected area remains more or less the same for some distance, and at the level where the antero-superior limb is free, the tip of the limb is degenerate in addition to the lateral half of the nucleus. The area of the nucleus involved progressively diminishes until at the middle of its dorso-ventral extent only the

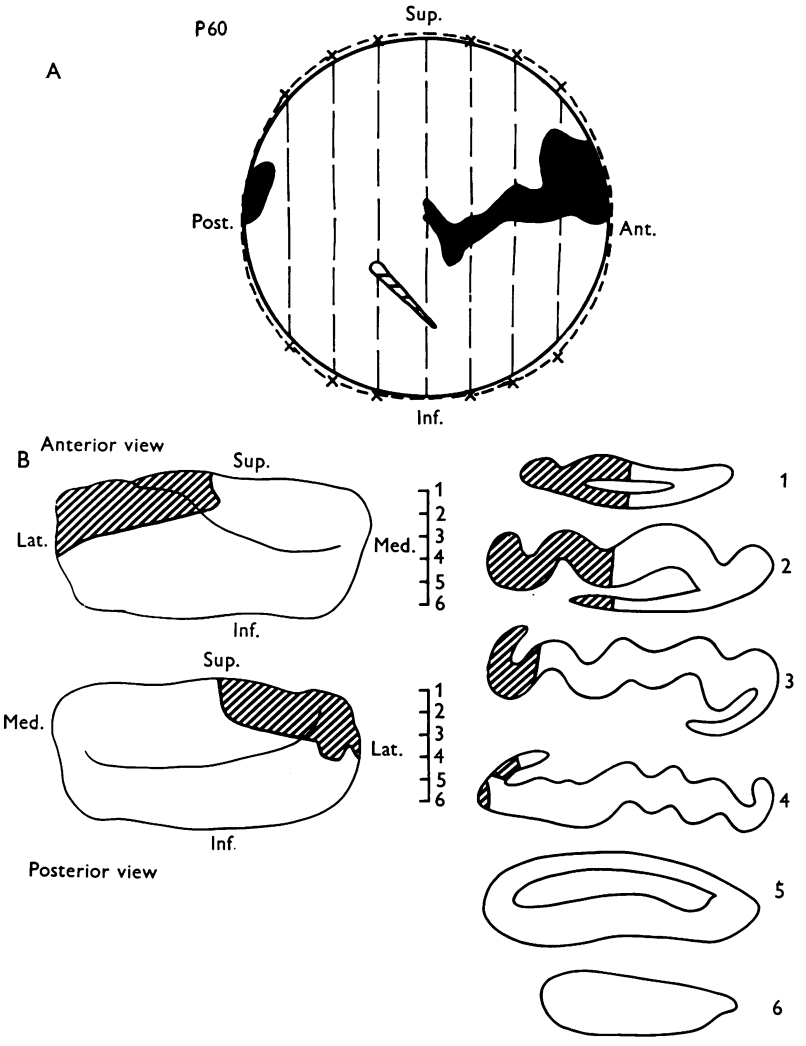

Fig. 3. The position of the lesion in the retina and the distribution of the degeneration in the isthmo-optic nucleus in Expt. P60.

junctional region between the postero-lateral limb and the body of the nucleus is affected. Ventral to this level there is a small area of cell shrinkage and partial cell loss affecting only the postero-lateral limb; the ventral third of the nucleus is quite free of degeneration (Fig. 3). As the area of damage at the point of entry of the electrode is comparable to that seen in several other experiments in which no

