STUDIES ON TRANSNEURONAL CELL DEGENERATION IN THE CENTRAL NERVOUS SYSTEM

BY

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"In presence of chemical, thermal, electrical, and traumatic stimuli the neuronal protoplasm responds with interesting intracellular or extracellular metamorphoses. Many scientists have devoted themselves to the analysis and exposition of such structural changes, and the sum of data and observations published in the last few years is already very great. Apart from their intrinsic interest, such observations have an important biological value. As we note below, they reveal some of the fundamental causes of neuronal form and growth, and thereby lay bare some of the mysteries of cellular life".

Cajal (1928)

CONTENTS

INTRODUCTION.

Transneuronal cell degeneration is the degeneration or atrophy of nerve cells which may ensue when they are deprived of part or all of their afferent connexions. It was first recognised as such in the dorsal nucleus of the lateral geniculate body after removal of the eye by Minkowski in 1913. Despite several claims for its occurrence at other sites in the central nervous system it is still generally considered that the lateral geniculate nucleus might be the only site for its unequivocal demonstration (c.f. Torvik, 1956). It is possible, however, that it may occur in the relay nuclei of other sensory pathways as well as in certain cerebral and brain-stem systems. This possibility is of interest for two reasons, one relevant to the degenerative process itself, and the other to its use as an experimental technique.

First, with regard to the degenerative process, little is known about either the factors which are responsible for initiating the degeneration or about those which influence the rate and severity of the cellular change. It has been shown that with this type of cellular degeneration, in contrast to retrograde cell degeneration (resulting from interruption of the axon of the cell) there are marked species differences; thus the cellular atrophy in the lateral geniculate nucleus following removal of the eye is both more rapid in

onset and more severe in the macaque than in the cat and rabbit (Matthews, Cowan and Powell, 1960). It is not known, however, whether the time-course and degree of cellular change is the same in different afferent sensory systems in the same species. It is also not clear to what extent the cellular change is affected by such factors as the morphology of the afferent nerve endings, the type of synapse between these and the nerve cell, and also the size and morphology of the cell itself. Again, there is no evidence to indicate whether the three main components of the neuron-dendrite, soma and axon - take part in the degenerative process, as all observations have so far been limited to the appearance and size of the cell body in Nissl-stained sections.

Secondly, in relation to its use as an experimental technique, if transneuronal cell degeneration were found to occur in other systems it might be possible to use the occurrence of such cellular changes, not only for analyzing more precisely the organization of the projection of the peripheral receptors upon the central relay nuclei, as was done by Le Gros Clark and Penman (1934) for the projection of the retina, but also for tracing connexions in systems where it is difficult to determine whether fibres are actually terminating or simply passing through a nucleus.

A study of transneuronal cell degeneration in the olfactory

bulb and auditory relay nuclei should provide answers to some of these questions. There are, for example, within the main subdivisions of the cochlear nuclei distinct differences in both the type of afferent nerve endings and in the size of the cells. The ascending branches of the primary auditory nerve fibres terminate as characteristic endings of Held on the cells of the antero-ventral cochlear nucleus, while the descending branches end around the cells of the posteroventral and dorsal cochlear nuclei in the form of boutons and pericellular plexuses. In the olfactory bulb, where the normal structure has been worked out in considerable detail by Cajal (1911), the synapse between the olfactory nerve fibres and the mitral cell is purely axodendritic, only the principal dendrite of the mitral cell being directly related to the incoming olfactory fibres. The accessory dendrites and cell body of the mitral cell, on the other hand, are exposed to other sources of afferent stimulation derived from the opposite bulb and certain forebrain structures. In addition, there are two other features of interest in the olfactory bulb relevant to this form of cellular degeneration; first, the olfactory nerve fibres make contact with other cells of different size and morphology, namely, the small periglomerular cells and the so-called tufted cells so that it would be of interest to determine whether all three of these neurons would be affected by deafferentation; secondly, as all parts of the mitral cell (dendrite, soma and axon)

can be stained and clearly identified with silver methods it should be possible to define the changes that might occur in all components of the neuron.

The question whether the time-course and severity of the cellular change is the same in different afferent sensory systems of the same species can only be investigated by the use of quantitative methods. In this respect an important contribution was made by Cook, Walker and Barr (1951) in their detailed quantitative study of the shrinkage in cross-sectional area of the cell body, nucleus and nucleolus of the neurons in the lateral geniculate nuclei of the cat and rabbit during transneuronal atrophy. For such quantitative studies it is preferable to be able to achieve more or less complete destruction of the major source of afferents, and the population of neurons to be studied should be relatively homogeneous. As these two requirements are satisfied better in the case of the individual cochlear nuclei than in the olfactory bulb, a quantitative study of the cellular degeneration in these nuclei in the cat or rabbit following destruction of the cochlea should provide results which can be compared with those of Cook et al (1951).

The value of the possible application of this cellular degeneration as an experimental technique for elucidating the connexions of the olfactory and auditory systems need hardly be stressed. Apart from the need for defining more precisely the topical organization of the olfactory mucosa upon the

olfactory bulb (c.f. Le Gros Clark, 1957) and of the basilar membrane upon the cochlear nuclei, it is necessary to determine the main sites of termination of the primary auditory nerve fibres (c.f. Galambos, 1954).

The preliminary observation which suggested that trans-neuronal cell degeneration occurs in sensory afferent systems other than the visual was made some years ago, during the course of a study of the projection of the olfactory mucosa upon the olfactory bulb in collaboration with Le Gros Clark (Le Gros Clark and Powell, 1957; Le Gros Clark 1957). It was found that the dendrites of the mitral cells of the olfactory bulb had undergone profound atrophy 24 days after removal of the olfactory mucosa. This finding, together with the reasons which have already been given, was the basis for the systematic study of this form of cellular change in the olfactory bulb and auditory relay nuclei. For the investigation of the olfactory system the rabbit was chosen as the experimental animal because of its readily accessible olfactory mucosa and bulb. The study of the auditory relay nuclei was done on the cat, however, because most experimental work - anatomical and physiological - on the auditory system has been done in this species, and also because quantitative data of trans-neuronal cell degeneration in the lateral geniculate nucleus of the cat given by Cook et al (1951) can be used for a comparison of the degeneration in two afferent systems in the same species.

The results of these respective studies constitute the first two sections of the Results. In the third section the findings of an investigation of the projection of the primary auditory nerve fibres done with different experimental methods are presented. This additional investigation was necessary for the interpretation of some of the results of the study of the cellular changes which occurred in the auditory relay nuclei following destruction of the cochlea described in the second section.

MATERIAL AND METHODS.

The observations on transneuronal cell degeneration in the olfactory bulb were made upon 32 young rabbits. The animals were operated upon at the age of 5-8 weeks, in order to minimize the occurrence of 'atrophic rhinitis' during the subsequent survival period (Le Gros Clark and Warwick, 1946). Pentobarbitone anaesthesia (with no supplementary ether) was used for all animals.

In 18 animals the bony nasal cavity of the right side was opened from above and the mucoperiosteum was detached from the walls of the nasal cavity without opening the latter and separated as completely as possible from the cribriform plate, but otherwise left in situ. The cavity was dusted with penicillin-sulphonamide powder and the periosteum and skin were closed over the bony defect. The rabbits were allowed to survive for periods ranging from 6 days to 200 days, and were then perfused, under pentobarbitone anaesthesia, through the ascending aorta with normal saline followed by 10% formol-saline. In 15 of these animals the brain was then removed from the cranial cavity and immersed in formol-saline; in the remaining 3 animals the cerebral hemispheres and olfactory bulbs were exposed but not removed, in order that the bulbs might be sectioned in undisturbed relationship to the nasal cavity, after decalcification.

It was found that the type of operation and the method

of fixation used in these 15 animals had two disadvantages. Firstly, because the mucoperiosteum was only detached from the walls of the nasal cavity and not completely removed, some of the olfactory nerve fibres escaped injury in a number of animals. Secondly, the fixation by formalin resulted in incomplete impregnation of the sections of the brains stained by the Bodian method. In order to overcome these difficulties a different procedure was adopted in 10 animals. The mucoperiosteum was removed entirely, together with the turbinate processes, from the posterior part of the right nasal cavity, again with careful scraping of the under surface of the cribriform plate. After survival periods ranging from 12 days to 184 days these animals were killed by an overdose of anaesthetic, and their brains were removed and fixed by immersion in 70% alcohol and 2% acetic acid. It should be emphasized that, apart from the more complete deafferentiation of the olfactory bulb and the better impregnation of the sections of these brains with protargol, there was no difference in the results in these two series of experiments.

In two rabbits in which the mucoperiosteum of the right nasal cavity had been removed and which survived for 48 and 96 days after operation, the removal of the brain was begun under anaesthesia and completed as soon as possible after death; the brain was then cut into three blocks which were placed at once in Golgi-Cox fixative (method given by Sholl, 1953).

In another two rabbits an attempt was made, by inserting a probe through the right nostril, to destroy selectively the vomero-nasal organ of this side. After survival periods of 49 and 98 days respectively, these animals were anaesthetised and perfused with saline and formol-saline.

From each brain, other than those treated by the Golgi-Cox method, a block consisting of both olfactory bulbs and peduncles with the foreparts of the cerebral hemispheres was embedded in paraffin wax and sectioned at 10μ or 25μ, usually in the coronal plane but in some cases sagittally or horizontally. Two regular series were mounted, at intervals of 5 or 10 sections, one for staining with thionin and the other for the protargol silver impregnation of Bodian. The sections of formalin fixed material were treated with acetic acid alcohol before staining by either method, as this was found to improve the differentiation.

Most of the observations were made qualitatively, but in two animals with 16 and 130 days' post operative survival, limited quantitative assessments were made of changes in the cross-sectioned area of various structures. Using a camera lucida, the outlines of the cell bodies of 50 mitral and 50 tufted cells of the main bulb, and 50 mitral cells of the accessory bulb, were traced from corresponding sites in thionin stained sections of each olfactory bulb in each animal. The tracings were made on mm. graph paper at a linear magnification of 1000 times, so that the number of mm. squares occupied by

each outline represented its area in μ^2. From each site outlines of the first 50 neurons encountered having a distinct nucleolus were traced, in the plane of focus of the nucleolus. In the animal with the longer survival, tracings were likewise made of the olfactory tract on each side from protargol preparations at 5 corresponding levels evenly spaced over the first 6 mm. of the tract, and in addition the outline of the olfactory bulb with its various layers was traced from one thionin-stained coronal section approximately half-way along each bulb, using a projection apparatus and a lower magnification. For each animal, the values of area or mean area obtained from these tracings in the two bulbs were compared in order to test for shrinkage and to give some measure of its degree; the figures obtained for the areas of the olfactory tracts are given in μ^2, but those for the different layers of the bulb in arbitary units.

A complication arising in relation to survival periods of 2 months or more was that 7 of the rabbits were found to have developed 'atrophic rhinitis' with severe or total loss of olfactory nerve fibres on each side, accompanied by shrinkage of the bulbs. It should be emphasized that all of the findings to be described are based on the brains of those animals in which this condition did not occur; 6 of these rabbits survived for 3 months or longer. Unexpectedly, the 'atrophic rhinitis' always spared the fibres of the vomero-nasal nerve, and the

accessory olfactory bulb to which they are distributed.

In the study of transneuronal degeneration in the auditory relay nuclei ten mature cats of both sexes were used. In all the animals the bulla was exposed on one side and the cochlea destroyed. After being allowed to survive for periods varying from 30 days to 359 days, (the interval having been chosen to correspond in general with those of Cook et al (1951)) the animals were killed and the brains and temporal bones fixed in 70% alcohol and 2% acetic acid. The brain of one normal animal was used as a control. Blocks of the brain stem were embedded in paraffin wax and cut transversely at 25μ. A 1 in 5 series of sections was mounted and stained with thionin.

The severity and the extent of the transneuronal cell degeneration and glial reaction were studied qualitatively in the cochlear nuclei, the nuclei of the superior olive, the trapezoid and lateral lemniscal nuclei. Quantitative studies were made of the degree of the cellular degeneration in all but the last of these nuclei in nine animals. In the tenth animal the cochlear nuclei had been inadvertently damaged at operation.

Cell shrinkage was estimated, as in the olfactory bulb, by drawing, on mm. graph paper with the aid of a camera lucida, the outlines of the cell, nucleus and nucleolus of 50 neurons on the normal and atrophied sides. The linear magnification was adjusted to exactly 1000 times so that a measurement in μ^2

was obtained of the nucleolar, nuclear and total cell area by counting the number of squares contained within each outline. The samples were taken at comparable levels on the two sides of the brain stem and at corresponding levels in each animal. Thus, for the medial trapezoid nucleus, the lateral superior olive and the cochlear nucleus two sections at the middle of the rostro-caudal extent of the nuclei were used, and for the postero-ventral and the antero-ventral nuclei the seventh and eighth sections from their caudal and rostral ends respectively were taken. Sample fields were taken at random throughout the cross-sectional area of the nucleus, and the only criterion used for selection of the cells was the presence of a distinct nucleolus.

In one experiment (survival period 319 days) an estimate was made of the total number of cells in the ventral cochlear nucleus and the lateral superior olive on the normal and atrophied sides. The areas of the nuclei in every section of the series (125μ apart) were measured from projection drawings on one inch graph paper at a magnification of 50 times. The volume of the nucleus was then calculated (Dornfield, Slater and Scheffe, 1942). Counts of cell density were made throughout the length of these nuclei using an oil immersion objective and a field of known diameter. Only neurons with a distinct nucleolus were counted, and the size of the sample was of the order of 2% of the total cell population of the ventral cochlear nucleus and 7% of that of the lateral superior olive.

In the female animals the nucleolar sex satellite was very conspicuous, and in two animals - one short and one long survival period - the position of the satellite in relation to the nucleolus or the nuclear membrane was determined in approximately 500 cells of the lateral superior olive and the ventral cochlear nucleus on the normal and atrophied sides. In order to determine the extent of the lesion the temporal bones from one animal (survival period 319 days) were decalcified and sectioned, and alternative series were stained with haematoxylin and eosin and Bodian's protargol method. The bones from other animals were not cut because the distribution of the degeneration in the brain stem was the same in all animals.

In the investigation of the central projection of the primary auditory nerve fibres eleven cats were used. Lesions were made in the cochlea on one side through an opening in the tympanic bulla. After periods ranging from 12 hours to 15 days the animals were anaesthetised with nembutal and perfused through the ascending aorta with saline followed by 10% formol-saline. After further fixation in formol-saline all but two of the brains were embedded in paraffin wax and sections of 15μ were cut in the coronal plane. From each brain four 1 in 10 series through the pons and medulla were mounted and stained with thionin, the Nauta and Gygax (1951) method, the Marsland, Glees and Erickson method (1954) or the Bodian

method (1936). Two brains were sectioned on a freezing microtome at 25μ and every tenth section stained by the Chambers, Liu and Liu (1956) modification of the Nauta technique (1954) and every eleventh section with the Glees method (1946).

Two brains of the previous series were stained by the Bodian method. In one of these brains the cochlea had been destroyed 359 days before death, and in the other brain in which the cochlear nuclei had been involved in addition to the cochlea, 60 days.

The temporal bones of some of the cats used in these experiments were decalcified and embedded in paraffin wax. The blocks were cut in the horizontal plane and series of sections were stained with haematoxylin and eosin.

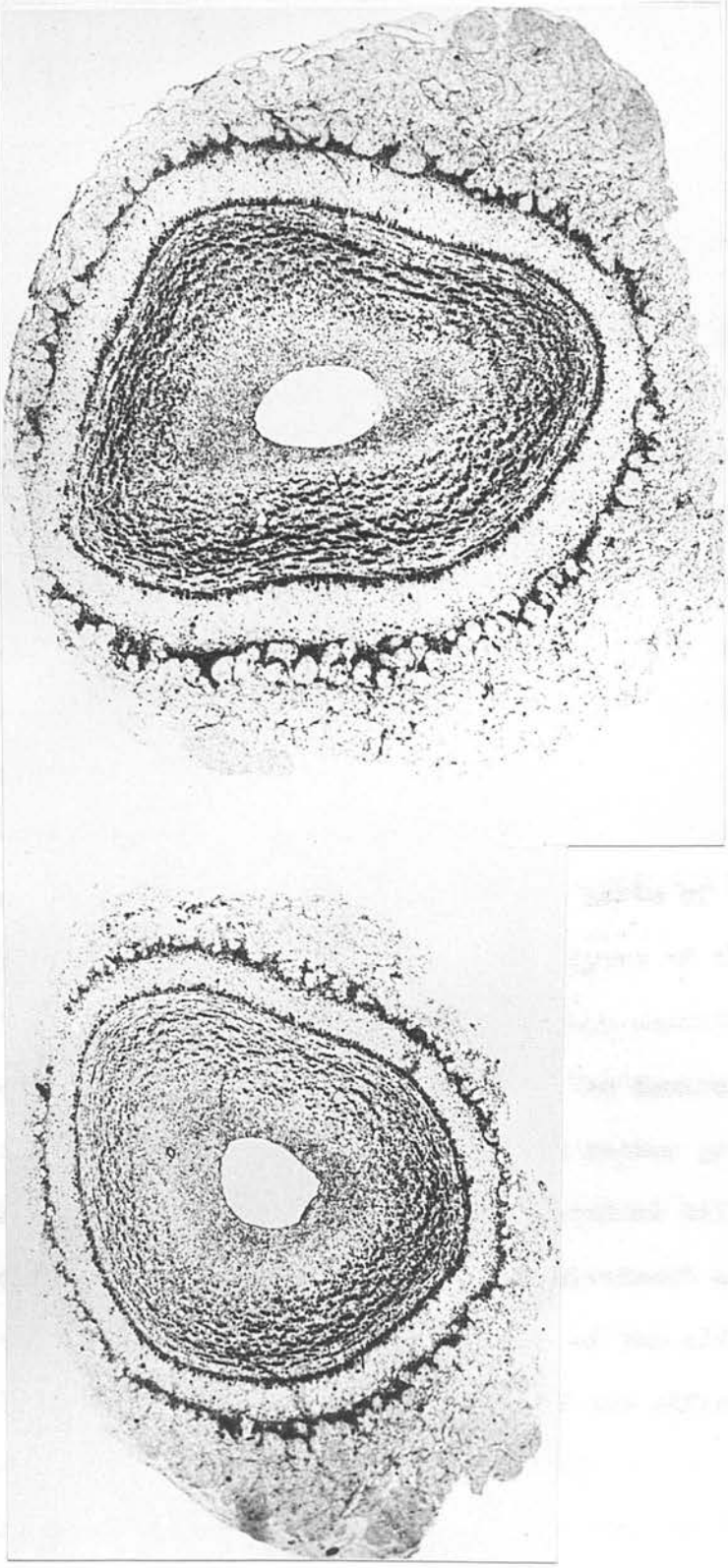


Figure 1. Photomicrograph showing the degree of shrinkage of the olfactory bulb 130 days after removal of the olfactory mucosa. Stained with thionin. x 28

RESULTS

1. Transneuronal cell degeneration in the olfactory bulb

In most experiments, some of the olfactory nerve fibres on the side of operation escaped injury, so that there was only partial loss of the plexus of olfactory nerve fibres on the surface of the olfactory bulb. The fibre loss varied in its severity from animal to animal, and often also from one region of the bulb to another in the same animal, but there was in all cases a sufficient degree of denervation to produce changes in the deeper structures of the bulb, after a long enough period of post-operative survival. Where there was more severe olfactory nerve depletion in some parts of the bulb than in others, then the changes in the various layers of the bulb were more or less severe from place to place, in approximate correspondence with the degree of denervation. The demarcation of such regions with different degrees of change was rather gradual.

One of the severest lesions was found in an animal killed 130 days post-operatively, and the results of this experiment are described in some detail. In this rabbit, most of the olfactory nerve fibres have disappeared from the surface of the olfactory bulb on the side of operation, except laterally where a number of bundles persist. This bulb is much smaller than that on the other side, which shows no evidence of any degenerative changes when compared with normally-innervated pairs of olfactory bulbs, and has been regarded as normal for purposes of comparison (Fig. 1

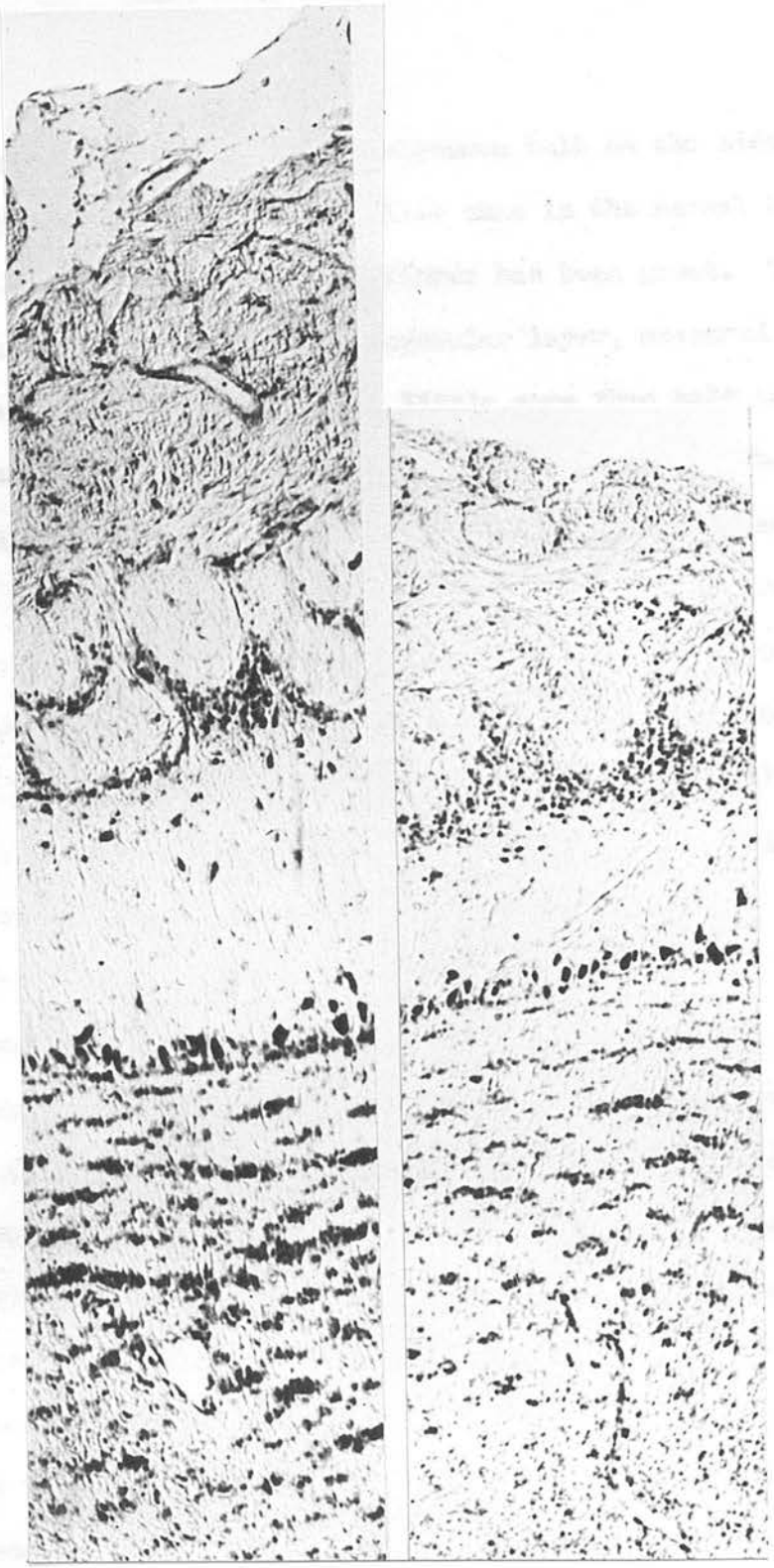


Figure 2. Photomicrographs to show shrinkage of the periglomerular, tufted, mitral and granule cells 130 days after removal of the olfactory mucosa (right) as compared with the normal side (left). Stained with thionin. x 105.