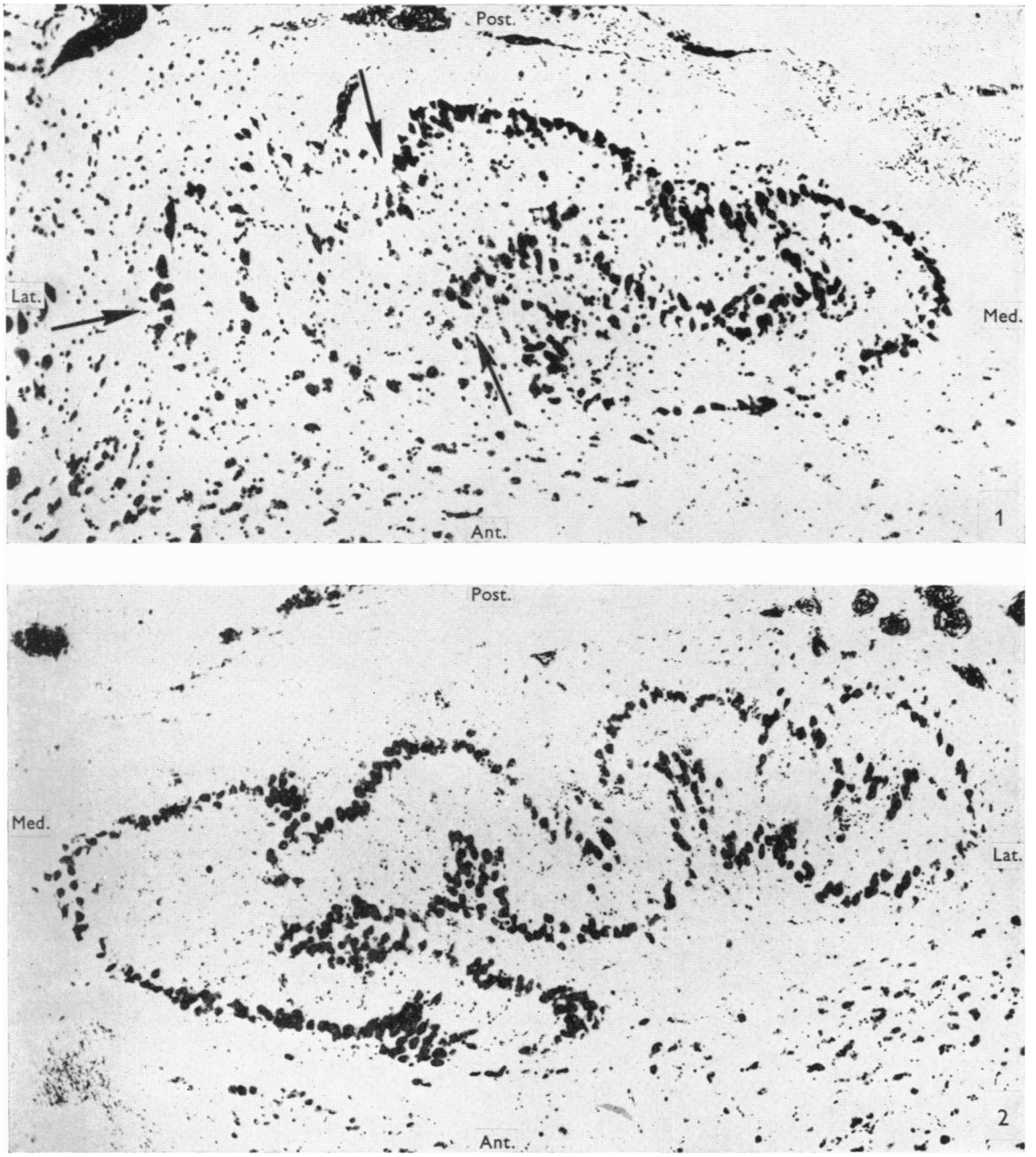

Fig. 4. (1) The cell loss in the lateral half of the body and postero-lateral limb (demarcated by arrows) of the affected nucleus of Expt. P 60, and (2) the nucleus of the normal side. Thionin, × 120.

degeneration was detectable in the isthmo-optic nucleus, it may be safely assumed that the degeneration in this experiment is due only to the lesion in the antero-superior and antero-inferior quadrants.

It is significant that in Expt. P60 the amount of degeneration in the ventral half of the nucleus was appreciably less than in P82 and this would agree well with the small amount of damage to the antero-inferior quadrant of the retina in P60. In the