The glomerular layer. In the shrunken bulb on the side of operation, the glomeruli are much smaller than in the normal bulb wherever the loss of olfactory nerve fibres has been great. The cross-sectional area of the entire glomerular layer, measured from one section midway along the bulb, is little more than half that at the corresponding site in the normal bulb (see Table 1). The protargol preparations show in these glomeruli not only the absence or depletion of the very fine terminations of the olfactory nerve fibres, which normally form a dense grey background, but also a change in appearance of the dendritic arborizations within the glomeruli. These are provided by the principal dentrites of mitral and tufted cells and by processes of the numerous peri-glomular cells which link adjoining glomeruli (Cajal, 1911). These dendritic arborizations are either virtually absent or are seen to be much reduced in calibre to very fine filaments or possibly fragments, unlike the coarse arborizations seen in well impregnated glomeruli of the normal bulb. In addition, in such shrunken glomeruli some, at least, of the arborizations must be considerably reduced in extent, for in protargol impregnations of normal glomeruli the branches of single dendrites frequently appear to reach all parts of the glomerulus, and in Golgi-Cox preparations these arborizations at glomerular level are seen to be both extensive and elaborate. The glomerular layer contains also a large population of periglomerular cells. In thionin or protargol preparations the nuclei of these cells, which are the only part clearly stained by either method, appear definitely smaller than normal in the severely denervated regions (Figs 2)

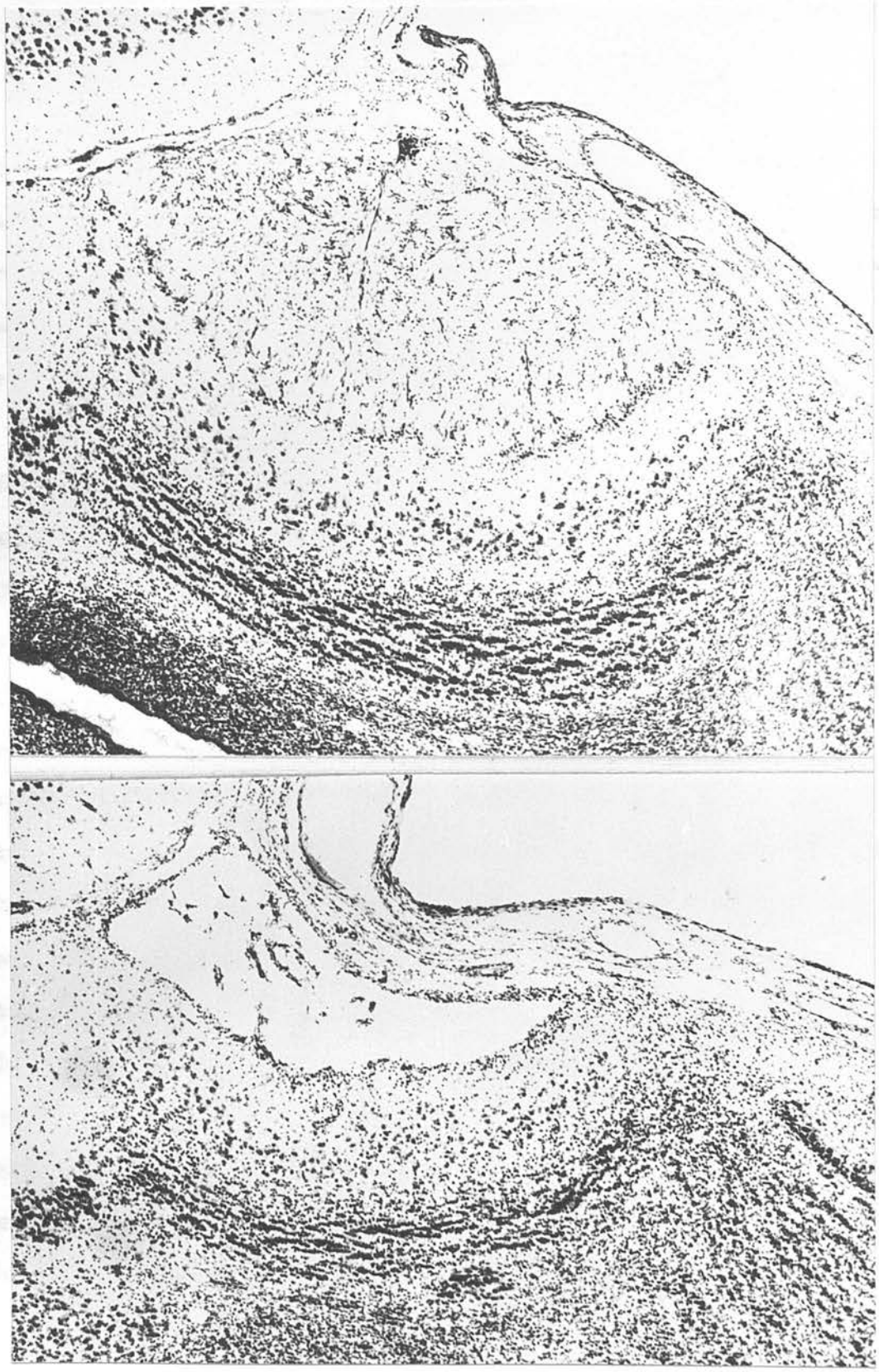


Figure 3. Photomicrographs of sagittal sections of the normal (above) and atrophied (below) accessory olfactory bulbs 96 days after destruction of the vomero-nasal nerve. Stained with thionin. x 70

The outer plexiform layer. This layer is narrowed to about half the width of that in the normal bulb, and in addition the diameter of the ring of mitral cells which forms its inner boundary is reduced. The cross-sectional area as measured in one section mid-way along the shrunken bulb is just over half that at the corresponding level on the normal side.

In thionin-stained sections the mitral cells are smaller in the more severely affected regions of the bulb, but do not appear appreciably paler than normal (Figs. 1 & 2). The tufted cells, gathered mostly in the outer part of the layer but some of which are placed more deeply within it and others just outside it among the peri-glomerular cells, are smaller than normal and have rather paler cyto-plasm, with their dendrites much less often filled with Nissl sub-stance, than in the normal bulb. Measurement of 50 mitral and 50 tufted cells from approximately mid-way along each bulb showed that the mean area of the cell body (figures given in Table 2) had de-creased by about 50% for the mitral cells and about 40% for the tufted cells. (These samples are small, and the figures are not intended to give more than a rough indication of the degree of shrinkage. Large samples would be required to decide whether the mitral cells have indeed shrunk relatively more than the tufted cells, and it would be important to seek confirmation in other experiments).

Other changes in the outer plexiform layer are revealed by the protargol preparations (Figs. 4 & 5). Its texture is lighter than normal, and the constituent fibres, including the main den-dritic stems, tend to be finer. The main dendrites of the mitral

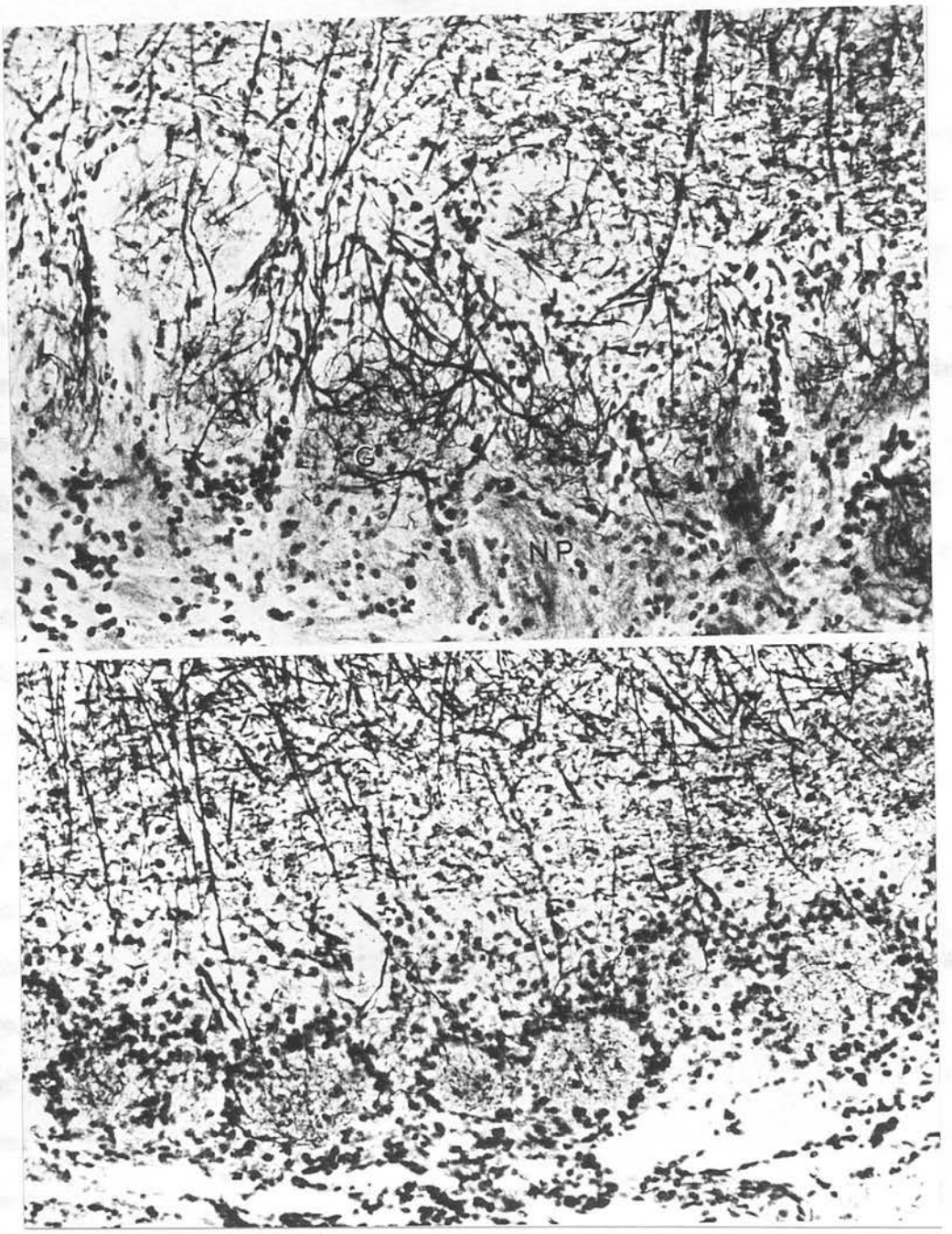


Figure 4. Olfactory nerve layer (NP) and glomeruli (G) of the normal bulb (above) and 24 days after destruction of the olfactory mucosa (below). On the operated side the glomeruli are shrunken, and the dendritic processes of the mitral cells are atrophied and fragmented. x 220

cells, which cross the layer to reach the glomeruli, do not obviously pursue a more wavy course than in the normal bulb, and therefore may well be shorter. The outer part of the outer plexiform layer is distinctly lighter than the inner part, and shows far fewer, or far finer fibres than usual. In some places, indeed, it is practically devoid of impregnated fibres. In the normal bulb, this region has a somewhat different appearance from the inner part of the layer. While the inner part consists largely of oblique and radial processes, which must be principally the main and accessory dendrites of the mitral cells, together with the peripheral processes of granule cells more deeply placed, the outer part contains a high proportion of more nearly tangential or horizontal fibres. The latter part is the region of greatest concentration of tufted cells, the dendrites of which, both main and accessory, have predominantly this orientation. It is therefore possible that the tufted cells may be more severely affected than the mitral cells, a suggestion which would be supported by the apparent loss of Nissl substance from the tufted cells, though not by the limited measurements of changes in area of cell body. It has not, however, proved possible to decide with certainty whether there is selective loss or sparing of any particular group of fibres in the outer part of the outer plexiform layer.

The internal plexiform layer. Deep to the mitral cells is the narrow internal plexiform layer, containing the axons and axon collaterals of the tufted cells and crossed by axons and recurrent axon collaterals of the mitral cells, and peripheral processes of granule cells (Cajal, 1911). This layer appears paler than normal

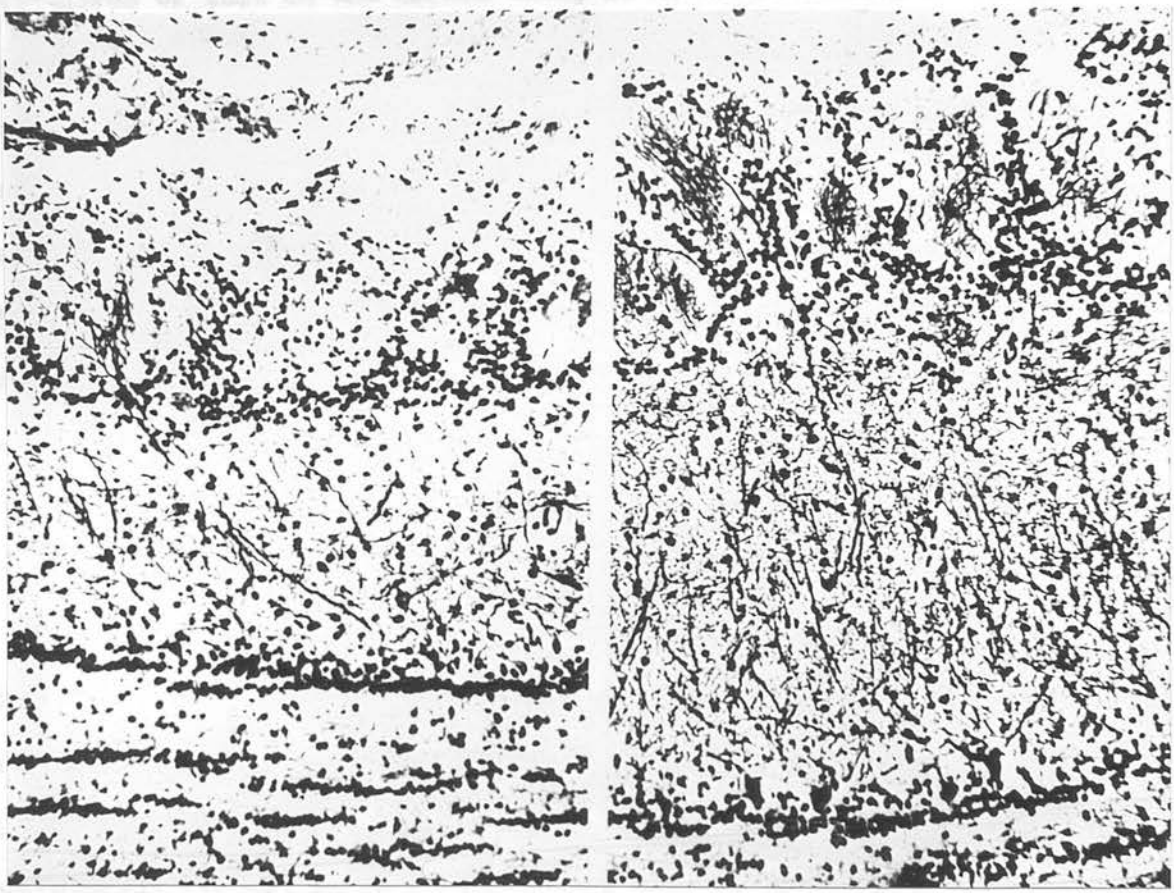


Figure 5. Photomicrographs to show the atrophy of the dendrites of the mitral and tufted cells in the glomeruli and outer plexiform layer 152 days after destruction of the olfactory mucosa (left) as compared with the normal side (right). Bodian's protargol method. x185.

in protargol preparations, when viewed at low magnification.

The layer of granule cells. This layer is distinctly narrower than in the normal bulb. Its area of cross-section is approximately two-thirds of that on the normal side, as measured mid-way along each bulb. In thionin-stained sections the clumps of granule cells are smaller and lighter-staining than on the normal side, and their nuclei (the cytoplasm is not stained by this method) are perceptibly smaller than normal wherever the denervation is severe (Fig. 2.) This applies also to the granule cells which surround the bases of the mitral cells, and are narrowly separated from the granule layer proper by the internal plexiform layer. In protargol preparations the outermost part of the perigranular fibre plexus, like the adjacent internal plexiform layer, appears rather paler than normal.

The periventricular layer has the same cross-sectional area as that on the normal side, and its appearance shows no obvious change either in the thionin or the protargol preparations. Both it and the perigranular fibre plexus contain many centrifugal and commissural fibres, which appear to end chiefly in the granule cell layer, perhaps also reaching the cell bodies of the mitral cells (Cajal, 1911; Allison, 1953), in addition to the axons of mitral and tufted cells and the processes of the granule cells and of other neurons scattered among them.

The changes in the main olfactory bulb, in addition to the loss of incoming olfactory nerve fibres and their terminations, are therefore seen in the cell bodies, dendrites and possibly the axons of the mitral and tufted cells (apparently accessory as well as principal dendrites), in the nuclei and possibly the processes of the periglomular cells, and in the nuclei of the granule cells.

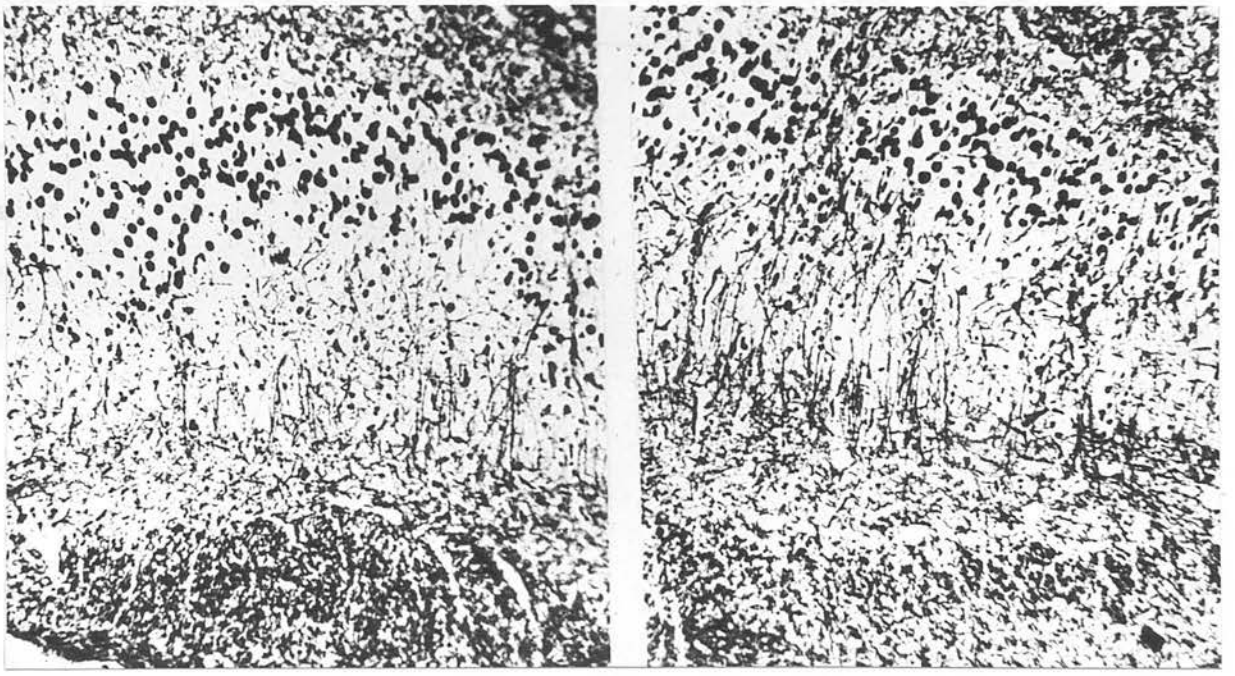


Figure 6. Photomicrographs to show the olfactory tract and the subjacent molecular layer on the normal (right) and the operated side (left) 49 days after destruction of the olfactory mucosa. The olfactory tracts (below) show no change, but in the outer part of the operated side a marked loss of fine fibres has occurred. Bodian's protargol method. x124.

The olfactory tract, in protargol preparations, looks very similar in texture on the two sides, and measurement at successive antero-posterior levels has shown no appreciable difference in area occupied by the tract, except towards the posterior end. This might indicate a more rapid tapering, or even a shortening, of some of the tract fibres on the side of operation, but the possibility of error due to the mis-matching of levels increases with distance from the bulb. A more striking change in relation to the olfactory tract is seen, however, in the subjacent molecular layer. On the normal side, the outer (and greater) part of this layer is of a darker grey than the inner part, and contains a denser plexus of very fine fibres. On the side of operation, this zone adjacent to the olfactory tract is of the same light grey as the inner zone, and its content of the finest fibres is reduced (Fig. 6). This is the site in which the collateral terminations of the olfactory tract fibres arborize in relation to the apical dendrites of the underlying pyramidal cells (Cajal, 1911; O'Leary, 1937; Le Gros Clark and Meyer, 1947); and since the pyramidal cells and the inner part of the molecular layer show no consistent change, it appears that the collaterals of the tract fibres are here deficient. As far as is known, the lateral olfactory tract is formed mainly by the axons of the mitral cells of the olfactory bulb (Cajal, 1911; Allison, 1953).

The accessory olfactory bulb. On the side of operation, the vomero-nasal nerve fibres have more or less completely disappeared from the surface of the accessory bulb, which is smaller than that on the normal side. The glomerular layer can

TABLE I

Measurements of area of cross section of various layers of the olfactory bulbs (mid-point of bulb) and of the olfactory tracts (5 corresponding levels, first 6 mm. behind bulb) in one rabbit, 1.14, 130 days after unilateral destruction of olfactory epithelium.

Region Measured	Area of cross section (approximate) Unoperated side	Side of operation
Glomerular layer	664	367 (arbitrary units)
Outer plexiform layer	950	526 "
(Granule cell layer (Internal plexiform layer	1368	872 "
Periventricular layer	317	321 "
Olfactory ventricle	86	76 "
Olfactory tract		
Level 1 (nearest bulb)	595	368 (μ^2)
Level 2	570	533 (μ^2)
Level 3	461	426 (μ^2)
Level 4	427	360 (μ^2)
Level 5	414	280 (μ^2)

TABLE 2

Mean areas of cell body for groups of 20 neurons from the 'normal' and 'degenerate' olfactory bulbs of two rabbits following unilateral destruction of olfactory epithelium.

Rabbit	Survival period in days	Cell type	Mean area of cell body (μ^2) Normal	S.E.	Degenerate	S.E.	Shrinkage %	Significance by t-test
0.8	16	Main bulb:						
		Mitral cell	168.6	7.91	138.7	8.25	17.7	5%
		Tufted cell	57.9	3.32	47.2	2.36	18.5	5%
		Accessory bulb:						
		Mitral cell	87.6	6.0	58.2	3.40	33.6	0.1%
0.14	130	Main bulb: Mitral cell	206.4	8.59	104.1	7.00	49.6	0.1%
		Tufted cell	58.6	3.4	35.6	3.13	39.3	0.1%
		Accessory bulb:						
		Mitral cell	88.5	3.90	54.1	2.37	38.8	0.1%

no longer be recognised: it is probably represented by a narrow superficial band of cells, some of which resemble shrunken periglomerular cells. The outer plexiform layer is narrowed, and the mitral cells scattered within it are smaller and rather paler than in the normal accessory bulb (Fig. 3). Measurement of 50 mitral cells from each accessory bulb indicates that the cell bodies have shrunk by about 40% on the side of operation (Table 2). The zone of granule cells is reduced in extent and the nuclei of these cells appear somewhat shrunken.

These results in one rabbit 130 days post-operatively are confirmed in various degrees, according to the extent of denervation, in the 5 other experiments in which a long survival period was used (96 to 200 days) with the same histological techniques.

The experiments involving shorter survival periods have shown that the various changes seen after the longer survival periods in thionin and protargol preparations appear at different times after operation. In one rabbit after 6 days, and in two rabbits killed 12 days post-operatively, the layer of olfactory nerve fibres on the affected olfactory bulb shows an increased cellularity and some reduction in thickness. Shrinkage of the glomeruli has begun, but no changes are to be seen in the periglomerular cells, or in any structure of the deeper layers, which appear unaltered in volume. At 16 days after an almost complete interruption of the olfactory nerve fibres in one experiment, the cross-sectional area of the bulb is obviously reduced deep to the greatly shrunken glomeruli. This change is in part due to a visible narrowing of the outer plexiform layer but in part also to shrinkage of deeper layers, probably of the

granule cell layer, since the mitral cell ring encloses a smaller area than on the normal side. The nuclei of the periglomerular cells appear reduced in size. The tufted cells on inspection seem possibly to be shrunken, the mitral cells not obviously so; but measurement of area of cell body for groups of 50 mitral and 50 tufted cells from corresponding sites in the two bulbs suggests an average reduction in area of about 18% for each of these classes of neurons (Table 2). No change is seen in the individual granule cells, but their clumps appear smaller. In protargol preparations very few olfactory nerve fibres appear on the surface of the bulb, and there is no longer any sign of their entering or ending in glomeruli. The dendritic arborizations in the glomeruli are much finer in calibre and less darkly impregnated than on the normal side, appearing sparse and possibly fragmented. The outer part of the outer plexiform layer is lighter than on the normal side, its constituent fibres, like the dendritic tufts, appearing sparser and of reduced calibre. In the accessory bulb hardly any fibres of the vomero-nasal nerve persist, and the whole structure is shrunken. The glomerular layer and the layer of vomero-nasal fibres are condensed together to a narrow, cellular band in which periglomerular cells cannot separately be distinguished. The mitral cells appear smaller than the normal, and measurement shows a shrinkage of the order of 30% (Table 2). As in the main bulb, the nuclei of the granule cells appear unchanged but lie in smaller clumps. The outer part of the molecular layer underlying the lateral olfactory tract of the operated side has begun to lose the darker staining with protargol which on the normal side distinguishes it from the inner part.