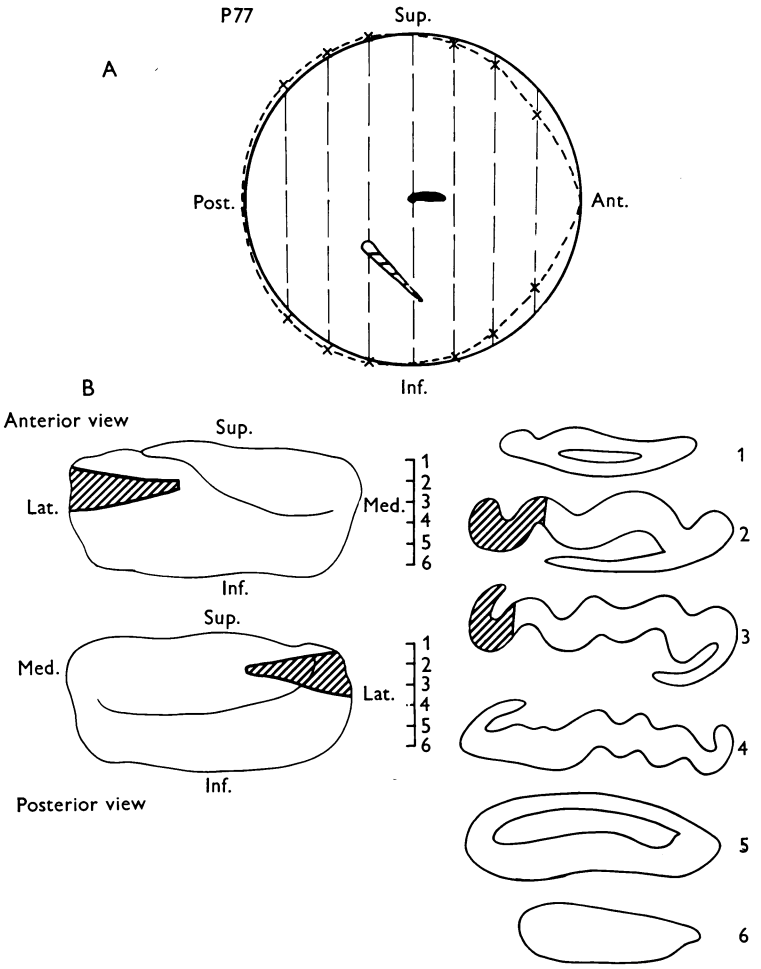

Fig. 5. The retinal lesion and the resulting cellular degeneration in the isthmo-optic nucleus in Expt. P77.

following experiment, P77, there has been no interruption of centrifugal fibres to the antero-inferior quadrant as the lesion lies immediately above the horizontal axis. Again, the entry of the electrode has caused an area of damage along the superior edge of the retina, but the definitive lesion is a small slit-like focus of destruction extending forwards from the fovea, the edge of which has been damaged (Fig. 5). The area of retrograde atrophy in the isthmo-optic nucleus in this experiment is more

restricted than in the previous cases and is limited to the dorso-lateral quarter of the nucleus. Here the cell shrinkage and partial cell loss affects the postero-lateral limb and the adjoining part of the body. The most dorsal sections are free of degeneration and the cellular atrophy stops abruptly at the middle of the dorso-ventral extent of the nucleus (Fig. 5).

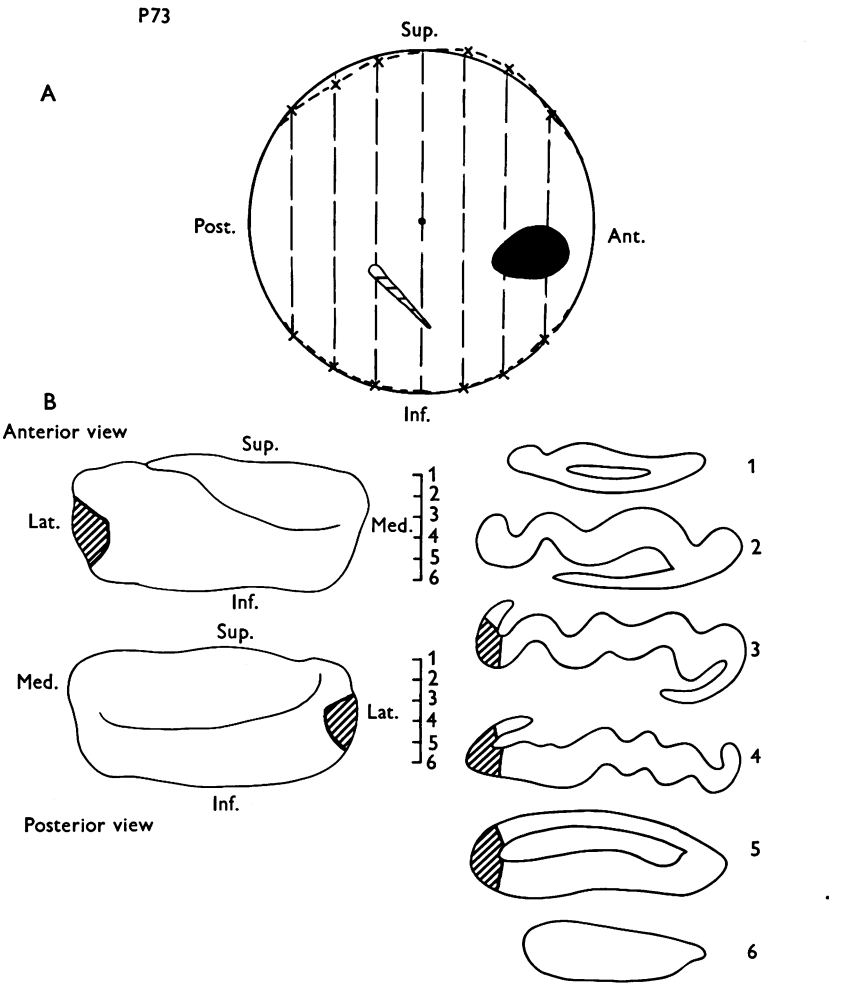

Fig. 6. The site and extent of the lesion in the retina and the distribution of the retrograde cell degeneration in Expt. P73.

The last three experiments which have been described are consistent in indicating that the antero-superior quadrant of the retina is in receipt of centrifugal fibres from the cells in the dorso-lateral quarter of the nucleus, and from a comparison of the amount of involvement of the antero-inferior quadrant by the lesions it seems reasonable to infer that the centrifugal fibres to this area have their origin in the ventro-lateral part of the nucleus. Direct evidence that this is so is provided by Expt. P73 in which there is a small, localized area of cell shrinkage and partial cell

loss with some gliosis near the lateral end of the body of the isthmo-optic nucleus and its junction with the postero-lateral limb at, and immediately below, the middle of the nucleus. This degeneration has resulted from a lesion of moderate size in the peripheral part of the antero-inferior quadrant of the retina just below the horizontal axis (Fig. 6).