Appearances closely similar to those described after 16 days are found in another rabbit, 20 days after a lesion of comparable severity. 24 days post-operatively, in an experiment in which the bulbs were left in situ and sectioned together with the decalcified nasal cavity, all the above changes are seen at slightly greater intensity. In protargol preparations the contrast between the inner and outer parts of the outer plexiform layer in the degenerate bulb is particularly sharp; and the internal plexiform layer and outer part of the perigranular plexus have begun to show a lighter texture, when viewed at low magnification. In addition, the nuclei of the granule cells of the main bulb appear shrunken for the first time. In this experiment a virtually complete denervation was achieved. Shrinkage of granule cell nuclei in the main bulb is not evident, however, at 32, 49, 63 or 73 days, despite lesions of comparable severity which have reduced the size of the clumps of granule cells and the extent of the granular layer as a whole. Atrophic rhinitis began to interrupt the series at 53 days, but the sparing of the accessory bulbs permitted the observation that the nuclei of the granule cells here did not appear to shrink within the first three months after complete denervation.

With this exception, relating to the granule cells, therefore, all the changes which have been found in the olfactory bulb after 130 days' survival appear to have begun by the end of the first month, provided that the destruction of olfactory nerve fibres has been nearly enough complete. In some of the rabbits with short survival periods, indeed, the lesion of the olfactory nerves was more severe than in those permitted to survive longer, and it is unfortunate that no rabbit with a complete lesion survived several months without

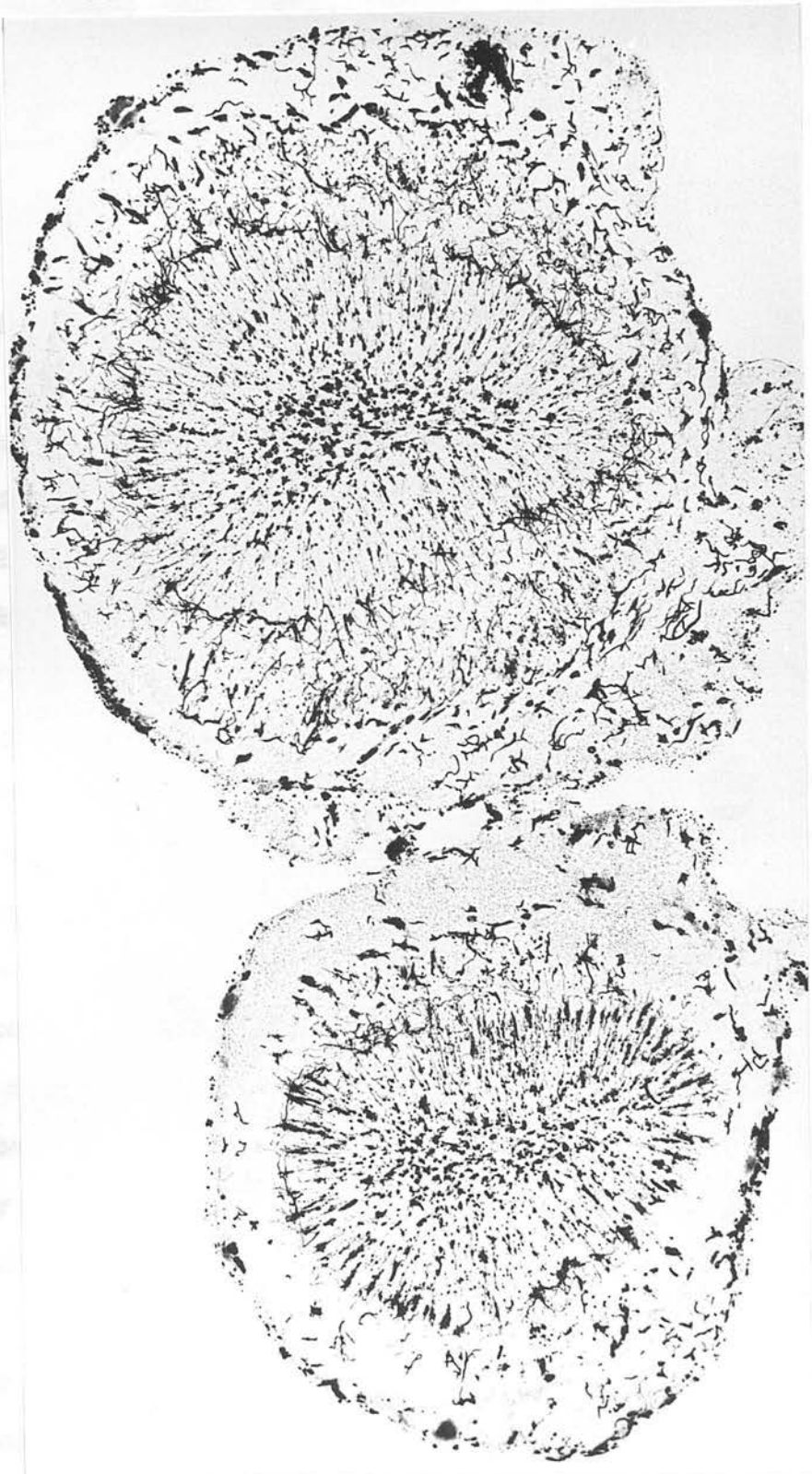


Figure 7. Photomicrograph showing the normal and atrophied bulbs 96 days after destruction of the olfactory mucosa. Golgi-Cox method. x32.

developing atrophic rhinitis. (It is, of course, impossible to judge the extent of the original denervation after this condition is established.)

A Golgi-Cox preparation made 96 days after operation, with rather severe resultant denervation and shrinkage of the affected olfactory bulb, gives other information, though this is not easy to interpret. In the normal bulb, numerous mitral, tufted and peri-glomerular cells are to be seen, with their dendrites and intra-glomerular arborizations (Figs. 7 & 8). In addition, many granule cells are impregnated, with their short basal dendrites and the long peripheral process extending radially into the outer plexiform layer. The shrunken bulb on the side of operation has in most regions a very different appearance, though on the medial aspect where the loss of olfactory fibres has been least severe it more closely resembles the normal bulb. In the rest of the main bulb very few indeed of the mitral, tufted and periglomerular cells are impregnated, and hardly any of the terminal dendritic tufts appear, so that in some sections no intraglomerular arborizations are to be seen, nor any of the neurons which form them (Figs 7 & 8). In those which are visible, however, no consistent differences have been detected from the range of the normal in size or in form. The granule layer is narrowed, as are the more superficial layers, but there are still quite numerous granule cells impregnated, together with their processes, and the appearance of these cells is within normal limits. Their peripheral processes are often the only nervous structures visible in the outer plexiform layer. A conspicuous feature of the shrunken bulb, wherever the mitral cells

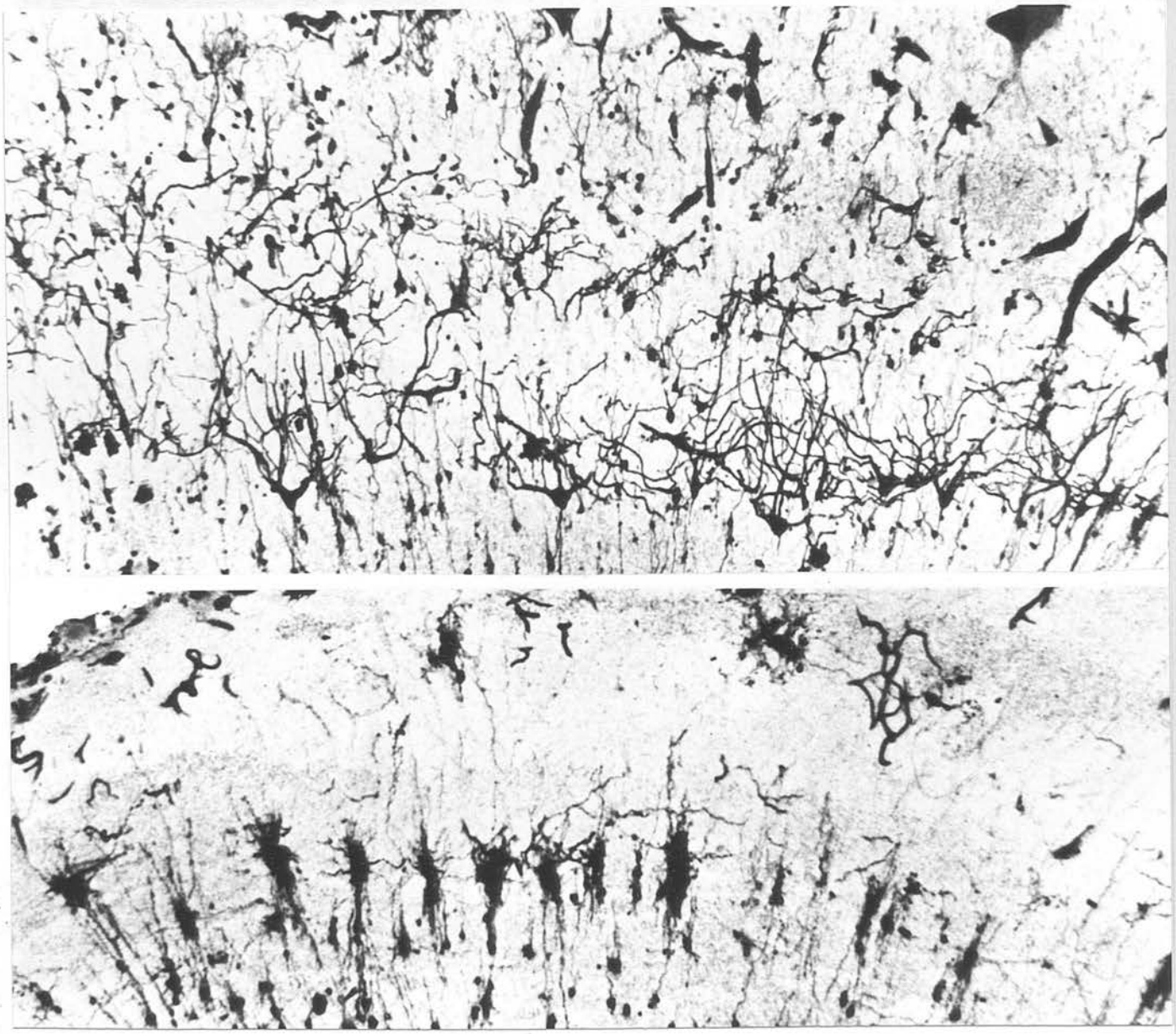


Figure 8. Photomicrograph to show the impregnation of the mitral, tufted and periglomerular cells on the normal side (above), and the corresponding area of the degenerate bulb (below). Golgi-Cox method. x84.

fail to impregnate, is a rather evenly-spaced ring of large structures resembling neuroglial cells at about the level normally occupied by the cell bodies of the mitral cells. The fine processes of these 'neuroglial cells' are mostly radial, but some run tangentially. In many instances the peripheral processes of granule cells lying deep to the 'neuroglial cells' appear to become enveloped by them in their outward course and even on occasion to deviate slightly in order to become so enveloped. This, however, may well be a normal relationship, for it is occasionally seen in the normal bulb where for a short distance no mitral cells have become impregnated, as sometimes happens dorsally or ventrally. In the shrunken accessory bulb of the operated side, there is almost complete failure of impregnation of mitral and periglomerular cells and of their dendritic tufts, but those neurons which are visible, as in the main bulb, are judged to be within the normal limits in size and form.

The second rabbit in which the Golgi-Cox method was used was allowed to survive for 48 days post-operatively. It gave evidence of greater damage to the olfactory nerves than in the 96-day animal: the affected bulb was more shrunken, especially in its superficial zones. There was even more complete failure of impregnation of mitral, tufted and periglomerular cells than after 96 days; but the occasional cells which had impregnated showed no gross abnormalities, and no new features were observed.

These experiments should not have interfered with the blood supply of the olfactory bulb, which is derived from the cerebral vessels, but indirect effects cannot absolutely be excluded. In order to test whether the type of change seen in the bulb could

occur independently of direct vascular lesions it was attempted in two experiments to produce an injury confined to the vomero-nasal organ of one side, from which fibres run to the accessory olfactory bulb. The vomero-nasal organ is placed far forward in the nasal cavity, at the lower edge of the nasal septum, and its blood supply is quite independent of that of the accessory bulb, which is dorsally situated at the posterior end of the main bulb. This attempt was successful in one case, and the accessory bulb 49 days later showed, in addition to considerable loss of vomero-nasal fibres and glomeruli, a narrowing of its outer plexiform layer with shrinkage of the mitral cells. Since the vomero-nasal epithelium and the accessory bulb so closely resemble the olfactory mucosa and main bulb, this is taken to support the view that the changes seen in the main bulb after mucosal lesions are attributable to loss of olfactory nerve fibres.

A striking and consistent feature of those experiments where transneuronal degeneration had occurred in the olfactory bulbs as the result of atrophic rhinitis was the complete preservation of the vomero-nasal nerve and the corresponding absence of any change in the accessory olfactory bulb. In one of these experiments where the degeneration in the olfactory bulb was very severe the nasal cavity was cut and the sections stained with haematoxylin and eosin and Bodian's protargol method. The olfactory mucosa on the unoperated side showed marked atrophy and a dense infiltration of lymphocytes and monocytes, but the mucosa of the vomero-nasal organ and the peripheral processes of the receptors appeared quite normal. Whether the preservation of

the receptors of the vomero-nasal organ, in contrast to those of the olfactory mucosa, is because of their more protected position or because of functional differences is not known.

2. Transneuronal Cell Degeneration in the Auditory Relay Nuclei

After destruction of the cochlea, with severe degeneration or complete atrophy of the cochlear nerve, unequivocal changes are found in the neurons of certain of the primary and secondary auditory relay nuclei. In some, but not in all, of the nuclei a glial reaction accompanies the neuronal degeneration. On the ipsilateral side of the brain stem cellular changes are seen in the ventral cochlear nucleus, in the lateral superior olive and in the preolivary nuclei. On the contralateral side the cells of the medial trapezoid nucleus and of the nuclei of the lateral lemnisous are affected. Although it is difficult to be certain about any neuronal degeneration in the dorsal cochlear nucleus definite gliosis is present.

The findings are presented in two sections. In the first section the appearance of both the normal and degerated nuclei are described and each nucleus is considered separately. Only a brief account of the normal morphology of these nuclei is given because detailed accounts are already available (Cajal, 1909 and Fuse, 1913); reference is made mainly to those points relevant to an understanding of the degenerative changes. The quantitative data are given in the second section in the form of tables and graphs, and here most of the nuclei are treated together.

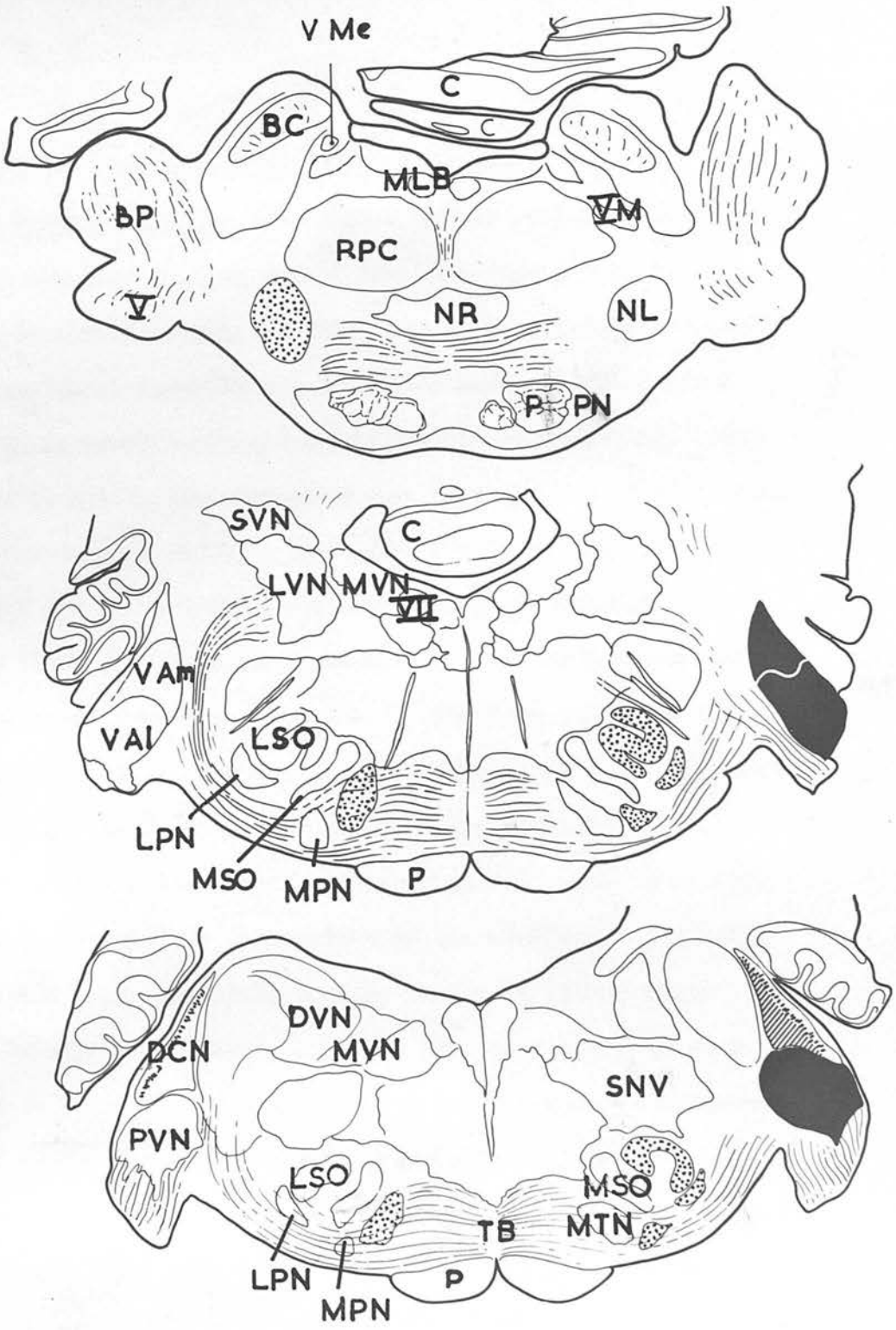


Figure 9. Diagrammatic representation of the distribution of the cellular degeneration and gliosis in the auditory relay nuclei after destruction of the cochlea. The drawings were made by means of a projection apparatus of tansverse sections of the brain-stem stained with thionin. The relay nuclei which show cellular shrinkage and gliosis are indicated in black, those in which cell atrophy occurs without accompanying gliosis are indicated by stippling, and those where gliosis is found without appreciable cell change by hatching.

Qualitative description of the degeneration.

The dorsal cochlear nucleus lies dorsal to the posterior part of the ventral cochlear nucleus and in Nissl-stained preparations is seen to be distinctly laminated (Fig. 10). Immediately beneath the outer ependymal layer is the molecular layer in which are a number of small round cells and a few medium-sized neurons. Deep (or ventral to it) is the characteristic 'spindle cell layer' which is two or three cells thick. The cells are spindle or pyramidal in shape and are quite deeply staining; they are arranged regularly with their long axes perpendicular to the surface and they have well-defined dendritic processes. The deep polymorph layer is the thickest but is sparsely populated; most of the cells are medium-sized but there is an occasional large pyramidal cell. No appreciable cellular degeneration is seen in this nucleus at any time up to 359 days after destruction of the cochlea. The cells of the spindle layer certainly show no change in either their depth of staining or in their size, but the possibility of some alteration in the cells of the polymorph layer cannot be excluded because of their sparse distribution and variation in size. There is, however a marked increase in the number of glial cells in the polymorph layer (Fig. 10). It is more pronounced after the shorter, survival periods and becomes progressively less intense after survival periods of several months.

Ventral cochlear nucleus. It is generally accepted that the ventral cochlear nucleus can be divided into an anterior and a posterior portion (Rose, Galambos and Hughes, 1959). The cells are of medium

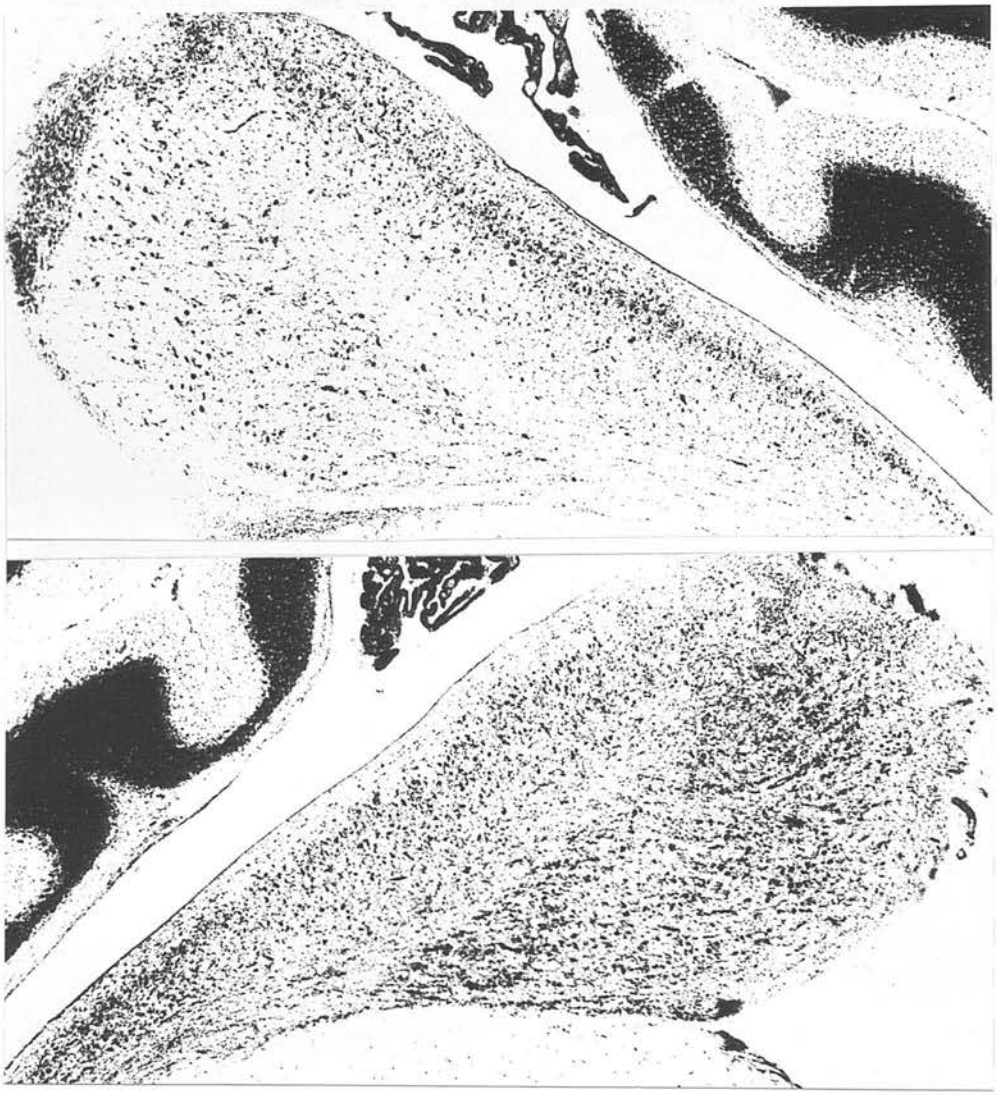


Figure 10. Photomicrographs of normal (above) and degenerated (below) postero-ventral and dorsal cochlear nuclei 60 days after destruction of the cochlea. Severe gliosis is seen throughout the postero-ventral nucleus and in the deep polymorph layer of the dorsal nucleus. x33.