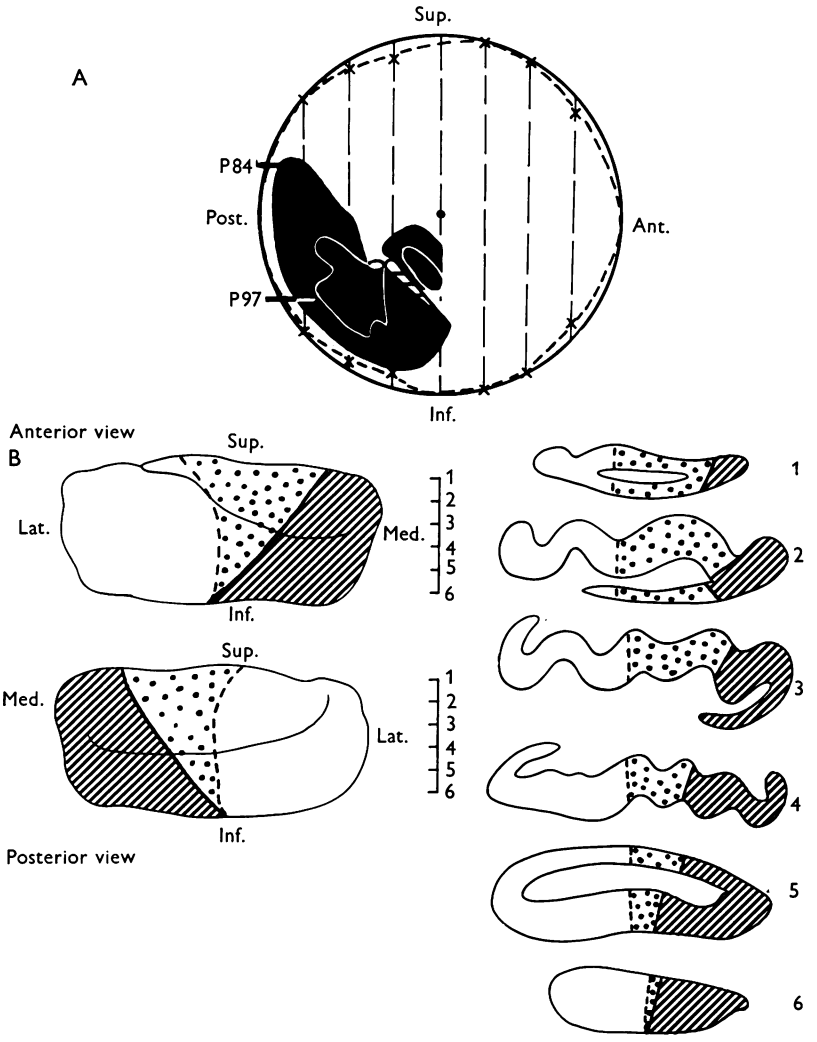

Fig. 7. The lesions in Expt. P84 together with that of P97 superimposed, and the cellular degeneration in the isthmo-optic nucleus of Expt. P84; the hatching represents the area showing severe cell loss and the dots the area of cell shrinkage.

The remaining two experiments to be described, P84 and P97, are concerned with the centrifugal fibres to the posterior half of the retina, and as they are similar in all respects they will be considered together. The lesion in P84 has effectively destroyed most of the postero-inferior quadrant of the retina, has partially involved the

postero-superior quadrant and has probably interrupted most of the fibres to the rest of this quadrant and some to the antero-superior quadrant (Fig. 7). The area of retinal destruction is in two parts which surround the posterior end of the optic disc; the larger part, below and behind the disc, extends right up to the disc margin but has not damaged the optic nerve head. The smaller part of the lesion occupies the area between the fovea and the postero-superior half of the disc but does not encroach upon the latter. In the isthmo-optic nucleus of the contralateral side there is unequivocal evidence of cell loss throughout its dorso-ventral extent. Both the extent and severity of the cell atrophy are more marked in the ventral half of the nucleus where it occupies both the body and the postero-medial limb. In the dorsal half of the nucleus the area of cell loss becomes progressively restricted to the medial end, but cell shrinkage can be found in the greater part of the medial half of the nucleus (Fig. 7). The cellular degeneration in the isthmo-optic nucleus of Expt. P97 is very similar in its distribution; differing only in that it extends a little farther medially and does not involve the most dorsal sections. This distribution agrees well with the site of retinal damage, because although the area of retinal damage is less than in P84 the two parts of the lesion have interrupted fibres from essentially the same area. In addition to the entry track of the electrode in the peripheral part of the antero-superior quadrant there is a narrow cut just above the upper border of the posterior half of the optic disc and a separate, moderately sized area of damage in a comparable position along the lower border of the disc.

Both these experiments complement the findings of the earlier group in showing that the posterior half of the retina receives its centrifugal fibres from the medial half of the isthmo-optic nucleus, and as the degeneration in both experiments is more extensive in the ventral half of the nucleus it seems likely that this part of the nucleus is related to the postero-inferior quadrant of the retina, which in both cases has suffered the greater damage. Unfortunately, we have no lesion of suitable size limited to the postero-superior quadrant which would provide direct evidence upon this point.

DISCUSSION

Although the use of the method of retrograde cell degeneration in this study illustrates many of the disadvantages inherent in this method there is no doubt that it is the only practicable technique available at present for defining the origin within the isthmo-optic nucleus of the centrifugal fibres to different parts of the retina. At the same time, the attempt to interpret these difficulties suggests certain features of the nature of the projection and particularly of the pattern of termination. The main problem concerns the absence of unequivocal atrophy of the cells in the isthmo-optic nucleus after small peripheral lesions of the retina. The first and most obvious explanation for this absence of degeneration is that there is, in fact, no centrifugal projection to the extreme periphery of the retina; this is unlikely on *a priori* grounds, and in a previous study (Cowan & Powell, 1963) it was shown that after destruction of the isthmo-optic nucleus fibre degeneration was seen extending out to the periphery of the retina. The second possibility is that which has been mentioned when describing the results, namely, that although a small amount of cellular degeneration does occur after these lesions it cannot be detected because of the small

size of the nucleus and the normal range of variation in cell density and in depth of staining. A third possibility is that the individual centrifugal fibres branch extensively before their termination, and because a small lesion would interrupt only a few of the terminal branches of a particular axon there would either be no cellular reaction or the change would be so slight as again not to be detectable. Such variation in the reaction of cells following section of some of the axonal branches is known to occur in other sites in the mammalian nervous system. There is good evidence to suggest that branching of the individual axons almost certainly occurs (Wallenberg, 1898; Cowan & Powell, 1963), but the area of distribution of the branches of any particular axon is not known. There may well be differences in the area of distribution of individual centrifugal fibres, and in particular between those passing to central and peripheral parts of the retina. In addition to any branching that may occur in or near the retina, it is possible that collateral branches are given off within the brain to the other relay nuclei along the visual pathway, and the persistence of some shrunken cells within an area of severe cellular degeneration following large retinal lesions or after eye enucleation must either be explained on this basis or on the supposition that the persisting cells are interneurons.