size, rounded, with short processes and have a relatively large amount of cytoplasm. In the majority of the cells the Nissl material is fine and evenly distributed throughout the cytoplasm, but in some it is aggregated into larger clumps. In the anterior part of the nucleus a further medial and lateral subdivision can be recognised in transverse sections of the brain stem. In the medial part, which is triangular in cross-section, the cells are smaller, rounder and paler-staining than in the lateral part and they are more compactly arranged (Plate 1 figure 1). In sagittal section Rose et al (1959) describe the oral part of the antero-ventral nucleus as having small cells which are more closely packed than in the caudal part of this element, and it is probable that this rostral portion corresponds to the medial subdivision described here. The cells of the lateral subdivision are of the same order of size as those in the postero-ventral part of the nucleus but they are more uniform in size. To facilitate the description of the results these two portions of the antero-ventral cochlear nucleus will be called the medio-ventral and latero-ventral nuclei respectively. Destruction of the cochlear nerve results in clear-cut changes in all subdivisions of the ventral nucleus (Fig.11). Thirty days after operation the cells are more compactly arranged but otherwise show no change; severe gliosis is present throughout the nucleus but is most obvious at the level of entry of the nerve. After sixty days the nucleus is distinctly smaller in its cross-sectional area and all subdivisions appear to be equally affected. The cells are more densely packed than on the normal side and are definitely shrunken

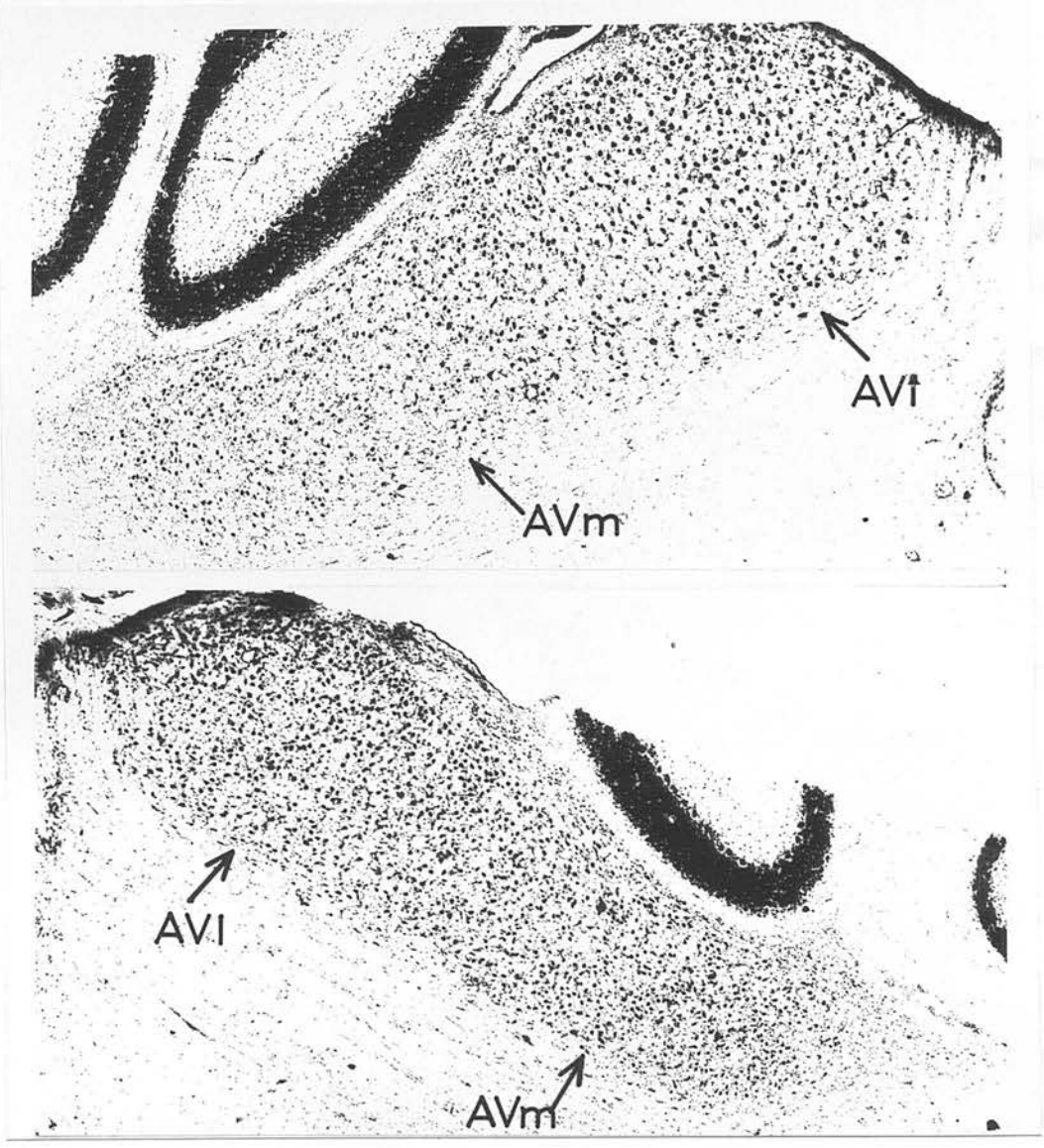


Figure 11. Low power photomicrographs to show the shrinkage of the antero-ventral cochlear nucleus and its constituent cells 359 days after destruction of the cochlea (below) as compared with the nucleus of the normal side (above). x38.

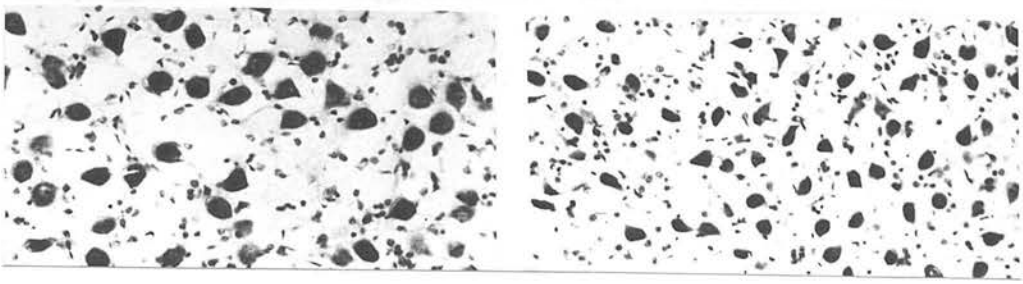


Figure 12. Photomicrographs of normal (left) and degenerated (right) cells of the antero-ventral nucleus 359 days after destruction of the cochlea. x180.

(Fig. 12). They are also more uniform in size and shape and have less prominent dendritic processes. Many of the cells are slightly paler than normal, and their Nissl material is more homogeneous in its distribution. The nucleus and nucleolus are shrunken but are not altered in position. There does not appear to be any cell loss. After longer survival periods the appearance of the cells remains essentially the same, but the glial reaction diminishes.

Superior olivary nuclei: The lateral superior olive is S shaped in transverse section and is found at a level corresponding approximately to the middle of the rostro-caudal extent of the antero-ventral nucleus (Fig. 9). Most of the cells are spindle-shaped, have prominent dendrites at each end and lie with their long axes at right angles to the corresponding part of the nucleus; a few of the cells are round or pyramidal in section (Fig. 13). In the cells there is a moderate amount of Nissl material in the form of fine granules evenly distributed throughout the cytoplasm. Thirty days after destruction of the cochlea this nucleus shows no appreciable change. After sixty days, however, severe shrinkage and pallor of the cells is quite obvious even at low magnifications. At higher magnifications the nuclei of the cells are also found to be smaller and the Nissl material is seen to be considerably reduced and to have a homogeneous 'matt' appearance. The nucleus as a whole shows no diminution in its cross-sectional area, and there is no gliosis. Not only is there no compacting of the cells but indeed they are more widely spaced apart. After longer survival periods the appearance of the nucleus remains the same, pallor and shrinkage of the neurons being the only change (Fig. 13). At no stage is there any sign of cell loss nor of any glial reaction.

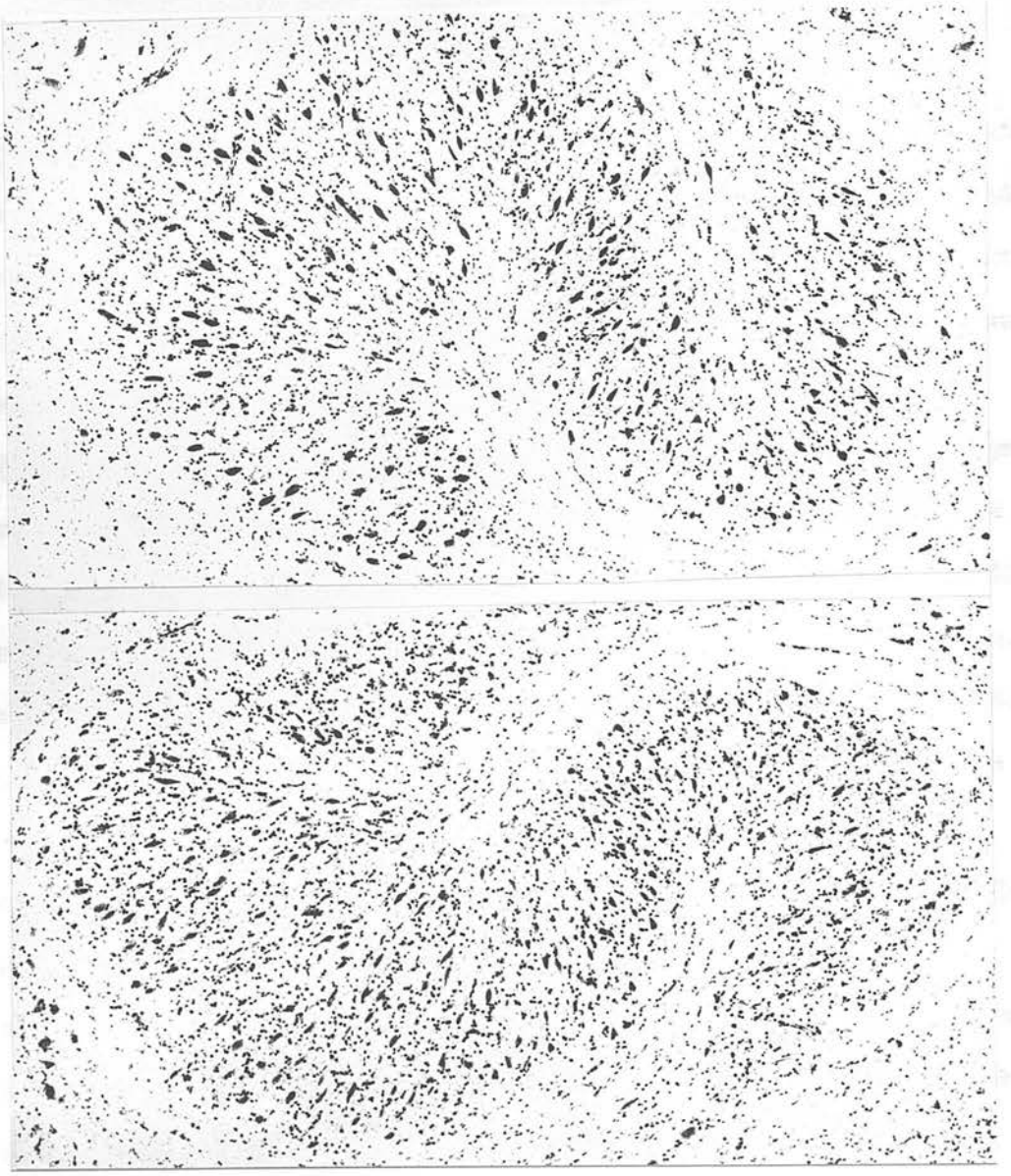


Figure 13. Photomicrograph to show the atrophy of the cells in the lateral superior olive 359 days after destruction of the cochlea (below) as compared with the normal side (above). x78

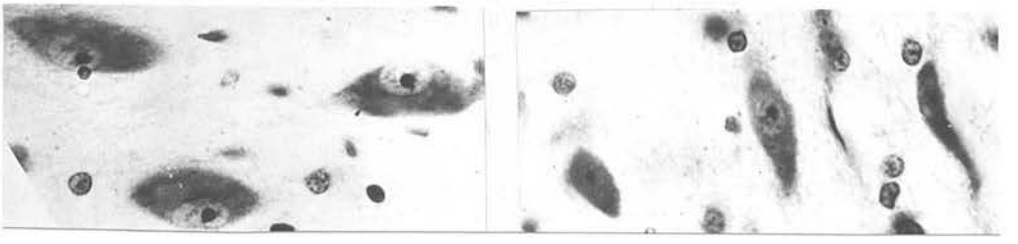


Figure 14. Normal (left) and atrophied (right) neurons of the lateral superior olive 359 days after destruction of the cochlea. x875

The Medial superior olive is formed of a narrow band of cells which extends obliquely in a ventro-lateral direction on the medial side of the lateral superior olive. Long, well-defined dendrites extend medially and laterally from the cell bodies. This nucleus shows no change on either side after destruction of the cochlea.

The medial trapezoid nucleus lies ventromedial to the medial superior olive, with its cells scattered amongst the deeper fibres of the trapezoid body. It is triangular in transverse sections with its medial border directed dorso-ventrally (Fig. 9). The neurons, which are quite deeply staining, are medium-sized and round or triangular in shape. In the nucleus of the contralateral side the neurons undergo definite changes following destruction of the cochlear nerve. After thirty days the cells are slightly smaller and paler than on the ipsilateral side and these changes become more pronounced from sixty days onwards (Fig.15). Examination at higher magnifications shows that the cell bodies are smaller but that there is no shrinkage of the nucleus, with the result that the nuclear-cytoplasmic ratio is considerably reduced. In this nucleus, as in the lateral superior olive, there is no compacting of the cells nor is there any gliosis. Furthermore, no gliosis can be seen amongst the fibres of the trapezoid body.

The ventral and lateral preolivary nuclei and lateral lemniscal nuclei. The preolivary nuclei are small groups of cells amongst the fibres of the lateral part of the trapezoid body and lie ventral to the medial and lateral superior olivary nuclei respectively (Fig.9). The dorsal and ventral nuclei of the lateral lemniscus are found more rostrally as two groups of cells

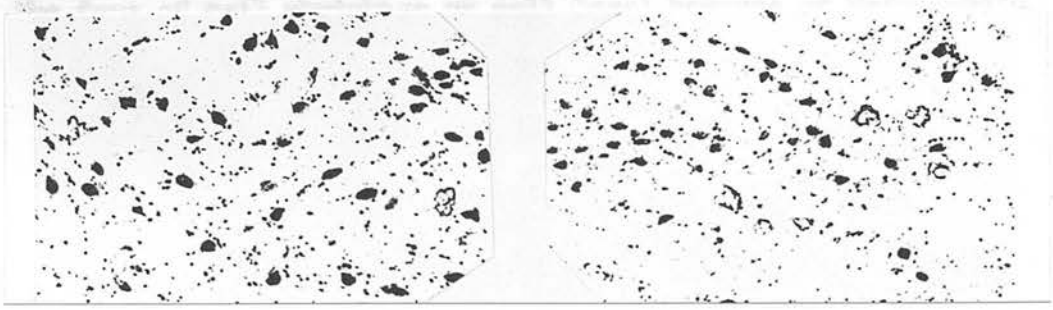


Figure 15. Photomicrographs to show the shrinkage of the cells of the contralateral medial trapezoid nucleus (right) 120 days after destruction of the cochlea as compared with those of the ipsilateral side (left). x86.

interspersed amongst the ascending lemniscal fibres. In the preolivary nuclei of the contralateral side the cells show changes similar in appearance and time course to those found in the contralateral medial trapezoid nucleus. Furthermore, these nuclei are similar in showing no gliosis nor any compacting of the cells.

A careful search was made in the region dorsal to the medial superior olive for any evidence of retrograde cell degeneration (in the form of cell shrinkage or cell loss) because of Rasmussen's observation (1946) that the centrifugal fibres to the cochlea originate here, but in none of the experiments was there any evidence suggestive of retrograde degeneration. The absence of any cell change in this region in our material is, of course, in no way in conflict with Rasmussen's evidence as the cells in question may be resistent to axonal section or they may have recovered within thirty days (the shortest survival period in this series). In all these experiments the vestibular nerve was not affected and there is no change in the cells of the vestibular nuclei.

In one experiment, A8, in addition to destruction of the cochlea, the dorsal and ventral cochlear nuclei were inadvertently destroyed. The survival period was sixty days. Following the involvement of the primary relay nuclei the proximal parts of the trapezoid body and of the dorsal and intermediate striae show intense gliosis; but as these tracts are traced towards and across the midline the gliosis decreases so that the contralateral lemniscus shows only a moderate, diffuse glial reaction. The

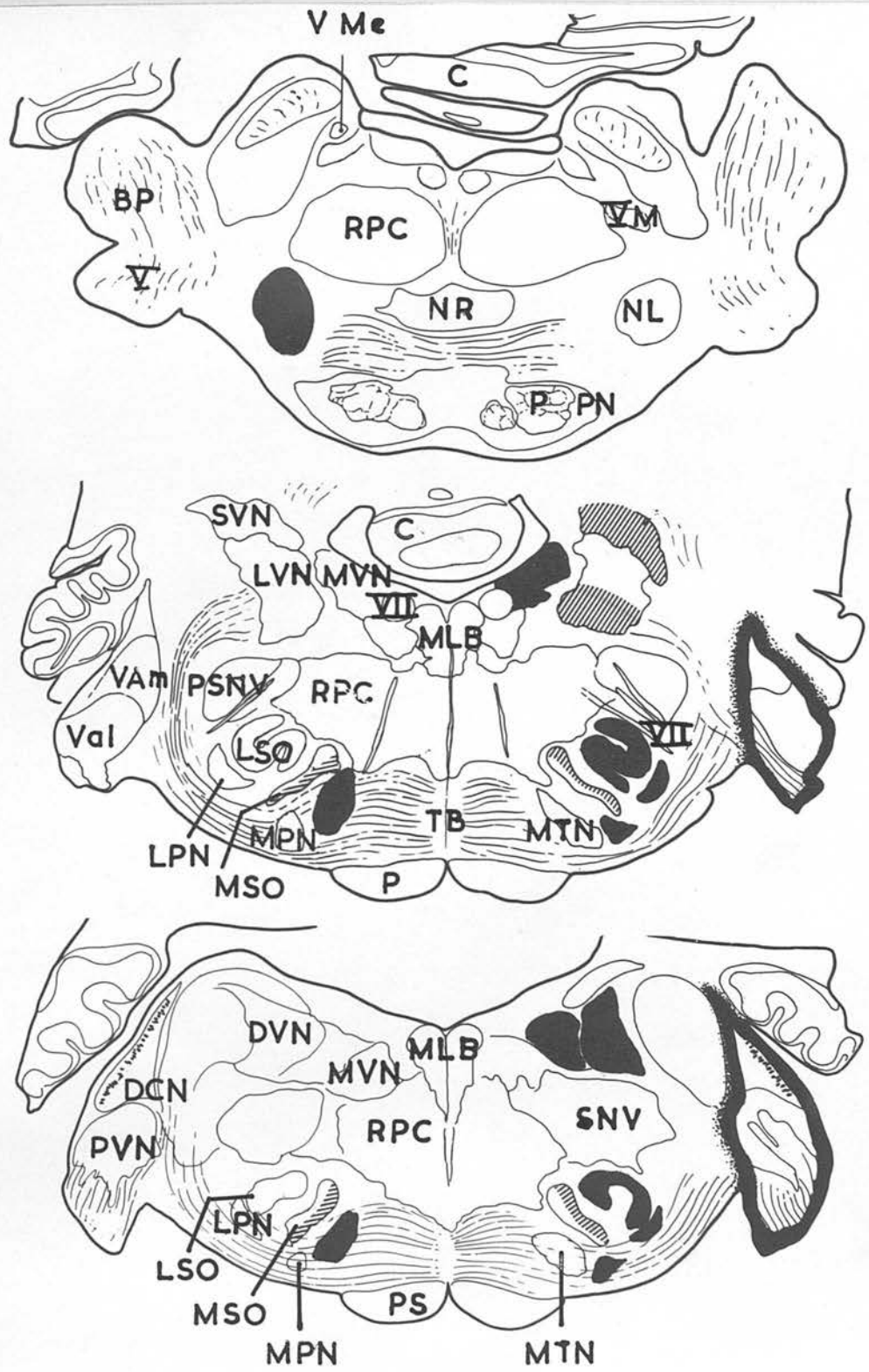


Figure 16. Diagrammatic representation of the distribution of the cellular degeneration and gliosis in the secondary auditory relay nuclei and in the vestibular nuclei after destruction of the cochlear nuclei and vestibular nerve. The extent of the lesion is indicated by a thick black line, the nuclei which show cell atrophy accompanied by gliosis in black, and those in which only gliosis occurs by hatching.

lateral superior olive and preolivary nuclei of the same side, and the medial trapezoid and lateral lemniscal nuclei of the opposite side all show essentially similar changes (Fig.16). These nuclei are all reduced in cross-sectional area and are severely gliosed; their constituent cells are shrunken and paler than normal and are more densely packed together. The gliosis in the contralateral medial trapezoid nucleus is particularly conspicuous. The changes in the medial superior olive of both sides is quite characteristic. The involvement of the cochlear nuclei has resulted in severe gliosis amongst the dendrites on the lateral side of the cell-bodies of the ipsilateral nucleus and amongst those on the medial side of the cell bodies on the contralateral side. The cells do not appear to be altered on either side as compared with the normal brain. In this experiment the vestibular nerve has been damaged and intense gliosis can be seen extending from the nerve into most of the cross-sectional area of the superior vestibular nucleus and into the ventral part of the lateral vestibular nucleus of the same side (Fig.16). A more diffuse and less severe glial reaction has occurred in the medial and descending nuclei. In the medial and descending nuclei definite shrinkage and pallor of the cells can be seen; in the affected part of the lateral nucleus some of the cells are smaller but the giant pyramidal cells show no change. There is no appreciable alteration of the cells of the superior vestibular nucleus.

Quantitative observations.

The data on the mean areas of the cells, nuclei and nucleoli, together with standard errors of the means, are presented in Tables 3 to 8. The figures for each nucleus are given separately and in each

Table 3 Postero-ventral nucleus

(50 neurons in each sample).

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Survival time	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
Control ↑⟶	348.7 Normal	14.21	352.3 Atrophied	13.03	74.48 Normal	1.57	77.5 atrophied	1.66	7.64 Normal	0.18	7.34 Atrophied	0.22
30 days	471.1	14.20	389.9	18.92	85.8	2.08	88.7	2.92	7.8	0.24	7.9	0.2
60 days	422.7	20.40	344.3	16.86	81.9	2.67	76.4	2.37	8.2	0.23	7.0	0.20
90 days	465.5	18.18	307.1	13.11	79.1	1.82	73.4	2.46	5.6	0.17	4.5	0.17
140 days	362.7	13.70	269.1	10.23	81.2	1.97	68.8	1.54	4.6	0.13	4.1	0.14
201 days	482.7	19.96	307.1	12.35	84.9	1.85	69.2	1.87	4.8	0.13	4.3	0.14
272 days	361.9	14.41	264.7	12.81	79.9	1.49	67.3	1.63	5.1	0.16	4.5	0.18
319 days	467.1	23.32	304.3	14.20	83.3	2.27	65.5	2.37	5.0	0.15	4.2	0.19
359 days	359.5	16.93	284.7	13.35	70.0	1.60	64.7	1.45	6.5	0.14	5.6	0.16

Table 4 Latero-ventral nucleus

(50 neurons in each sample)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Survival time	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
Control	349.1 Normal	9.20	333.5 Atrophied	9.21	83.6 Normal	1.47	79.7 Atrophied	1.51	6.9 Normal	0.14	6.8 Atrophied	0.17
30 days	337.9	8.81	304.7	7.90	85.0	1.44	88.3	1.42	4.5	0.10	4.2	0.12
60 days	355.1	13.82	233.1	7.37	73.8	2.55	59.7	1.55	4.7	0.15	4.0	0.13
90 days	417.9	11.11	315.1	11.22	88.6	1.81	75.9	1.76	5.3	0.14	4.6	0.16
120 days	355.9	11.80	267.1	8.05	82.1	2.15	64.4	1.58	4.2	0.08	3.6	0.12
140 days	375.5	12.63	265.9	6.11	91.7	1.99	75.4	1.25	6.04	0.19	5.2	0.15
272 days	335.7	8.67	229.7	6.48	72.7	1.73	59.3	2.14	4.31	0.10	3.5	0.12
319 days	409.9	17.67	265.9	10.26	88.9	3.07	65.4	2.46	5.3	0.21	3.9	0.12
359 days	330.7	16.42	263.1	10.45	93.7	2.64	71.1	2.07	5.8	0.19	4.6	0.16

Table 5 Medio-ventral nucleus

(50 neurons in each sample)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Survival time	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
Control	263.5 Normal	8.0	267.5 Atrophied	6.69	68.3 Normal	1.42	67.0 Atrophied	1.29	6.4 Normal	0.21	6.4 Atrophied	0.19
30 days	262.7	8.81	257.1	9.1o	71.3	2.03	65.5	1.55	6.2	0.19	6.1	0.20
60 days	253.5	9.40	215.1	7.81	77.6	1.49	64.6	1.33	6.2	0.19	5.9	0.18
90 days	329.9	8.54	240.3	7.20	75.4	1.16	62.4	1.14	6.0	0.19	5.1	0.15
120 days	259.5	8.63	207.5	5.42	63.4	1.66	56.8	1.16	5.4	0.16	5.0	0.15
140 days	331.9	7.24	216.3	6.24	79.7	1.45	64.1	1.24	4.8	0.14	4.0	0 10
201 days	247.9	6.33	122.5	5.50	55.9	1.53	46.3	1.37	3.8	0.09	3.3	0.10
272 days	249.1	8.02	205.5	6.08	76.5	1.65	66.4	1.09	4.8	0.12	4.2	0.12
319 days	310.3	12.98	217.9	6.31	70.6	2.19	63.0	1.43	4.6	0.16	4.1	0.12
359 days	225.9	5.61	169.5	4.49	61.3	1.26	52.4	1.18	5.0	0.19	4.5	0.20

Table 6 Lateral Superior Olive

(50 cells in each sample)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Survival time	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
Control	227.5 Normal	8.66	235.1 Atrophied	7.91	55.9 Normal	1.63	55.4 Atrophied	1.40	6.7 Normal	0.18	6.3 Atrophied	0.19
30 days	237.9	9.10	207.9	7.22	53.1	1.86	48.4	1.52	4.5	0.10	4.2	0.12
60 days	278.7	11.34	197.1	7.81	66.6	2.30	49.2	1.85	7.4	0.28	6.2	0.21
90 days	258.3	8.36	201.1	7.18	56.1	1.43	45.9	1.38	7.9	0.23	6.8	0.22
120 days	204.3	6.73	151.1	4.73	52.9	1.61	41.4	1.15	6.6	0.21	5.4	0.21
140 days	182.3	6.16	129.5	6.46	41.0	1.51	34.7	1.53	5.4	0.16	4.9	0.17
201 days	231.1	10.49	166.9	7.47	42.3	1.68	33.5	1.43	4.0	0.9	3.3	0.12
272 days	192.7	8.26	149.1	4.79	44.0	1.73	37.0	1.18	4.6	0.18	3.9	0.16
319 days	208.3	13.05	140.7	6.9	42.1	1.68	32.9	1.57	5.0	0.19	3.7	0.14
359 days	205.5	9.84	159.5	12.34	39.1	1.95	31.0	1.64	4.1	0.16	2.8	0.14

Table 7 Medial Trapezoid Nucleus
(50 cells in each sample)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Survival time	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
Control	328.7 Normal	9.50	314.3 Atrophied	7.54	45.6 Normal	1.52	44.1 Atrophied	1.48	5.5 Normal	0.14	5.8 Atrophied	0.15
30 days	390.6	12.38	316.0	10.68	66.1	1.83	67.0	1.97	4.4	0.09	4.1	0.09
60 days	368.7	11.43	307.9	9.52	56.0	1.81	59.5	1.70	4.0	0.08	3.7	0.13
90 days	392.7	12.66	301.5	9.49	58.2	2.36	59.6	1.43	6.4	0.23	6.7	0.18
120 days	370.7	11.79	286.3	9.96	58.4	1.87	57.6	1.47	3.6	0.10	3.8	0.13
140 days	308.3	11.93	254.7	10.17	49.4	1.77	50.1	1.37	3.5	0.11	3.7	0.14
201 days	318.3	11.33	261.5	10.12	36.2	1.97	37.2	1.48	4.0	0.14	4.0	0.14
272 days	273.9	11.98	213.1	6.63	40.2	1.97	40.7	1.60	3.7	0.15	3.7	0.12
319 days	341.5	12.30	258.3	10.31	45.6	1.64	48.1	1.76	4.2	0.12	4.4	0.12
359 days	321.3	14.19	218.5	9.38	62.9	2.83	59.7	2.33	3.5	0.18	3.5	0.14

Table 8 Dorsal Cochlear Nucleus
(50 cells in each sample)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Survival time	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
319 days	383.5	20.95	364.3	17.87	73.9	2.58	79.0	2.21	5.5	0.19	5.3	0.23
359 days	365.1	16.23	363.1	14.0	90.2	2.98	89.6	2.50	6.6	0.26	6.5	0.24

case they are arranged in order of increasing survival periods. Measurements of the cells in the postero-ventral nucleus after 120 days, and in the latero-ventral nucleus after 200 days, were not made because the lateral parts of these nuclei were damaged during removal of the brain. The cellular degeneration in the preolivary and lateral lemniscal nuclei was not studied quantitatively. In Tables 9 to 11 the percentage changes in the areas of the cells, nuclei and nucleoli at each survival period are given, together with the results of the t-test for the significance of each comparison. Each percentage change is calculated with reference to the unaffected nucleus in that experiment. The accompanying graphs (Fig.17) showing the time-course of the degeneration in the auditory relay nuclei are constructed from these data, and, for the sake of comparison, a similar graph has been drawn for the lateral geniculate nucleus from the results of the study by Cook et al (1951).

These tables show that although the mean values of the areas of the normal cell, nucleus and nucleolus in the auditory relay nuclei vary between different animals, the relative sizes of these structures usually remain similar. The largest cells are found in the postero-ventral nucleus and the smallest in the lateral superior olive; the cells in the latero-ventral nucleus, those of the spindle cell layer of the dorsal cochlear nucleus and those of the medial trapezoid nucleus are all of the same order of size. In general, the quantitative data confirm the qualitative impressions of the degeneration in the respective nuclei, and a comparison of the qualitative descriptions with Table 9 makes it clear that a shrinkage of approximately 20% of the cell body must occur before any diminution in size can be detected

Table 9 Percentage change of cell area from normal

Survival time	Postero-ventral nucleus	Latero-ventral nucleus	Medio-ventral nucleus	Lateral superior olive	Medial trapezoid nucleus	Dorsal cochlear nucleus
Control	+ 1.0	- 4.6	+ 1.5	+ 2.4	- 4.5	
30 days	-17.2^{xx}	- 9.8^{xx}	- 2.1	-12.6^{x}	-19.1^{xxx}	
60 days	-18.5^{xx}	-34.3^{xxx}	-15.1^{xx}	-29.4^{xxx}	-16.5^{xxx}	
90 days	-34.0^{xxx}	-24.6^{xxx}	-27.1^{xxx}	-22.1^{xxx}	-23.2^{xxx}	
120 days		-24.9^{xxx}	-20.0^{xxx}	-26.0^{xxx}	-22.7^{xxx}	
140 days	-25.8^{xxx}	-29.1^{xxx}	-34.8^{xxx}	-28.9^{xxx}	-17.3^{xx}	
201 days	-36.3^{xxx}		-30.4^{xxx}	-27.7^{xxx}	-17.8^{xxx}	
272 days	-26.8^{xxx}	-31.6^{xxx}	-17.5^{xxx}	-22.6^{xxx}	-22.1^{xxx}	
319 days	-34.8^{xxx}	-35.1^{xxx}	-29.7^{xxx}	-32.4^{xxx}	-24.3^{xxx}	-5.0
359 days	-20.8^{xxx}	-20.4^{xx}	-24.9^{xxx}	-22.4^{xxx}	-32.0^{xxx}	-0.6

Table 10 Percentage change of nuclear area from normal

Survival time	Postero-ventral nucleus	Latero-ventral nucleus	Medio-ventral nucleus	Lateral superior olive	Medial trapezoid nucleus	Dorsal cochlear nucleus
Control	+ 4.0	- 5.0	- 1.8	- 0.9	-3.4	
30 days	+ 3.3	+ 4.0	- 8.1^{x}	- 9.0	+1.2	
60 days	- 6.7	-19.0^{xxx}	-16.2^{xxx}	-26.1^{xxx}	+6.1	
90 days	- 7.2^{x}	-14.3^{xxx}	-17.3^{xxx}	-18.1^{xxx}	+2.4	
120 days		-21.4^{xxx}	-10.0^{xx}	-21.7^{xxx}	-1.3	
140 days	-15.1^{xxx}	-17.7^{xxx}	-19.5^{xxx}	-15.2^{xx}	+1.4	
201 days	-18.5^{xxx}		-17.1^{xxx}	-20.8^{xxx}	+2.5	
272 days	-15.6^{xxx}	-18.3^{xxx}	-11.8^{xxx}	-15.8^{xx}	+1.2	
319 days	-21.4^{xxx}	-26.0^{xxx}	-10.7^{xx}	-21.8^{xxx}	+5.4	+6.9
359 days	- 7.5^{x}	-24.0^{xxx}	-14.3^{xxx}	-20.8^{xxx}	-5.0	-1.3

Table 9/ Percentage change of nucleolar area from normal

Survival time	Postero-ventral nucleus	Latero-ventral nucleus	Medio-ventral nucleus	Lateral superior olive	Medial trapezoid nucleus	Dorsal cochlear nucleus
Control	+ 8.1^{x}	- 0.9	0	- 5.3	+4.4	
30 days	+ 1.2	- 7.0^{x}	- 1.0	- 4.8	-7.1^{x}	
60 days	-13.9^{xxx}	-13.9^{xx}	- 5.1	-17.1^{xxx}	-6.0	
90 days	-19.8^{xxx}	-13.1^{xx}	-14.8^{xxx}	-13.6^{xx}	+5.9	
120 days		-14.6^{xxx}	- 7.0	-17.2^{xxx}	+3.2	
140 days	-11.3^{x}	-12.9^{xx}	-15.4^{xxx}	-10.2^{x}	+3.9	
201 days	-10.3^{x}		-13.2^{xx}	-15.5^{xxx}	0	
272 days	-11.5^{x}	-17.5^{xxx}	-11.6^{xx}	-15.5^{xx}	0	
319 days	-16.9^{xxx}	-25.1^{xxx}	- 9.9^{x}	-26.0^{xxx}	+3.2	-2.9
359 days	-14.2^{xx}	-20.0^{xxx}	-10.0^{x}	-30.1^{xxx}	0	-0.9

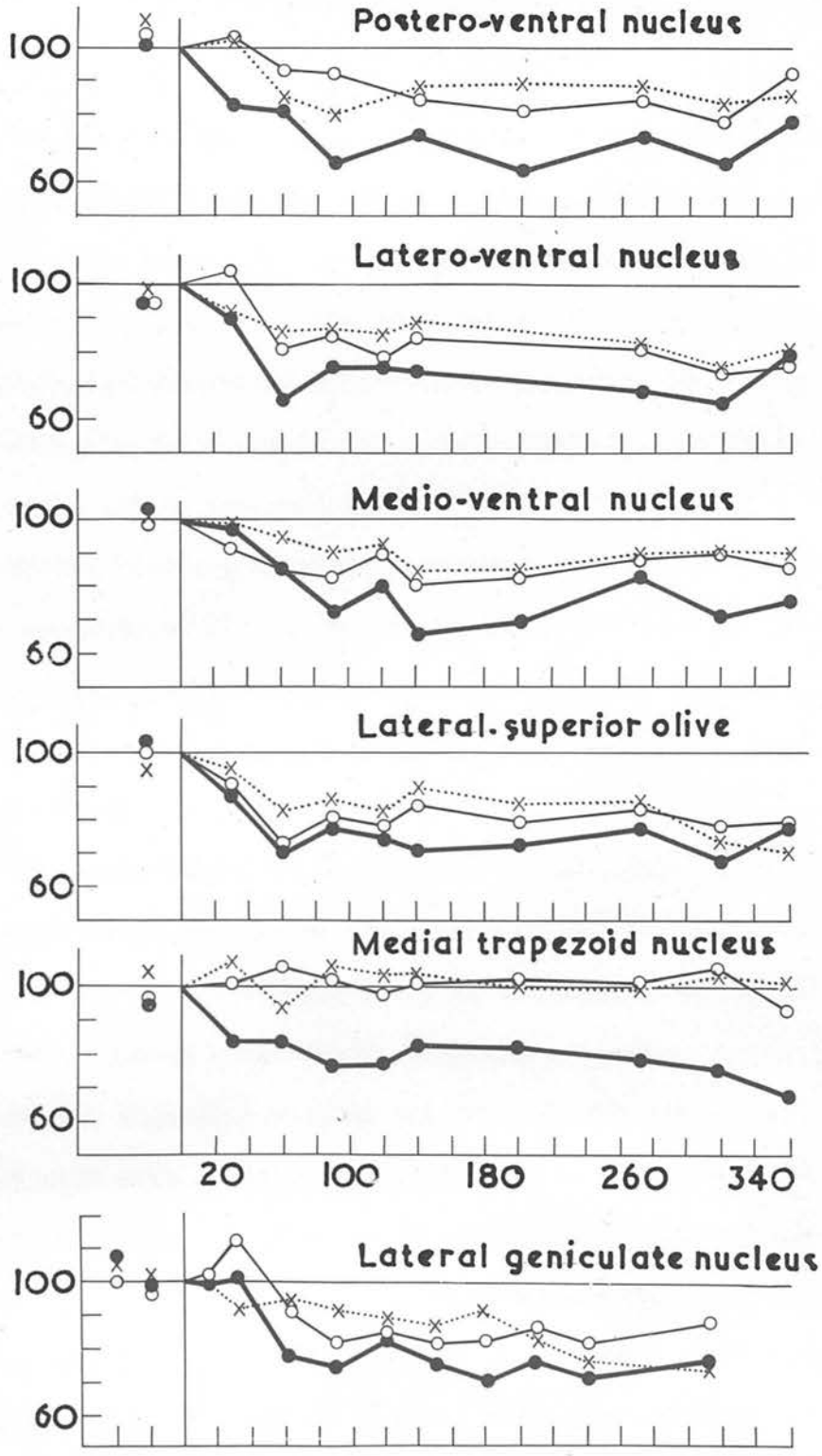
/. Table 10

Nucleus	Volume (c. mass)			Mean number of cells per unit volume			Estimated total cell count		
	normal	atrophied	% difference	normal	atrophied	% difference	normal	atrophied	% difference
Ventral cochlear nucleus	11.46	7.53	-34.3	4.78	7.6	+59	75,897	79,210	+4.2
Lateral superior olive	1.21	1.08	-10	4.86	5.22	+7.4	7,787	7,505	-3

qualitatively. With the exception of the medial trapezoid nucleus, in which the nuclei and nucleoli do not undergo any shrinkage and which will be considered separately, the degenerative process in the cells of the different relay nuclei follows the same time-course and is of the same degree of severity. The slight differences found between individual nuclei in the same animal and between the degree of shrinkage of the cells of the same nucleus at different survival periods (after the appearance of degenerative changes) can probably be accounted for in part by technical difficulties of sampling and measuring, by 'biological variation' and possibly by minor differences in the extent of the lesion.

Apart from the postero-ventral nucleus, unequivocal shrinkage of the cell body, nucleus and nucleolus is only found after survival periods of 60 days or longer. After survival periods of longer than 60 days, even up to one year, the shrinkage is more or less constant. In the postero-ventral nucleus, however, a shrinkage of 17% of the total cell area is found as early as 30 days after destruction of the cochlea, and after the same period a slight but significant degree of shrinkage occurs in the cells of the latero-ventral and lateral superior olivary nuclei amounting to approximately 10%.

In all the nuclear groups for which quantitative data are available, and at almost all survival periods, the shrinkage of the total cell area is proportionately greater than that of the nucleus or nucleolus. For the ventral nuclei and the lateral superior olive this decrease in size amounts to between 20 and 35% of the normal area. At most of the intervals studied after 60 days the nuclear shrinkage is found to be between 10 and 25% and is usually more severe in the lateral superior



Text figure 17. Graphs to show the time-course of the changes in the mean cell, nuclear and nucleolar areas in the ventral cochlear, lateral superior olivary and medial trapezoid nuclei following destruction of the cochlea, and in the lateral geniculate nucleus after eye removal. Each mean value for the atrophied neuron is plotted as a percentage of the mean normal value for that animal. The group of the lateral geniculate nucleus is constructed from the results of Cook et al (1951).
● = cell area, O = nuclear area, x = nucleolar area.

olive than in the other nuclear groups. In two nuclear groups, the postero-ventral and latero-ventral, a slight increase in size similar to that found in the lateral geniculate nucleus by Cook et al (1951) is seen after 30 days. The time-course of the nuclear change in the cells of a particular nucleus usually parallels that of the cell area. Most commonly the nucleolus is found to undergo the least degree of shrinkage, and it was usually between 10 and 15%. It should be emphasized, however, that these estimates of nucleolar area are the least accurate of all the measurements partly because of the difficulty in outlining the nucleolus precisely, and partly because of the relatively large error in measuring such small areas outlined. No double nucleoli were observed either in the normal or atrophied cells.

The measurements of the neurons of the medial trapezoid nucleus show that there are distinct differences in the degenerative process at this site. The most obvious difference is that at almost all of the intervals studied there are no significant changes in the size of the nucleus and nucleolus despite the unequivocal and constant shrinkage of the cell area. Although the degree of shrinkage of the cell area is slightly less than that in the cells of the other nuclei, being between 15 and 25%, the time of onset of the degeneration of these cells appears to be earlier as they are found to have undergone a decrease in size of 19% at 30 days. Because of these exceptional findings many of the measurements of the cells of this nucleus were repeated, but with the same results.

The characteristic spindle cell layer of the dorsal cochlear nucleus show no change in area of the cells, nuclei or nucleoli after the two longest survival periods, thus confirming the qualitative impression that these cells do not undergo transneuronal atrophy.

The data of the volumes and cell counts of the normal and degenerate ventral cochlear nucleus and lateral superior olive at 319 days are presented in Table 12. The volume of the ventral cochlear nucleus on the operated side is reduced by 34.3%, but the mean cell density, i.e. the number of cells/unit volume is increased by 60% so that the total cell counts differ by the statistically insignificant figure of 4.2%. The decrease in volume of the lateral superior olive is much less, being only 10% of the normal, and as the mean density of the cells increased by only 7.5% the difference in the total population of the cells between the normal and atrophied nuclei is only 3% which is again not significant. In these nuclei in the cat, therefore, no cell loss occurs up to 300 days after destruction of the primary auditory nerve fibres. The counts of the nucleolar sex satellite in these two nuclei in female cats at 319 and 60 days also show no difference in incidence or in position. The satellite is present in between 90 and 94% of cells, and most frequently it is found immediately adjacent to the nucleolus.

The sections of the temporal bones of the cat which survived 319 days after operation show that the basal parts of the cochlea have been completely destroyed and that the basilar membrane of the middle and apical turns is degenerated. The Bodian-stained sections show clearly that the cochlear division of the 8th cranial nerve is almost totally degenerated, only a few fibres remaining in the nerve and a comparable number of bipolar cells in the ganglion. The vestibular division is quite unaffected.

3. The projection of the cochlea

In the previous section on transneuronal cell degeneration in the auditory relay nuclei following destruction of the cochlea one of the most significant findings was the occurrence of distinct changes in the cells of the lateral superior olive, the medial trapezoid and so-called preolivary nuclei. These nuclei are commonly considered to be composed of third-order neurons (Barnes, Ranson and Magoun, 1943; Stotler, 1953), but because the severity and time-course of the degenerative changes so closely parallel those in the ventral cochlear nucleus two alternative interpretations of their cause have to be considered. The first possibility is that these changes are secondary to those in the primary relay nuclei as most of the evidence indicates that all the primary auditory fibres terminate in the dorsal and ventral cochlear nuclei. On the other hand, the possibility that some primary auditory fibres pass to the lateral superior olive and trapezoid nuclei cannot definitely be excluded; such a connexion was suggested by Held (1893) and Cajal (1909) and experimental evidence for this projection to the medial trapezoid nucleus has been presented by Lewy and Kobrak (1936).

Another finding in the previous section was the absence of any change in the characteristic spindle cell layer of the dorsal cochlear nucleus. As the other possible explanations for this lack of change will be considered in the discussion, only the conflicting evidence for a direct projection of primary auditory nerve fibres to this nucleus is relevant here. All the earlier workers (cf. Cajal, 1909) agree, from a study of both normal and experimental material, that the dorsal cochlear nucleus receives such primary fibres, and Lorente de No

(1933 a, b) and Lewy and Kobrak (1936) have confirmed this connexion. On the other hand, it has been called in question by two recent experimental studies. In a brief report Stotler (1949), using his modification of the Bodian method, failed to find degeneration in this nucleus following destruction of the cochlea, and Rasmussen (1957), using yet another variant of the protargol method developed specifically to show terminal synapses, states quite definitely that no primary afferents terminate in the dorsal nucleus. As Rose, Galambos and Hughes (1959) point out, however, the failure to find a direct projection to the dorsal cochlear nucleus is difficult to reconcile with their own electrophysiological evidence. In a single unit analysis of the cochlear nuclei they found essentially similar responses in the dorsal and the ventral posterior cochlear nuclei.

The present study of the projection of the cochlea was undertaken primarily in order to resolve these discrepancies. The material also, quite incidentally, provided some evidence on the question of the topographical organization of the projection of the cochlea upon the different parts of the cochlear nuclei. A detailed investigation on the organization of this projection is outside the scope of the present study, but the finding of localized degeneration in all three cochlear nuclei in experiments in which the cochlea was only partially destroyed indicates that this problem is amenable to study with the techniques used.

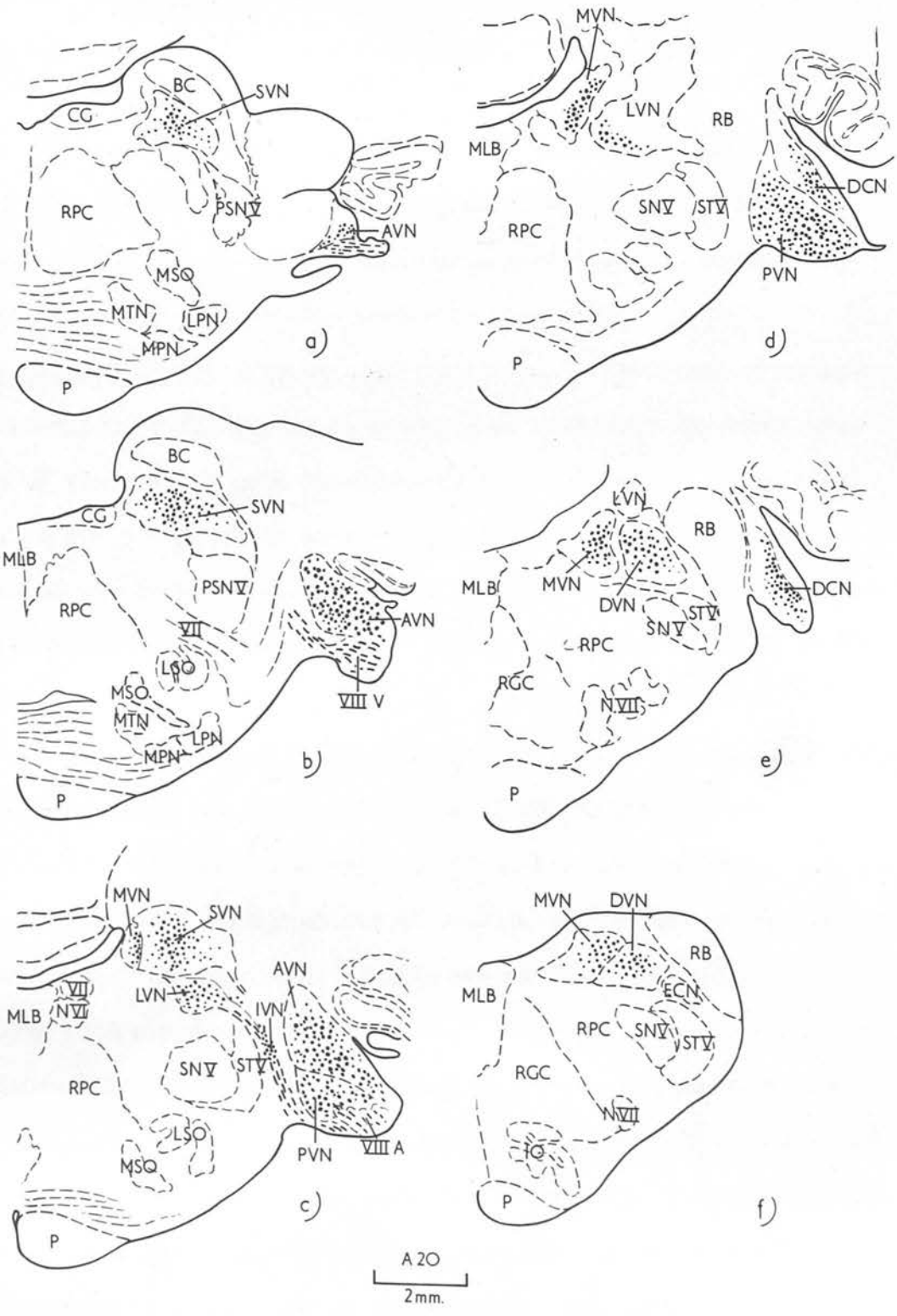


Figure 18. The distribution of degenerating fibres (indicated by dashes) and terminal degeneration (dots) in outlines of successive antero-posterior sections of the brain-stem in experiment A 20, in which both the cochlear and vestibular divisions of the VIIIth cranial nerve were destroyed.