Despite these difficulties it has been possible to show that there is a topical organization in the projection of the isthmo-optic nucleus upon the four quadrants of the retina. Thus the cells of the medial half of the nucleus send their axons to the posterior quadrants of the retina and those in the dorsal half of the nucleus to the superior quadrants (Fig. 8). This basically simple arrangement allows the fibres from each topographical quarter of the nucleus to pass directly to the corresponding part of the retina of the contralateral eye. The significance of this topical organization is to be found in the organization of the afferents to this nucleus from the tectum. As was shown in the first part of this study (McGill *et al.* 1966) the precise representation of the retina upon the tectum is maintained in turn in the projection of the tectum upon the isthmo-optic nucleus, so that each part of the nucleus is influenced, through the tectum, by a localized part of the retina. That is to say, the retina may be regarded as being secondarily represented in the isthmo-optic nucleus, the superior quadrants being represented dorsally and the posterior quadrants medially. This means that each quarter of the nucleus is related to the same quadrant of the retina both from the point of view of its afferents (through the tectum) and on the basis of its efferent projection via the isthmo-optic tract. As was pointed out in the previous study (McGill *et al.* 1966) the retino-tectal projection is such that the retinal representation is inverted in both the vertical and horizontal dimensions. Fig. 9 shows that in the tecto-isthmo-optic projection there is complete re-inversion in the vertical dimension and a partial (approximately 90°) rotation in the horizontal dimension so that the anterior and posterior parts of the tectum respectively are represented medially and laterally in the isthmo-optic nucleus. The results of the present study make it clear that in the return projection of the isthmo-optic nucleus to the retina the only topical change which occurs is that the lateral and medial parts of the nucleus project to the anterior and posterior halves of the retina, with the result that the partial re-inversion which occurs at the tecto-isthmo-optic level is completed.

Whether the reciprocal relationship holds true between small subdivisions within

each quarter of the nucleus and the corresponding part of each retinal quadrant cannot be determined with present techniques, but that this is highly likely is suggested by the finding of such a precise relationship in both the retino-tectal and

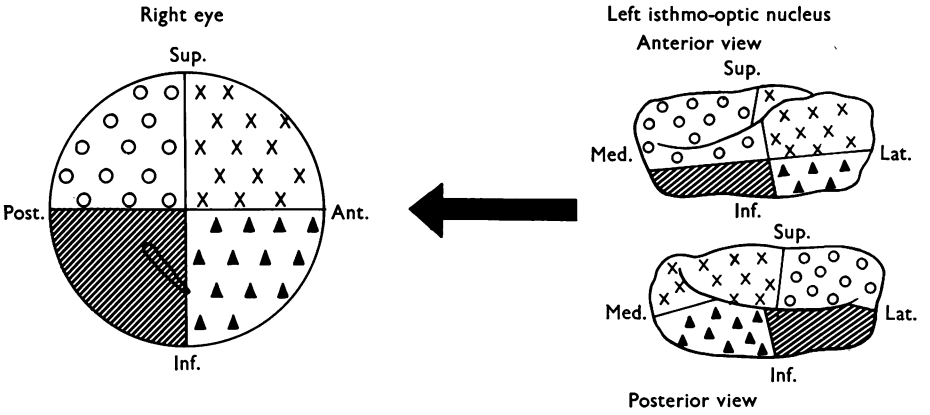

Fig. 8. Composite diagram to illustrate the results of the present study of the projection of the isthmo-optic nucleus upon the contralateral retina.