No account of the normal morphology of the auditory relay nuclei will be given as they have been briefly described in the previous section. The position of these nuclei in successive transverse sections of the brain-stem is illustrated in figure 18

Projection of the primary auditory fibres. The first experiment to be described, A 21, serves to demonstrate virtually the total projection of the cochlea upon the brain-stem. This animal was allowed to survive for 7 days after destruction of the cochlea and frozen sections of the entire brain-stem were stained. There was no involvement of the rest of the labyrinth or of the vestibular division of the VIIIth nerve.

The Nauta preparation of this brain show that at its level of entry into the brain-stem the cochlea division of the VIIIth nerve has almost completely degenerated. Against a clear background the fibres are seen to be severely fragmented and in regular rows of intensely stained droplets. Only an occasional spindle-shaped fibre is seen. In accordance with previous descriptions of the branching of the auditory nerve fibres (Cajal, 1909) degenerating fibres can be traced passing to the two divisions of the ventral cochlear nucleus, and as successive sections of the ventral cochlear nucleus are examined in either rostral or caudal directions from the level of entry of the nerve, the bundles of degenerating fibres are found progressively more medially. Because of their arrangement in relatively distinct bundles the degenerating fibres can be distinguished quite easily from the finer pericellular preterminal degeneration.

Around the cells of the anteroventral cochlear nucleus severe preterminal degeneration is seen in the form of fine droplets arranged

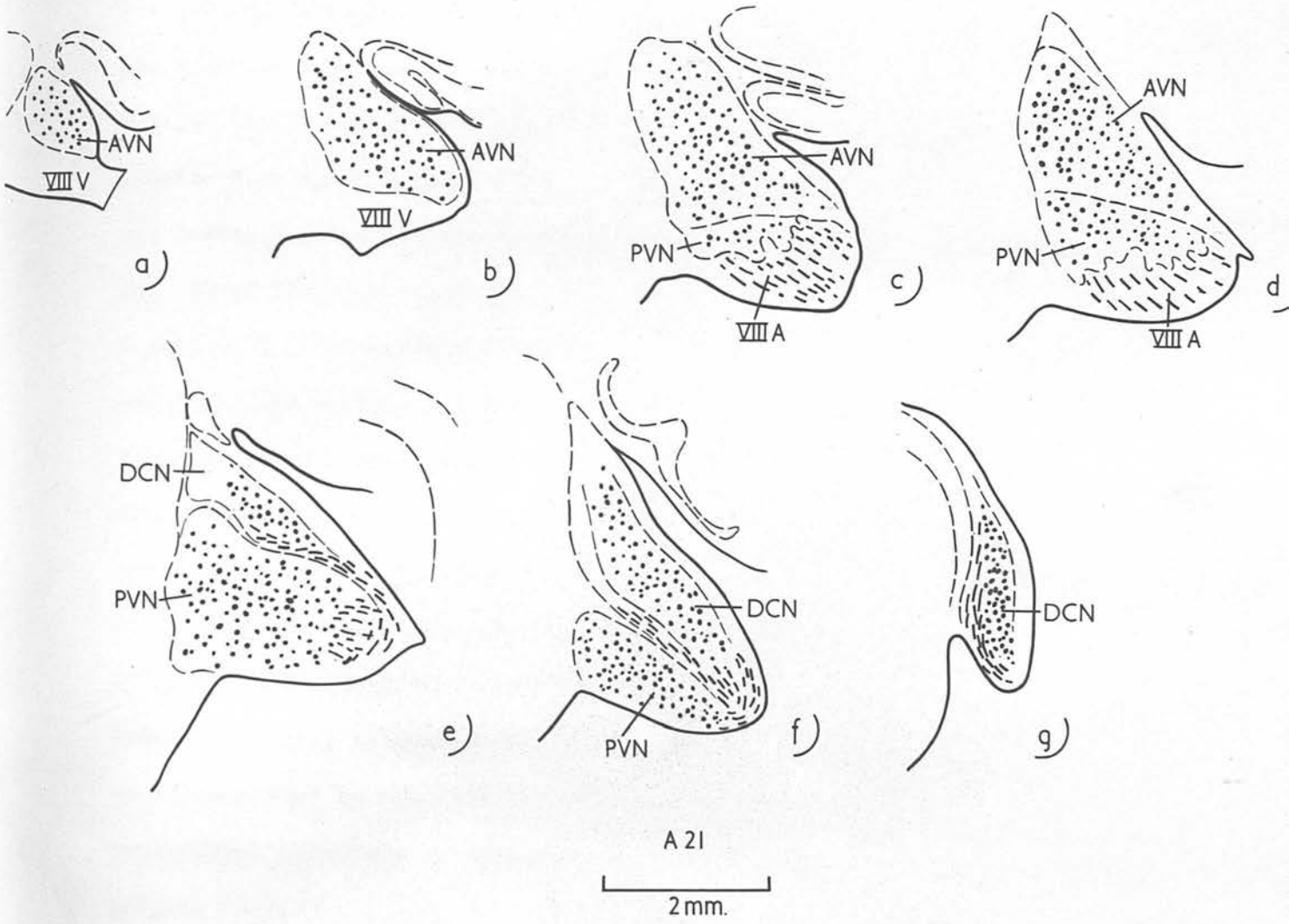


Figure 19. The distribution of fibre and preterminal degeneration in the cochlear nuclei in an experiment in which the cochlea was completely destroyed without involvement of the vestibular nerve (experiment A 21).

in clusters around the cells. The most rostral and medial parts of the nucleus contain less degeneration, and this is probably due to the incompleteness of the cochlear lesion. The appearance of the posteroventral nucleus is essentially the same, apart from the somewhat greater density of degenerating fibres, but here it is the most caudal and medial parts which are less severely affected. In addition to the coarse fibre degeneration in the posteroventral nucleus there is a good deal of much finer fibre degeneration which can be traced through and along its ventral and lateral margins into the dorsal cochlear nucleus. While some degeneration is found along the entire ventral margin of the dorsal cochlear nucleus, its most caudal and lateral parts are the more severely affected. In the deepest layer of the nucleus there is a profuse plexus of degenerating fibres which extends dorsally up to the characteristic spindle-cell layer; around the bodies of these cells less intense preterminal degeneration is found. There is no degeneration in relation to their superficial dendrites so that the superficial plexiform or molecular layer is entirely free of degeneration (figure 29).

Careful examination of serial sections from this brain has shown that after a virtually complete destruction of the cochlea, fibre and preterminal degeneration is confined to the cochlear nuclei. In particular it should be emphasized that no bundle of degenerating fibres passing to the medial trapezoid nucleus, comparable to that described by Lewy and Kobrak (1936), has been seen in our material, and in all the brains the medial trapezoid nucleus and superior olivary complex are entirely free of degeneration.

In sections stained according to the Glees method the degenerating

fibres are broken up into larger argyrophilic fragments, and in close relation to the cells of both divisions of the ventral cochlear nucleus there are large, solid end-bulbs and boutons, many of which are connected to short segments of the terminal part of a fibre. The endings of Held which can be clearly seen in the anteroventral nucleus of the normal side are hardly distinguishable on the operated side where they are irregular and partially fragmented. In addition to the fibre break up in the deep polymorph layer of the dorsal cochlear nucleus, there are numerous degenerating boutons most of which have lost their ring-like form and are already solid. The only feature of the Bodian preparations which needs to be described is the appreciable fibre loss which has occurred in the nerve and neuropil of the cochlear nuclei at this early stage, so that in the affected areas the dendrites of the cells stand out conspicuously.

Another example of almost complete destruction of the cochlea without involvement of the vestibular division of the VIIIth nerve is experiment A25. The findings in this brain will be described briefly as the animal was allowed to survive for a longer period (15 days) and the brain was embedded in paraffin and stained with a number of "on the slide" silver methods. In the sections stained according to the original Nauta and Gygax (1951) method as applied to paraffin sections the fibre degeneration has proceeded to distinct droplet formation and the endings of Held in the anteroventral nucleus are distinctly swollen and partially fragmented. In the posteroventral nucleus preterminal degeneration is seen in the form of fine droplets around the cells and in addition there are numerous solid end bulbs in contact with the cells. The neuropil of the deep polymorph layer of the dorsal cochlear nucleus

is broken up but little degeneration can be seen in the immediate vicinity of the cell bodies of the spindle-cell layer. In the Bodian preparations there is marked fibre loss both in the cochlear nerve and in the neuropil of the cochlear nuclei; indeed, the only distinctly normal cell processes seen in the nuclei are the thick dendrites of the cells.

The findings in the present material from experiments with short survival periods, are confirmed by a study of Bodian preparations of the brain of a cat which had been allowed to survive for 359 days after destruction of the cochlea. The cellular changes in this brain have been described in the previous section. In both divisions of the ventral cochlear nucleus marked loss of fibres has occurred, but this is not so obvious at low magnification as it is after short survival periods because of the gliosis and shrinkage of the nucleus as a whole. At higher magnifications there are two striking differences between the nuclei on the normal and operated sides. First, on the operated side no distinct endings of Held can be seen in the anteroventral nucleus and there is a marked decrease in the number of boutons in the posteroventral division. Second, the dendritic processes of the cells, particularly in the posteroventral nucleus stand out more prominently in the degenerated nucleus and many of these could be traced over a distance of several hundred micra. In the dorsal cochlear nucleus there is some fibre loss in the deep polymorph layer, but it is not so clearly seen as in the ventral nucleus because of the greater density of the intrinsic neuropil. In both the lateral superior olive of the same side and the contralateral medial trapezoid nucleus, the cells show secondary transneuronal changes in Nissl preparations, but there is no apparent reduction of the afferent fibres or of the intrinsic fibre plexus. Although it is difficult to

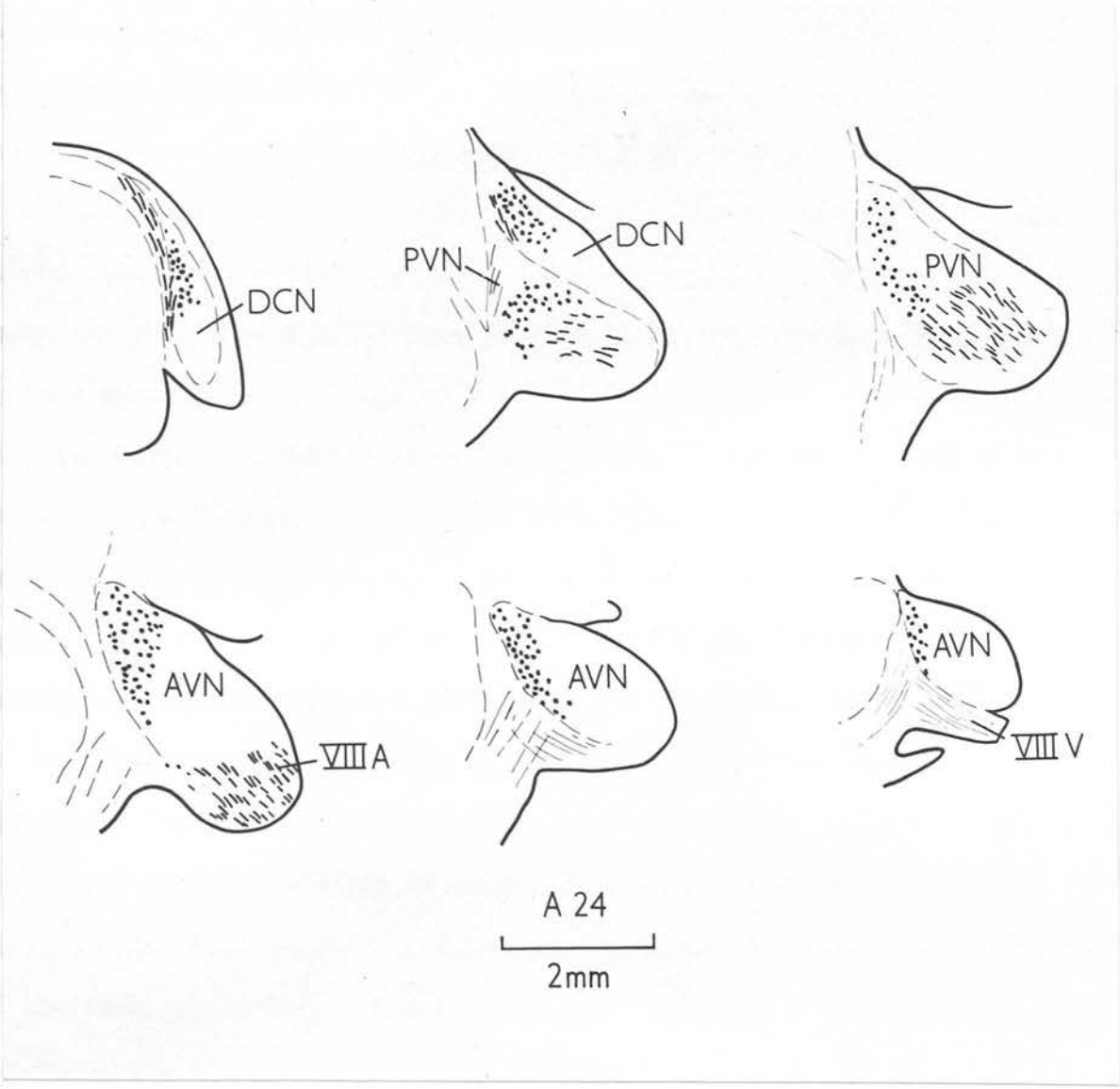


Figure 20. The distribution of fibre and terminal degeneration in experiment A 24 in which there was a partial lesion of the cochlea involving only the basal coil.

be certain of a distinct difference between the two sides because of the low density of the cells, the endings of Held in the contralateral medial trapezoid nucleus appear to be more heavily impregnated.

Experiment A 24 is the only example of a partial lesion which will be described as the distribution of the degeneration in all the other experiments with partial lesions is essentially similar in appearance. In this experiment the basal coil of the cochlea alone was damaged but some involvement of the cochlear nerve cannot be excluded. The partial nature of the degeneration is clearly indicated by the fact that in some sections of the brain-stem no degeneration in the auditory nerve is apparent at its level of entry. The course of the degenerating auditory nerve fibres within the cochlear nuclei and the distribution of the terminal degeneration are shown in text-figure 20. The degenerating fibres are seen to pass progressively more medially as the serial sections are traced either rostrally or caudally from the level of entry of the nerve and terminal degeneration is only found in the medial third of each of the cochlear nuclei. The intensity of the degeneration and the extent of its distribution through the cross-sectional area of the nuclei also become progressively less as the rostral and caudal poles of the ventral cochlear nuclei are approached. Finer degenerating fibres can be seen passing along the medial aspect of the caudal half of the ventral nucleus into the fibrous lamina between the posteroventral and dorsal cochlear nuclei. From here they enter the medial part of the dorsal cochlear nucleus at about the level of the junction of its rostral and middle thirds. Rostral to this level no degeneration is found in the deep polymorph layer of the nucleus but more caudally typical terminal degeneration is seen in this layer over an increasing sector of its

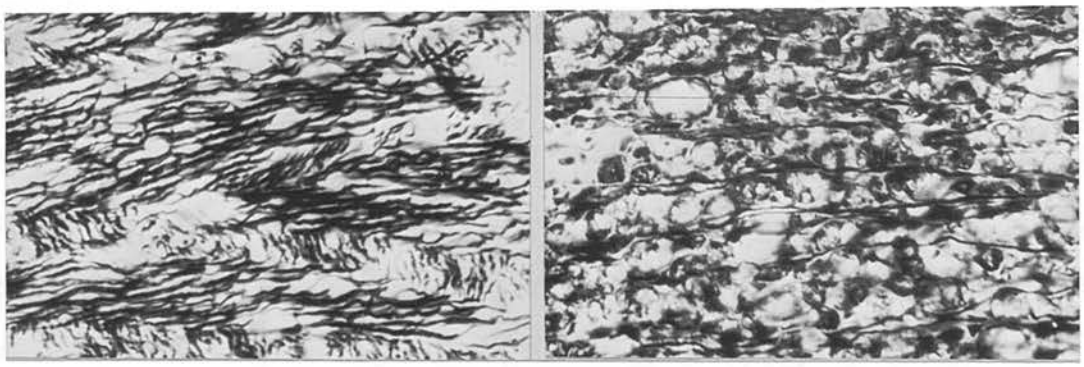


Figure 21. The appearance of normal (left) and degenerating (48 hours, right) fibres in the cochlear division of the VIIIth cranial nerve. Marsland, Glees and Erickson method. x460

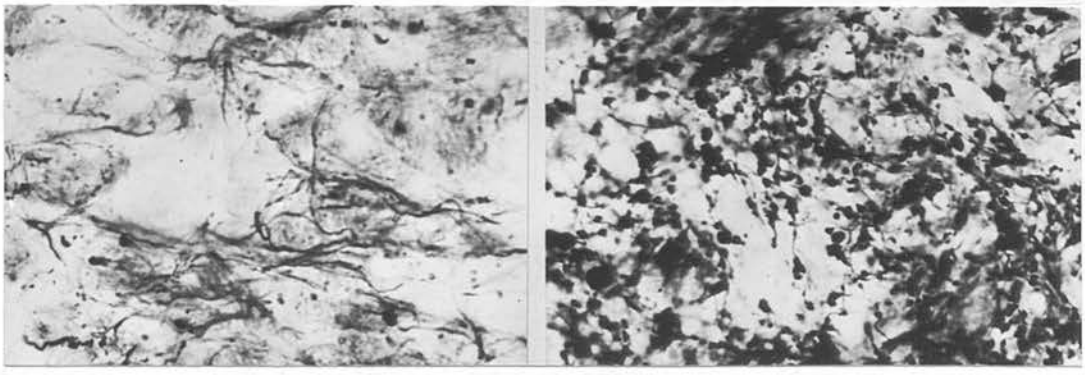


Figure 22. Nauta preparations of the postero-ventral cochlear nucleus on the normal (left) and the operated side (right). (2 day survival; frozen sections) x 460

medio-lateral extent. This description applies to the paraffin sections stained by the Nauta and Glees methods, but in the Bodian preparations marked fibre loss is seen to have occurred at this survival period (12 days). With this method the affected areas of the three nuclei are clearly delimited by the marked loss of neuropil, even at low magnifications. At higher magnifications an interesting feature is the prominent appearance of the larger dendrites of the cells, many of which can be traced over considerable distances.

In the cat in which the cochlear nuclei were inadvertently destroyed in addition to the cochlea, the Bodian sections provide evidence of the projection of the second-order neurons. On the ipsilateral side there is a complete loss of fibres between the cochlear nuclei and the trapezoid body, and in the proximal parts of the dorsal and intermediate striae. As the striae and trapezoid body are traced towards and across the midline the number of normal fibres present increases progressively. The lateral superior olive of this side has lost most of its afferent fibres, and among the cells there is a profound loss of neuropil so that the nucleus as a whole appears distinctly paler than that of the opposite side. There is a similar loss of neuropil in the two preolivary nuclei of the operated side. No change is seen in the medial trapezoid nucleus of the operated side, but on the contralateral side the extent of the nucleus is clearly delimited by the marked reduction of the intrinsic neuropil and by the absence of the coarse fibres which in normal preparations can be seen to turn dorsally from the trapezoid body to terminate on the cells of this nucleus. None of the characteristic endings of Held are preserved on this side (figure 31). In the medial superior olive changes are seen on both sides: on the ipsilateral side the

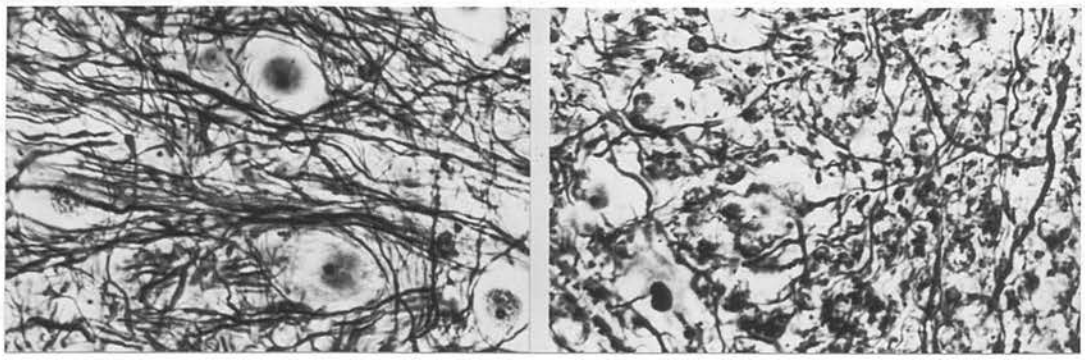


Figure 23. Photomicrograph to show the fibre plexus in a Bodian preparation of the normal postero-ventral nucleus (left) and 2 days after destruction of the cochlea (right). x460

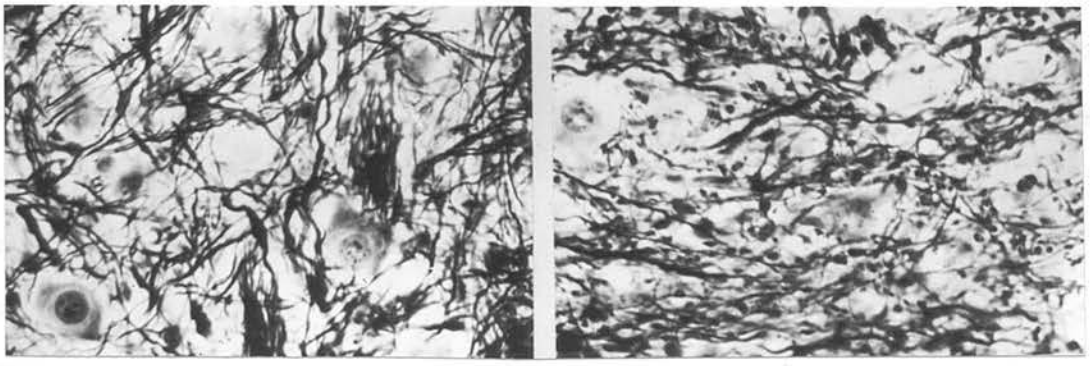


Figure 24. The fibre plexus of the ventral cochlear nucleus in a paraffin section stained according to the Nauta and Gygax (1951) method. Normal (left) and 48 hours after destruction of the cochlea (right). x460

lateral half of the nucleus is distinctly pale staining, and at higher magnifications the laterally directed dendrites stand out clearly because of the loss of afferent fibres and terminal boutons: in the contralateral medial olive the medial half of the nucleus shows equivalent to changes following the deafferentation of the medially directed dendrites. No appreciable difference is seen between the lateral lemniscal nuclei of the two sides or in the inferior colliculi.

In one cat, A 20, with a survival period of five days, the vestibular division of the VIIIth nerve was involved in addition to an almost complete destruction of the cochlea. This experiment will be described briefly because it provides confirmatory evidence for the distribution of the primary vestibular afferents as recently described by Walberg, Bowsher and Brodal (1958). The distribution and appearance of the degeneration in the cochlear nuclei is identical with that found in the earlier experiments with comparable lesions (figures 18). The degeneration in the vestibular nerve can be clearly traced dorsomedially around the spinal tract of the Vth nerve to the four vestibular nuclei. The interstitial nucleus of the nerve shows very heavy preterminal and terminal degeneration in the form of solid boutons and pericellular fragmentation (figures 28). The distribution of the terminal degeneration in the vestibular nuclei is shown in figure from which it can be seen that the projection of the primary vestibular fibres is limited to the central part of the superior nucleus, to the rostral and ventral parts of the lateral nucleus and the lateral part of the medial nucleus. In the descending nucleus, however, the degeneration is found throughout its entire medio-lateral extent. As Walberg et al (1958) have pointed out, the majority of the large cells of the lateral vestibular nucleus do

Figure 25. Photomicrographs showing the appearance of the normal fibre plexus of the anteroventral cochlear nucleus (left) and the corresponding region of the operated side 5 days after destruction of the cochlea. Paraffin sections. Nauta and Gygax method (1951). x460.

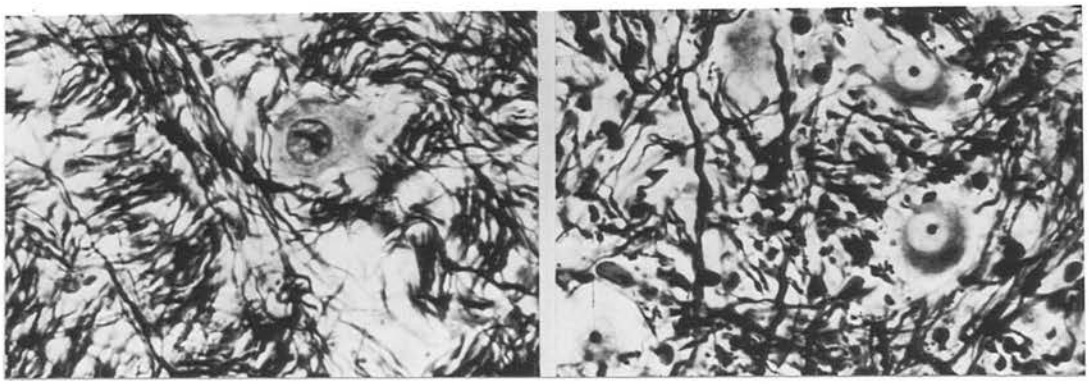


Figure 26. To show the normal (left) and degenerated (right) fibre plexus of the postero-ventral cochlear nucleus. Paraffin sections. Nauta and Gygax (1951). x460

not appear to be in direct contact with primary vestibular afferents as their perikarya and dendritic processes are singularly free of terminal degeneration.

As was stated in the previous section no evidence was found one or more months after destruction of the cochlea of retrograde cell degeneration in the region from which the olivo-cochlear bundle is said to arise. (Rasmussen, 1946). In the present material with survival periods between 2 and 15 days there has been no evidence of chromatolysis in any element of the superior olivary complex or in the area dorsal and medial to the medial olivary nucleus. While these observations should not be interpreted as being incompatible with Rasmussen's description of the origin of this bundle, it is interesting to note that Fernandez (1951) found no loss of fibres in this pathway after destruction of the organ of Corti by acoustic trauma.

A study of the appearance of the terminal degeneration after relatively short survival periods was considered essential after examination of Glees and Marsland, and Bodian preparations of the cats which had survived for 5 days or longer. In the Bodian preparations there is, even at 5 days, a marked loss of the finer preterminal nerve fibres and severe fragmentation of the coarser fibres while in the Glees and Marsland sections it was surprising to find the advanced state of degeneration of boutons and preterminal fibres. These findings suggested that a study of the cochlear nuclei after even shorter survival periods might demonstrate more clearly the sequence of degenerative changes in the boutons and their relation to the cells of the cochlear nuclei. For this purpose four immature animals were operated upon and allowed to survive for 12, 24, 48 and 90 hours respectively. At 12 and 24 hours no

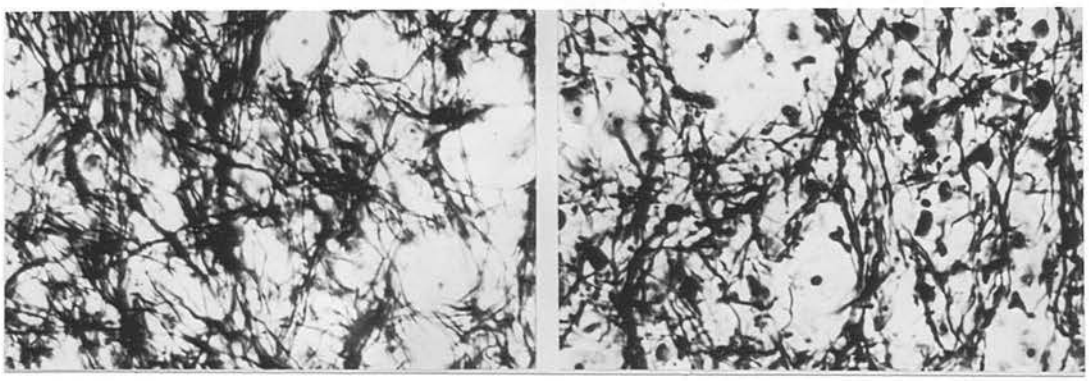


Figure 27. The normal fibre plexus of the deep polymorph layer of the dorsal cochlear nucleus (left) and the corresponding area of the operated side 5 days after destruction of the cochlea. Paraffin sections. Nauta and Gygax (1951) method. x460

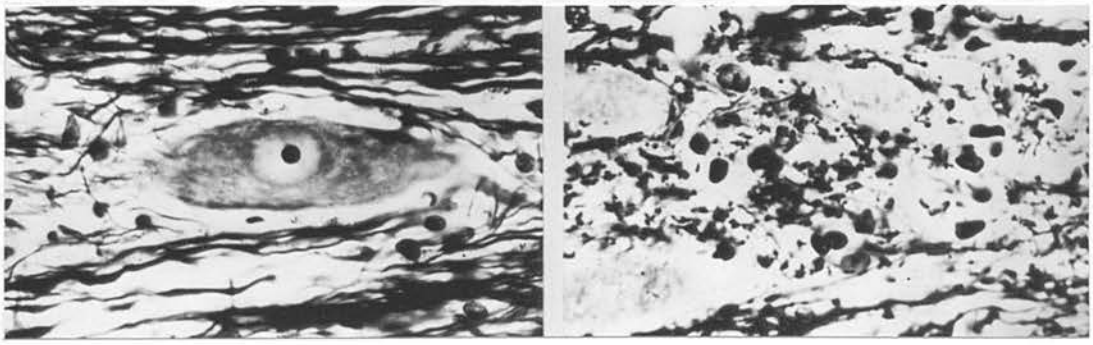


Figure 28. The appearance of the fibre plexus in the interstitial nucleus of the vestibular nerve of the normal (left) and operated sides (right). 5 day survival. Paraffin Nauta sections. x460

changes were seen in either the auditory nerve or the cochlear nuclei with any of the techniques used. At 48 hours, however, a remarkable degree of fibre degeneration had already occurred; because of the somewhat unusual features of the degeneration at this stage the findings in this animal, A 29, will be described in some detail. The Bodian preparations are most striking in the way they show the advanced state of the degeneration and also the degree of precision with which it is localized within the cochlear nuclei. The difference in the size of the fibres terminating in the ventral and dorsal cochlear nuclei is also clearly shown in this material. The coarser fibres in the ventral cochlear nucleus are broken up into conspicuous fragments each of which appears to be coiled upon itself, giving the appearance of irregular whorls (figure 23). Under higher magnifications these are seen to be composed of dense aggregates of fine granules. Passing into the ventral aspect of the dorsal cochlear nucleus are finer fibres which are simply fragmented so that the neuropil of the degenerated part of the deep plexiform layer stands out with remarkable clarity. The endings of Held in the anteroventral nucleus are swollen and irregular while the boutons in the posteroventral nucleus are enlarged, distinctly irregular and frequently connected to a short terminal part of an axon.

The appearance of the terminal degeneration and of the fine fibres passing to the dorsal cochlear nucleus is essentially the same in the Glees and Marsland preparations, but the appearance of the coarse auditory nerve fibres is unusual and will be described in some detail as it has not (as far as we are aware) been recognised in the more recent literature on fibre degeneration. At its entry into the brainstem advanced fibre degeneration is seen amongst the incoming auditory nerve

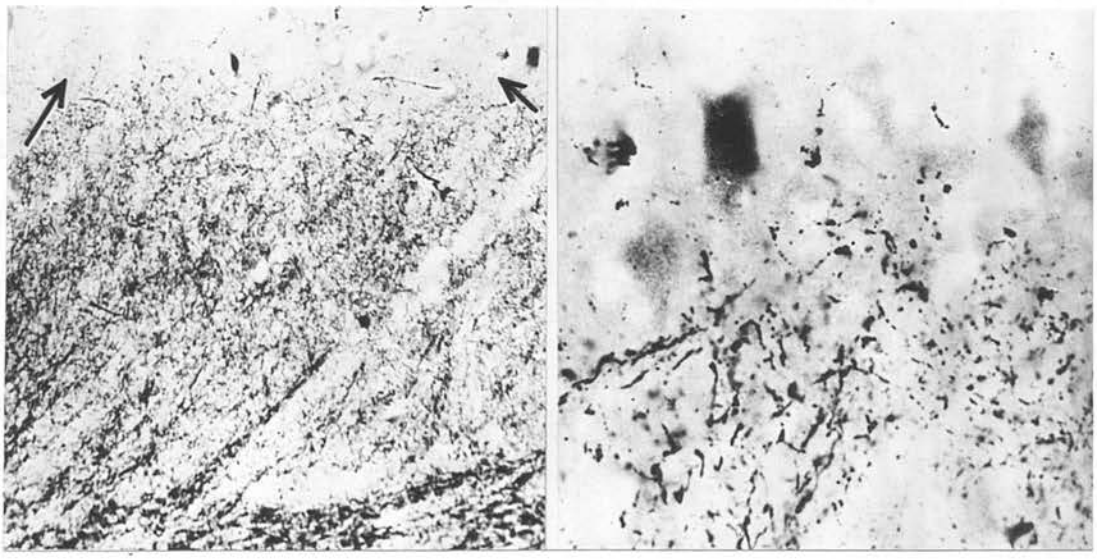


Figure 29. Photomicrographs of degeneration in the deep polymorph layer of the dorsal cochlear nucleus. On the left (x 100) the entire thickness of the polymorph layer is seen to be affected, but the degeneration ends abruptly at the level of the spindle-cell layer (arrows). On the right (x 460) the degeneration is seen around the deep dendrites and bodies of the spindle cells.
Frozen Nauta preparation.

fibres in the form of large, ring-like structures about the size of small neurons, arranged in regular rown between the normal fibres from uninjured parts of the cochlea (figure 21). Many of these large rings have a finer trabeculated internal structure which varies in density from ring to ring. Others are more or less solid and are intsnsely argyrophilic. There can be little doubt that these rings represent a more completely impregnated form of the 'whorls' seen in the Bodian preparations. In the paraffin Nauta sections the degenerating fibres present an essentially similar but less striking appearance, many more of the rings having a solid structure (figure 24). In the Nissl-stained sections there is no appreciable gliosis amongst the cochlear nerve fibres. In the frozen sections of cat A 30, which had the same survival period, stained according to the Glees and Nauta methods the appearance of the degeneration is characteristic of these techniques; i.e., in the Glees sections, the fibres are swollen, argyrophilic and partially fragmented; in the Nauta sections the fibres appear to be more severely fragmented the degeneration consisting of regular rows of argyrophilic droplets (see figure 22).

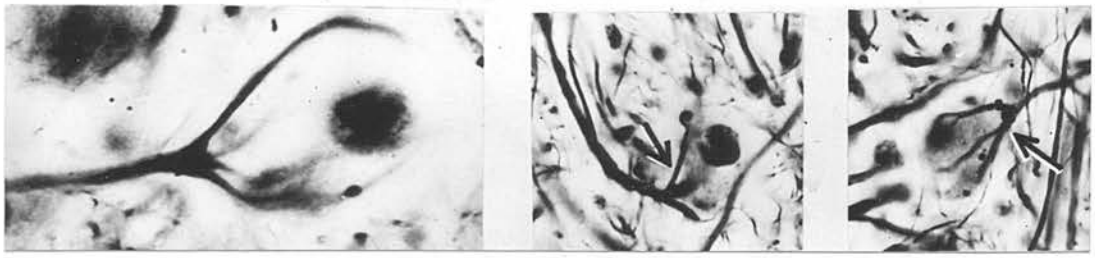


Figure 30. Photomicrographs of normal endings of Held in medial trapezoid nucleus (left, x 1040) and the antero-ventral cochlear nucleus (right, x 460). Bodian method.

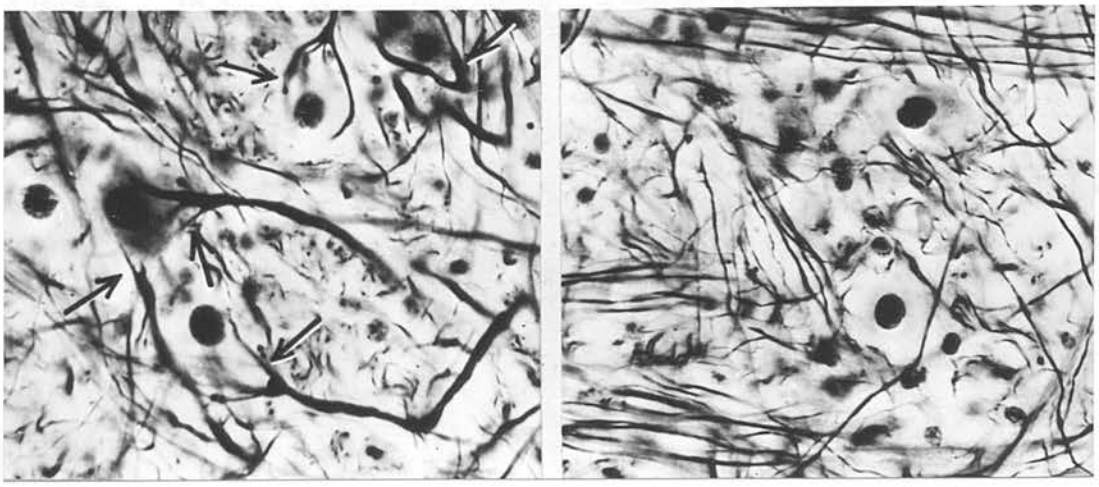


Figure 31. Endings of Held and fibre plexus in normal medial trapezoid nucleus (left) and absence of endings and coarse afferent fibres (right) 2 months after destruction of contralateral cochlear nuclei. Bodian method. x460.

DISCUSSION

The results of these studies have shown that following destruction of the primary afferent fibres transneuronal degeneration occurs in the cells of the olfactory bulb of the rabbit and in the auditory relay nuclei of the cat. In addition, they have provided information relevant to some of the problems raised in the introduction, and they have also helped to clarify some of the discrepancies in the literature concerning the projection of the primary auditory fibres. In this discussion of the findings the morphological changes in the neuron undergoing transneuronal degeneration will be summarized first; next, the details of the degeneration in the olfactory bulb and auditory relay nuclei will be considered; and finally some of the general features of the degenerative process will be discussed.

Morphological changes. During transneuronal degeneration the cells of the olfactory bulb and of the auditory relay nuclei, as seen in Nissl-stained preparations, undergo essentially the same changes as have been described in the lateral geniculate nucleus of the cat and rabbit (Cook et al, 1951), of the monkey (c.f. Matthews et al, 1960), and of man (Goldby, 1957). All three of the major components of the soma - the cytoplasm, nucleus and nucleolus - are reduced in size, the cytoplasm showing the maximum degree of change and the nucleolus the least. There is also a slight reduction in the amount of Nissl material,

and the granules become finer and have a more homogeneous 'matt' appearance. From the evidence available at present it may be concluded that in all sites in the central nervous system of adult animals transneuronal atrophy appears qualitatively to be the same. There are two possible qualifications, however, for Cook et al (1951) described distinct vacuolation of the cells of the lateral geniculate nucleus of the cat and rabbit 8-10 months after eye enucleation, and Penman and Smith (1950) found that the medium-sized cells of the spinal nucleus of the trigeminal nucleus in man had undergone swelling and chromatolysis 4 months after alcohol injection of the nerve.

From the quantitative data which have been recorded for the cat and monkey it is apparent that the cytoplasm, nucleus and nucleolus do not behave uniformly during transneuronal degeneration. The degree of shrinkage of the total cell area is proportionately greater than that of the nucleus or nucleolus. In the lateral geniculate body and auditory relay nuclei of the cat this decrease in size amounts to between 20 and 35% of the normal area. The nuclear shrinkage in these groups of neurons is found to be between 10 and 25%, and its time-course usually parallels that of the cell area. Most commonly the nucleolus shows the least degree of change, and the shrinkage is usually between 10 and 15%. Comparison of the data for the degree of change in the cells of the auditory relay nuclei with those given for the lateral

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geniculate nucleus of the same species by Cook et al (1951) indicates that, in two, at least, of the main afferent sensory systems in a given species the severity of the degenerative process is the same. The shrinkage in cell area in the olfactory bulb of the rabbit was appreciably greater, however, than in the lateral geniculate nucleus of this animal. In the bulb the shrinkage was between 40-50% while in the lateral geniculate nucleus it was approximately 20% (Cook et al, 1951). The greater severity of change in the bulb can probably be accounted for by the use of 6 week old animals for these experiments, as Torvik (1956) has shown that both the rate of onset and the degree of change are more pronounced in young animals. In the lateral geniculate nuclei of different species, on the other hand, distinct differences have been found in the severity of the degeneration. Thus in this nucleus in the cat the shrinkage of the cell area is between 20 and 35% and in the rabbit it is slightly less; in the macaque it amounts to 30-40% (Matthews et al, 1960) and in man it is slightly more (Goldby, 1957). Not only the cell areas but also the nuclear and nucleolar areas show more marked changes in the monkey than in the cat (20-30% compared with 10-25%).

The observations in the Bodian sections of the olfactory bulb indicate that the three main components of the neuron- dendrite, soma and axon - are all affected by the degenerative process. The marked atrophy of the dendritic processes of

the cells is quite apparent, and from the changes described in the molecular layer of the pyriform cortex subjacent to the olfactory tract it would appear that a marked reduction has occurred in the number of the collaterals given off by the axons of the mitral cells. While the Golgi-Cox preparations of the bulb have to be interpreted with caution they do suggest that cells undergoing transneuronal degeneration are resistant to impregnation by this method. The significance of this change must remain speculative without further evidence, but it would be interesting to determine whether or not all neurons undergoing this form of degeneration behave in this way, and also the precise time of its onset. It is possible that the increased resistance to impregnation might be found before any change is seen in Nissl-stained sections.

The evidence available for adult cats, rabbits and monkeys indicates that the process of transneuronal degeneration does not proceed to death of the cell. In the present material there was no qualitative impression of neuronophagia in either the olfactory or auditory systems, and this was confirmed by the cell counts of two of the auditory nuclei. Using infant animals, Polyak (1957) and Torvik (1956) have found complete cell loss during transneuronal degeneration in the lateral geniculate nucleus of the monkey and in the pontine and inferior olivary nuclei of the cat respectively. It appears, therefore, that the occurrence of cell loss in these species is mainly a factor of the age of the animal at the time

of operation. The validity of this deduction could be tested experimentally by a study of the degeneration in the lateral geniculate and cochlear nuclei in kittens or in the pontine and inferior olivary nuclei of mature cats. In man, however, Goldby (1957) found that in the atrophic laminae of the lateral geniculate nucleus there was a cell loss of approximately 50% many years after removal of the eye, and in the main sensory nucleus of the trigeminal nerve Penman and Smith (1950) described 'a definite reduction in the number of neurons'. The fact that in transneuronal degeneration in adults appreciable cell loss has only been described in man might suggest that here species is a determining factor.

Olfactory bulb. This work has shown what appears to be true transneuronal degeneration of mitral, tufted and periglomerular cells of the olfactory bulb. It thus extends the earlier observation by Le Gros Clark and Powell (Le Gros Clark, 1957) of 'fragmentation and dissolution' of dendritic ramifications in the glomeruli, 24 days after unilateral destruction of the olfactory mucosa. The present effects consistently followed interruption of olfactory nerve fibres, and were severest in regions of the bulb where the loss of afferent fibres was most complete. The possibility, however, that they were caused by some interference with the blood supply of the bulb can probably be excluded, for the similar shrinkage of mitral cells in the accessory olfactory bulb following a remote lesion in the vomero-nasal organ does suggest that the changes

in the cells of the main bulb were entirely due to the operative deafferentation.

For none of these three types of neuron (mitral, tufted or periglomerular cells) can the deafferentation achieved have been complete. It was probably most nearly complete in the case of the periglomerular cells, for their dendrites are confined to glomeruli, and the only afferent fibres entering glomeruli in the rabbit apart from the olfactory nerves appear to be the axons of periglomerular cells in adjoining glomeruli (Cajal, 1911; Le Gros Clark and Meyer, 1947; Allison, 1953). Towards the centre of a region of total loss of olfactory fibres, therefore, the glomeruli may well have ceased to receive any afferent impulses, unless from spontaneous activity in periglomerular cells (which might have either an excitatory or an inhibitory effect). Shrinkage of periglomerular cells, therefore, is not such a very surprising result. Less to be expected, however, was the discovery that the changes in the mitral and tufted cells were not confined to their intraglomerular dendritic tufts, though perhaps severest there, but involved also the accessory dendrites, cell body and probably the axon. This suggests that activity in the principal dendrites is of great importance for maintaining the integrity of the whole of the neuron. The atrophy of the accessory dendrites and the cell body can hardly be attributed to the presence nearby of degenerating terminals or to the disruption of the synaptic contacts upon them, for they are not reached by any olfactory

nerve fibres. In addition, the accessory dendrites and the cell bodies are still exposed to possible sources of afferent impulses from the various structures in the outer plexiform layer. These include, first, the recurrent collaterals of their own axons; second, the peripheral processes of granule cells, the basal dendrites of which are thought to receive commissural and centrifugal impulses; third, any centrifugal or commissural fibres which may pass directly into the outer plexiform layer (Le Gros Clark and Meyer, 1947; Allison, 1953); fourth, and perhaps important, the electrical fields produced by activity in other dendrites. Some of these influences might be inhibitory (c.f. Kerr and Hagbarth, 1955) but the net effect on the mitral and tufted cells from all these sources could be considerable. In Golgi-Cox preparations their accessory dendrites are seen to have a wide lateral spread: for example, one horizontal dendrite of a tufted cell was at least 600u in length. Possibly the cells cannot be excited to discharge action potentials by activation of the accessory dendrites alone, or possibly there is insufficient activity in these, in the absence of the normal olfactory inflow, to cause discharge of the cell. At any rate, cessation of activity in the principal dendrite led to distinct atrophy of the neuron as a whole, and not just to that of the principal dendrite itself.

The shrinkage of the granule cell layer, with reduction in size of the clumps of granule cells, is not easily accounted

for in terms of deafferentation. For they do not receive any olfactory fibres, although it is not known whether they are activated by axon collaterals from mitral and tufted cells; but they probably receive many terminations of commisural and centrifugal fibres. The changes in the granule cells were of later onset than those in other cell types, and were also less severe, for their nuclei did not in general become visibly smaller and (so far as this is adequate evidence) they were much more often impregnated than the other neurons in the Golgi-Cox preparations. The atrophy of the layer might, indeed, be due to some indirect interference with its blood supply, consequent upon compression from the densely felted outer plexiform layer in its shrinkage. There was no evidence, however, that serious compression had occurred: no shrinkage was detected in the periventricular layer, nor any collapse of the olfactory ventricle, and the degenerate bulb, instead of taking on a more rounded form, often retained in all its layers contours closely resembling those of the normal bulb. It may finally be noted that the granule cells are peculiar in lacking any process with the characteristic morphology of an axon (Cajal, 1911), and that their function is far from clear; it is therefore not surprising that the slight atrophic changes in them are difficulat to interpret. It might be reasonable, however, to speculate that these changes may reflect the altered conditions in the environment of the peripheral process in the outer plexiform layer.