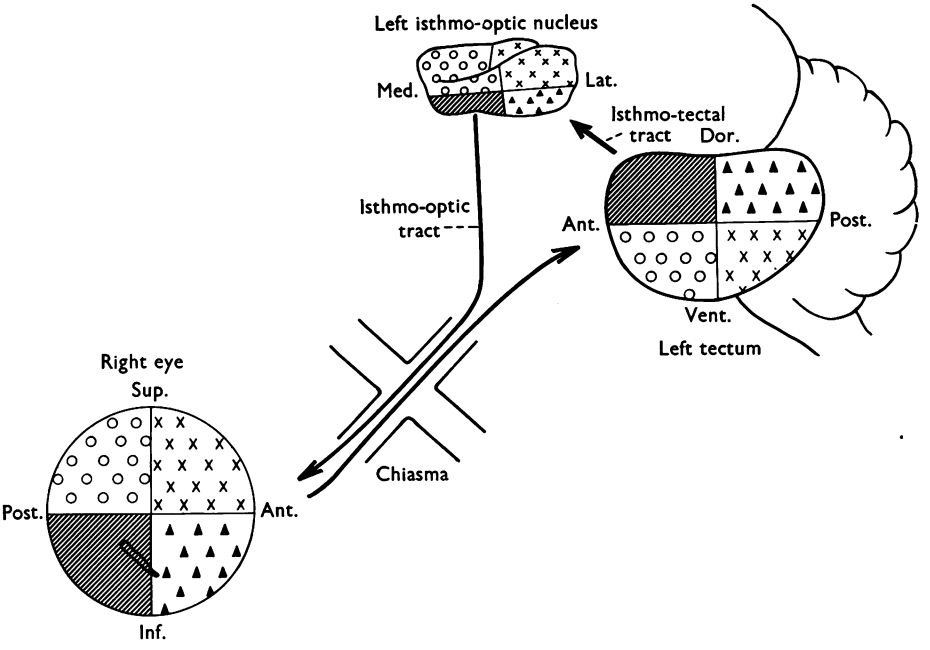

Fig. 9. The organization in the relationship between the retina, optic tectum and isthmo-optic nucleus and the projection of the centrifugal fibres from the latter upon the retina.

in the tecto-isthmo-optic projection (Hamdi & Whitteridge, 1954; McGill *et al.* 1966). In this respect it would be of particular interest to determine experimentally the details of the relationship between the centrifugal fibres and the fovea, and especially

whether the isthmo-optic nucleus sends a relatively larger number of fibres to the fovea than to the surrounding area of the retina as described in normal material by Cajal (1889), and in view of Whitteridge's (1961) finding that the magnification factor for the foveal representation upon the tectum 'is about 0·25 mm/°, whereas that for most of the peripheral field more than 30° out is only 0·04 mm/°'. This problem must await investigation with electrophysiological techniques with which it should be possible to establish not only the detailed relationship between the isthmo-optic nucleus and the retina but also the functional significance of this centrifugal fibre system. At present it would be idle to speculate as to what the functional importance of such a reflex pathway may be, but two important factors which have to be taken into account are the site of action and the distribution of the centrifugal fibres in the retina. From what is known of the termination of these fibres (principally upon the amacrine cells, Cajal, 1889; Dogiel, 1895; Cowan & Powell, 1963) they are capable of influencing activity at all levels within the retina. On the other hand, nothing is known of the extent of the retina which a single centrifugal fibres may influence. Present concepts of the functional organization of the retina (Kuffler, 1953) suggest that three alternative patterns must be considered. First, the axon of any individual centrifugal neuron may be distributed only to the area including the ganglion cells from which it received, through the tectum, its afferent activation. Secondly, it may be distributed only to ganglion cells in the area adjoining those with which it is related on the afferent side, and thirdly, it may have a wide distribution affecting both these groups of ganglion cells. As the total number of centrifugal neurons in the pigeon is only of the order of 1% of the ganglion cells (Cowan & Powell, 1963), the field of distribution of each fibre must include many ganglion cells, and conversely there must be a considerable degree of convergence of ganglion cell activity (through the tectum) upon these centrifugal neurons. It should perhaps be pointed out that by concentrating upon the relationship between the ganglion cells and the centrifugal neurons we are ignoring the interaction which almost certainly occurs in the retina and the optic tectum, the structural complexity of which surpasses that of any other part of the avian nervous system.

SUMMARY

The projection of the isthmo-optic nucleus (the nucleus of origin of centrifugal fibres in the avian visual pathway) upon the contralateral retina has been studied using the method of retrograde cell degeneration. The lateral half of the nucleus projects to the anterior (or nasal) quadrants, and the superior half of the nucleus to the upper retinal quadrants. These findings are discussed in relation to the whole reflex pathway between the retina, optic tectum and isthmo-optic nucleus of which the centrifugal fibres form the efferent limb.

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