All the changes which have been found to occur in the olfactory bulb as the result of removal of the olfactory mucosa begin within one month of operation. The rather early onset of transneuronal degeneration in the olfactory bulb is in contrast to that in the lateral geniculate nucleus of the rabbit where Cook et al (1951) found no changes until 5-6 months after removal of the eye. Although it is known that the time course of this form of neuronal degeneration in the same afferent system may differ considerably in different species, this finding of a distinct difference between the time of onset of the degeneration in the olfactory bulb and lateral geniculate nucleus might indicate that the time course of the degeneration also varies in the same species in different groups of neurons. As it is known, however, that transneuronal degeneration is more severe in young animals (Torvik, 1956) the earlier onset of the changes in the olfactory bulb could be due to the denervation being done on immature animals.

The findings in the Golgi-Cox material should be interpreted with caution in view of the marked capriciousness of the technique. For several reasons, however, it seems likely that the differences in impregnation of the mitral, tufted and periglomerular cells between the two sides are due to experimental denervation: the two bulbs were prepared simultaneously in one block; no differences were found between the adjoining frontal poles of the two hemispheres; on the medial aspect of the bulb of the operated side where some olfactory fibres were preserved

a few of these cells were apparently normally impregnated; and finally, the appearance of the bulbs on the operated sides was essentially the same in the two experiments with different survival periods. Those cells in the olfactory bulb which are undergoing transneuronal degeneration therefore appear to be more resistant to impregnation by this technique. It is difficult to explain why this should be so when so little is known about the factors which govern the impregnation of only a small proportion of normal neurons. It is interesting to conjecture whether this difference in reaction would be seen in cells undergoing transneuronal degeneration at other sites, and relevant to this is the finding of Jones and Thomas (1956) who found, with the Golgi-Cox technique, that there was a marked reduction in the number of dendritic branches arising from the pyramidal cells in the pyriform cortex of the rat 100 days after removal of the olfactory bulb.

Auditory relay nuclei. Before discussing the cellular changes in these nuclei following destruction of the cochlea it is necessary to comment upon the findings of the study of the projection of the cochlea.

The primary purpose of this investigation was to determine the projection of the cochlea upon the brain-stem. From the results of these experiments it appears that no fibres pass beyond the dorsal and ventral cochlear nuclei. In view of the recent claims of Stotler (1949) and Rasmussen (1957) that no primary auditory fibres terminate in the dorsal cochlear nucleus

and because no degenerative changes were seen in the characteristic spindle cell layer of this nucleus, it should be emphasized that in the present material unequivocal degeneration has been traced into this nucleus as well as into the two divisions of the ventral cochlear nucleus. On the other hand, no evidence has been found to confirm the findings of Lewy and Kobrak (1936), in a Marchi study of the cochlear projection in the rabbit, of primary auditory nerve fibres passing directly to the medial trapezoid nucleus of the opposite side; similarly, the present findings do not support the suggestions of earlier workers (Held, 1893) for a direct projection to the lateral superior olive. The occurrence of fibre loss restricted to the cochlear nuclei in the protargol-stained sections of the brain-stem of an animal which survived for nearly a year after destruction of the cochlea excludes the possibility that the absence of degeneration in other sites after shorter survivals, is due to the refractoriness of these fibres to degeneration. It was noticeable that the gliosis and shrinkage of the cochlear nuclei in this experiment rendered the fibre loss less conspicuous than after the shorter survival periods. This observation, together with the fact that the changes in the dorsal cochlear nucleus were much less obvious than in the ventral nucleus, might account for Stotler's (1949) failure to find evidence for a direct projection to this nucleus (using a modified Bodian method), but in the absence of information of the post-operative survival periods in his experiments

this point cannot be established with certainty. It is more difficult to explain the discrepancy between the present findings and the statement of Rasmussen (1957) that "the tuberculum acusticum suffered no detectable depopulation of argyrophilic particles" even after complete destruction of the cochlea. The only possibility is that his method does not impregnate the finer fibres projecting to the dorsal cochlear nucleus or the terminals in its deep polymorph layer.

The absence of a direct projection to the superior olivary complex and trapezoid nuclei is significant for the interpretation of the cellular changes found in these nuclei after destruction of the cochlea. Although the severity and the time course of the degeneration in these nuclei parallel those seen in the ventral cochlear nucleus the results of the present investigation make it clear that they are secondary to the transneuronal atrophy of the cells in the ventral cochlear nucleus.

Our material has not been suitable to demonstrate the branching of the central processes of the spiral ganglion as described and illustrated by Cajal (1909) and confirmed by Lorento de No (1933, a,b) but the distribution of the terminal degeneration after partial and complete lesions of the cochlea indicates that each part of the cochlea is represented in all three components of the cochlear nuclear complex (i.e. the dorsal, anteroventral and posteroventral nuclei). The classical accounts of the synaptic organization of these nuclei have been confirmed, and it has been shown that the endings of Held in the anteroventral nuclei degenerate in essentially the same

way as the boutons and pericellular fibre plexuses in the posterior division of this nucleus. Experimental confirmation has also been provided of the mode of termination of afferents in the dorsal cochlear nucleus around the cells of the deep polymorph layer and on the deep dendrites of the spindle cells; comparatively little degeneration has been seen in relation to the bodies of the spindle cells and none has been observed in the superficial molecular layer or around the superficial dendrites of these cells. In addition to the difference in the synaptic endings in these three nuclei there is an appreciable difference in the calibre of fibres to the dorsal and ventral cochlear nuclei. The fibres to both divisions of the ventral cochlear nuc;eus are relatively coarse while the portion of the descending branch which passes to the dorsal cochlear nucleus is distinctly finer.

Although a systematic study of the organization of the projection of the cochlea upon the three nuclei of the primary relay group has not been made the findings in Experiment A 24 illustrate two points. First, they confirm the description of Lewy and Kobrak (1936) that there is a topical organization in the projection, and in particular that the basal turn of the cochlea projects to the medial parts of the ventral nuclei. Secondly, the clarity of the localization of the degeneration in this experiment shows that, given sufficiently small and varied lesions of the cochlea, the details of the relationship between the basilar membrane and the cochlear nuclei could be determined by the use of these techniques, especially if more

than one of the latter were used on the same material.

The findings in the experiments which have been described in the second section of the Results show that the cells of some of the auditory relay nuclei of the cat undergo transneuronal degeneration after destruction of the cochlea. The most obvious atrophy occurs in the cells of the ipsilateral ventral cochlear and lateral superior olivary nuclei, but definite degenerative changes are also found in the cells of the ipsilateral preolivary nuclei and in those of the contralateral medial trapezoid and lateral lemniscal nuclei. The morphological changes in the cell bodies of the neurons undergoing transneuronal degeneration in the ventral cochlear and lateral superior olivary nuclei are essentially similar to those which have been described in other sites in the cat and other species. It should be understood, however, that apart from these changes the cells appear remarkably normal so that the degeneration would almost certainly not be detected if the normal cells of the corresponding group of the other side were not available to serve for comparison. In the ventral cochlear nucleus these cellular changes are accompanied by gliosis which is most severe after the shorter survival periods and which becomes progressively less intense after longer intervals. In the lateral superior olivary nucleus, on the other hand, the cellular atrophy occurs without any gliosis being seen at any interval after operation. In the contralateral medial trapezoid nucleus (and possibly in the affected preolivary and lateral lemniscal nuclei

where quantitative studies were not made) the changes in the size of the cell bodies and in the Nissl material are similar to those already described, but the nucleus and nucleolus differ in showing no shrinkage.

The data obtained from the quantitative studies indicate that both in the time course and in the degree of the shrinkage, the cells of the different auditory relay nuclei closely resemble each other and those of the lateral geniculate nucleus of the same species. In both these afferent systems of the cat little appreciable change is seen in the cells before a period of approximately 60 days has elapsed, and after this period little further change occurs, the degenerative process appearing to remain more or less stationary. The finding that the time-course and severity of the degeneration of the cells in the different subdivisions of the ventral nucleus and the lateral superior olive are also essentially the same, indicates that neither the size of the cell body nor the morphology of the afferent terminals influence appreciably the degenerative process. The distinct differences in the size of the cell body in the subdivisions of the ventral cochlear nucleus and lateral superior olive are shown in Tables 3 to 6, from which it can be seen that although the cells of the postero-ventral nucleus are normally considerably larger than those in the medio-ventral and lateral superior olive, transneuronal degeneration in both types of cells is affected to the same degree.

Furthermore, it is known that the auditory nerve fibre

bifurcates upon entering the brain-stem into two branches. The ascending branch passes to the antero-ventral nuclei to end as the characteristic endings of Held (Held, 1891, 1893, Cajal, 1909) and the descending branch terminates on the cells of the postero-ventral nucleus as pericellular endings and boutons. This difference in the morphology of the afferent nerve terminal is probably not a significant factor in determining the onset or severity of the degeneration, however, as the only difference between the degeneration of the cells of the different subdivisions of the ventral cochlear nucleus, of the lateral superior olive and of the lateral geniculate nucleus is the slightly earlier onset of change in the postero-ventral nucleus. It has already been mentioned that this difference could well be within the limits of error of the technique of cell measurement particularly in view of the greater variation in the size of the cells in the postero-ventral nucleus as compared with those of the other nuclei.

There is no qualitative evidence of any cell loss in the auditory relay nuclei even one year after destruction of the cochlea, and this is confirmed by the counts of the ventral cochlear and lateral superior olivary nuclei. This agrees with the observations, in the same species, of Cook et al (1951) on the lateral geniculate nucleus and Hamlyn (1954) on the superior cervical ganglion. In view of these findings it is difficult to explain the statement of Carpenter, Bard and Alling (1959) that chromatolysis and cell loss are

found in the cochlear nuclei 13-17 days after labyrinthectomy.

While the finding of transneuronal degeneration in the cells of the ventral cochlear nucleus is not particularly surprising, the absence of any such change in the characteristic spindle cell layer of the dorsal cochlear nucleus is one of the unexpected features of these experiments. Because of their variable size and low density the absence of transneuronal changes cannot be excluded for the cells of the deeper polymorph layer of this nucleus. There are at least three possible explanations for the lack of occurrence of this form of atrophy in the spindle cells. The first is that there are no primary auditory fibres projecting to this nucleus as Stotler (1949) and Rasmussen (1957) have claimed. That the classical description of the descending branch of the auditory nerve fibre going on to terminate in the dorsal nucleus is the more correct, however, is confirmed by the finding of definite gliosis in the deeper polymorph layer in these experiments, by the presence of preterminal degeneration in the same layer after shorter survival periods as well as by the recent electrophysiological evidence of Rose et al (1959). The second possibility is that the primary auditory fibres which pass to the dorsal nucleus terminate exclusively in its deep polymorph layer and do not make direct contact with the spindle cells. Against this interpretation is the description by Cajal (1909) of Golgi material, that the terminals of the descending branch form an elaborate plexus in relation to the

deep dendrite and soma of the spindle cell, and this is confirmed by the experimental studies with silver methods. The third and most likely explanation is that although the central processes of the spiral ganglion do terminate upon the spindle cells they form only a small proportion of the total number of afferents to these cells. For transneuronal cell degeneration to occur it is probable that a high proportion of the afferents must be destroyed. The exact figure is not known - and this may vary with the site in the nervous system and the relative functional importance of the afferents - but Torvik (1956) found cellular degeneration in the inferior olivary nuclei of kittens, even though approximately 50% of the afferent fibres were still intact. In this connexion it is significant that in Golgi material Lorente de No (1933) has described many centrifugal fibres to the dorsal nucleus.

The occurrence of transneuronal degeneration in the lateral superior olive is another unexpected finding because it is generally accepted that this is a secondary relay nucleus (c.f. Stotler, 1953) and not directly related to the auditory nerve fibres (Held, 1893). The remarkable similarity in the time-course and in the severity of the degeneration in the cells of this nucleus and those of the cells in the ventral cochlear and lateral geniculate nuclei might be taken as evidence for a primary projection to this nucleus. That this is incorrect, however, is suggested by two important differences between the degeneration in this nucleus and the ventral cochlear

nucleus: the absence of gliosis at any interval after destruction of the cochlea is particularly striking, and in addition there is a lack of compacting of the cells and shrinkage of the nucleus as a whole. Furthermore, the fibre degeneration studies, after long and short survival periods, have failed to show any direct connexion to this nucleus, and the findings in the brain in which the cochlear nuclei were destroyed confirm the results of Barnes, Ranson and Magoun (1943) and Stotler (1953) in showing that this nucleus receives the majority of its afferents from the cochlear nuclei. It must be concluded, therefore, that the cellular changes in this nucleus after destruction of the cochlea are not due to the interruption of direct afferents but that they are secondary to the changes which have occurred in the cochlear nuclei. In other words, the cells of the lateral superior olive have atrophied following a lesion which is separated from them by two synapses. It is surprising that the time course and the severity of the degeneration are so similar to those in the cells separated by only one synapse, but the criteria for degeneration used here - of change in size and depth of staining - are relatively crude, and it is possible that electronmicroscopic of electrophysiological studies might show differences between the degenerated cells of these two nuclei.

A similar problem arises in the interpretation of the changes found in the contralateral medial trapezoid and lateral lemniscal nuclei and the homolateral preolivary nuclei. The

possibility of a direct projection of some auditory nerve fibres to the contralateral medial trapezoid nucleus was admitted by Cajal (1909) and Winkler (1921), and Lewy and Kobrak (1936) produced evidence from Marchi experiments in the rabbit for such a connexion. On the other hand, no fibre degeneration was found in this nucleus at either short or long survival periods after destruction of the cochlea, and furthermore, the presence of dense gliosis in all three of these nuclei together with loss of afferent fibres to the preolivary and medial trapezoid nuclei after additional involvement of the cochlear nuclei indicate that the latter nuclei are indeed the origin of their afferent fibres as described by Stotler (1953). It would appear, therefore, that the cellular degeneration found in these nuclei after destruction of the cochlea is similar to that found in the lateral superior olive in being secondary to that occurring in the cells of the ventral cochlear nucleus or to the deafferentation of the dorsal cochlear nucleus, It is also interesting to note that the degenerative process is qualitatively different, as shown by the absence of change in their nuclei and nucleoli.

The only secondary relay nucleus in which cellular changes do not occur on one or other side is the medial superior olive. The probable explanation for the absence of change here lies in the approximately equal proportion of afferents which this nucleus receives from the cochlear nuclei of each side (Stotler

1953). In view of Torvik's (1956) finding of transneuronal degeneration in the inferior olive of kittens even though at least one half of the afferents are intact, it would be of interest to repeat these experiments in younger animals.

In addition to the finding of unequivocal transneuronal degeneration in the auditory relay nuclei, there is the incidental observation that this type of degeneration also occurs in two of the vestibular nuclei after interruption of the primary vestibular fibres.

General features of transneuronal degeneration. From these and other studies during the last decade it may now be accepted that transneuronal cell degeneration can occur at more sites in the central nervous system than was formerly considered. Apart from those neurons in which its occurrence has been disputed, as, for example, in the anterior horn cells of the spinal cord (c.f. Cook et al, 1951), this type of degeneration has now been demonstrated to occur in most of the main afferent sensory systems - visual, olfactory, auditory, vestibular and trigeminal - and in the pontine and inferior olivary nuclei. Indeed, if the survival periods after operation in experimental animals were made longer, if young animals were used more frequently, and if the possibility of this type of degeneration occurring were considered it is almost certain that it would be found to occur even more widely. It is probable, also, that more experience with this form of cellular degeneration

would enable its occurrence to be used as an additional technique for tracing connexions in the central nervous system, especially if used in conjunction with silver methods for demonstrating degenerating axons. These predictions are confirmed by some preliminary observations which have recently been made: distinct shrinkage of the cells of the pontine nuclei and the substantia nigra has been found in rabbits and monkeys at survival periods of two months after lesions of the cerebral cortex, and the distribution of the cellular changes in these nuclei is found to vary according to the site and extent of the lesion.

Despite all the work which has been done in recent years, the factors which determine whether or not a cell will degenerate after removal of some or all of its afferent connexions are not clearly known. Although the experiments which have been described in the present study have yielded little positive evidence of the exact cause of the degeneration, they have, nevertheless, served to eliminate some of the possible causes which have been suggested. First, they have shown that the morphology of the afferent nerve ending, of the synapse and of the cells themselves is of little importance in influencing either the severity or the time-course of the degeneration. Second, the finding of this type of cellular change in the granule cells of the olfactory bulb and in the lateral superior olivary and medial trapezoid nuclei of the brain-stem after interruption of the primary olfactory and auditory nerve fibres respectively

shows that it can occur in cells which are separated by two synapses from the interrupted afferent fibres; the degeneration of the cells is therefore not due to the liberation of some trophic substance by the degenerating nerve terminals. Third, the occurrence of shrinkage of the cells in the lateral superior olivary and medial trapezoid nuclei without any accompanying gliosis or compacting of the cells indicates that neither the gliosis nor the increased density of the cells is significant, as Torvik (1956) and Goldby (1957) have postulated. It is generally accepted, although without any real quantitative evidence, that the size of the cell body is proportional to the amount of its axoplasm. It is also possible that the size of the soma and the extent of its dendritic processes are proportional to the number of afferent fibres terminating upon them, and that the shrinkage found in transneuronal degeneration is simply a reflection of the decrease in the number of afferent fibres terminating upon the neuron.

The severity and the time-course of the degeneration appear to depend upon at least three main variables. All other forms of neuronal degeneration which have been adequately studied so far have proved to be more severe in younger animals; Torvik's findings (1956) of rapid dissolution of the cells of the pontine and inferior olivary nuclei following lesions of the brain-stem in kittens (1956), and the occurrence of profound cell loss in the lateral geniculate nucleus of a monkey from which an eye had been removed at birth (Polyak, 1957) indicates

that transneuronal atrophy is no exception to this rule. It is probable, also, that the earlier and more serious changes found in the olfactory bulb of the rabbit in the present experiments are explicable on this basis. The second factor of importance is the species of the animal used. Its effect upon the time-course is illustrated by the marked difference in the time of appearance of transneuronal changes in the monkey, cat and rabbit; in the monkey definite changes became apparent within a few days of deafferentation (Matthews et al,1960), but in the cat and rabbit the changes can only be seen after about two months (Cook et al. 1951). In consequence of such differences of time-course, it is obvious that the duration of the survival period required to demonstrate unequivocal trans-neuronal degeneration may vary markedly from species to species. What is surprising, and difficult to understand, is the slow development of detectable morphological change in the lateral geniculate and auditory relay nuclei of the cat and rabbit. The nerve fibres and afferent terminals to these nuclei degenerate within a few days (Glees, 1941, De Robertis, 1956, and the present study). It is difficult to explain why the cells do not atrophy soon after the break up of the afferent fibres, or, alternatively, what factors are operating to cause the atrophy when it ultimately does appear. The differences in the severity of neuronal changes between cat, rabbit, monkey and man at the longest survival periods for which data are available have been mentioned above; it would appear that for the first two species , once transneuronal degeneration is

established, further survival (up to one year in the cat) makes little difference to the degree of cellular shrinkage. It should be emphasized, that even after survival periods of one year, the affected cells appear remarkably healthy so that degeneration would almost certainly not be detected if the normal cells of the opposite side of the brain were not available for comparison. The third variable which may be considered is the nuclear formation in which transneuronal degeneration is studied. Here the evidence available suggests that in adult animals of the same species, transneuronal degeneration will probably be found to show very much the same time-course and severity in all regions in which it occurs. Thus in the present study the transneuronal degeneration in the cells of the auditory relay nuclei of the cat following section of the primary auditory nerve fibres has been found to show a time-course and a degree of neuronal shrinkage virtually identical with those described for the lateral geniculate nucleus in this species. In a study of the superior cervical ganglion of the rabbit after preganglionic nerve section, Hamlyn (1954) found cell shrinkage of up to 18% after 3-4 months, which is probably of the same order as that described qualitatively by Cook et al (1951) in the rabbit lateral geniculate nucleus. Further confirmation of the similarity ofthe time-course and severity of the degeneration within a particular species could be obtained by the quantitative study of transneuronal degeneration in the superior cervical ganglion of the cat and monkey, and in the auditory nuclei of

the rabbit and monkey. Structural or functional differences between groups of neurons may, however, produce some differences in transneuronal atrophy, as indicated, for example, by the differences in the time-course of the changes between crossed and uncrossed laminae of the monkey lateral geniculate nucleus (Matthews et al, 1960), and in the ultimate severity of the degeneration between the large and small neurons of the lateral geniculate nucleus in man (Goldby,1957) and in the monkey. The question of the region affected by transneuronal degeneration in any given species may not be important as far as the time-course and severity of the degeneration are concerned; the site certainly is significant, however, in determining whether or not transneuronal degeneration does occur after interruption of the afferent connexions. The factor critical in determining the occurrence of this form of degeneration is almost certainly the extent to which afferents have been destroyed. The precise proportion of the afferent fibres which has to be destroyed for degeneration to occur is unknown, and may vary from nucleus to nucleus. The only evidence bearing on this point is Torvik's statement for the young kitten that "heavy degeneration is observed even though at least one half of the afferents are intact (as evidenced by the bilateral changes in the olive in unilateral lesions)."

It has already been pointed out that the criteria for degeneration which have been used up to the present - of change in size and depth of staining - are relatively crude. There is

now sufficient knowledge of this degenerative process to allow the study of it by more refined techniques such as electron-microscopy. The olfactory bulb should be a very suitable site for such studies as the type of cell and dendritic process could be recognised without undue difficulty. There is no doubt that the results of these investigations would lead to a better understanding of the process of transneuronal cell degeneration.

SUMMARY

1) A study has been made of transneuronal cell degeneration in the main and accessory olfactory bulbs of the rabbit, after destruction of the olfactory mucosa, and in the auditory relay nuclei of the cat following destruction of the cochlea. To facilitate the interpretation of the cellular changes in the auditory relay nuclei an additional investigation of the projection of the cochlea was done by the use of silver methods for demonstrating degenerating nerve fibres and terminals.

2) Following deafferentation all layers of the olfactory bulb, except the periventricular layer, show severe shrinkage, and the periglomerular, tufted, mitral and granule cells undergo transneuronal atrophy.

3) Bodian-stained preparations of the olfactory bulb show that the dendrites of the mitral and tufted cells atrophy, and that there is a loss of the fine collaterals of the mitral cell axons in the outer part of the molecular layer subjacent to the lateral olfactory tract.

4) Golgi-Cox preparations of the bulb show that periglomerular tufted and mitral cells which are undergoing transneuronal degeneration are more resistant to impregnation by this method.

5) The primary auditory nerve fibres of the cat terminate in the anteroventral, posteroventral and dorsal cochlear nuclei. No fibres end in the superior olivary and medial trapezoid nuclei.

6) In one experiment in which the cochlear nuclei were destroyed it was found that these two nuclei project to the two preolivary nuclei and the lateral superior olive of the same side, to the medial trapezoid and lateral lemniscal nuclei of the opposite side and to the proximal halves of the medial superior olive of both sides.

7) Following destruction of the cochlea transneuronal cell degeneration is seen in the ventral cochlear, lateral superior olivary and preolivary nuclei of the same side, and in the medial trapezoid and lateral lemniscal nuclei of the opposite side. The cells of the two divisions of the ventral cochlear nucleus and the lateral superior olive show the most obvious atrophy. No change is seen in the spindle-cell layer of the dorsal cochlear nucleus.

8) In both the time-course and severity of degeneration the cells of the different auditory relay nuclei closely resemble each other and those of the lateral geniculate nucleus of the same species. Little change is found after survival periods of less than 60 days, and after this period the degenerative process appears to be more or less stationary.

9) There is no evidence of any cell loss in the auditory relay nuclei up to 359 days after destruction of the cochlea.

10) The cellular atrophy which occurs in the ventral cochlear nucleus is due to the interruption of the direct auditory afferent fibres which terminate in this nucleus. The cellular changes which are found in the other auditory relay nuclei, however, are considered to be secondary to those in the ventral cochlear

nucleus.

11) In one experiment in which the vestibular nerve was incidentally involved, transneuronal cell degeneration was found in the medial and descending vestibular nuclei.

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Abbreviations.

AVN	Anteroventral cochlear nucleus
BC	Brachium conjunctivum
BP	Brachium pontis
C	Cerebellum
CG	Central gray
DCN	Dorsal cochlear nucleus
DNLL	Dorsal nucleus of lateral lemniscus
DVN	Descending vestibular nucleus
ECN	External cuneate nucleus
IC	Inferior colliculus
IO	Inferior olive
IVN	Interstitial nucleus of the vestibular nerve
LPN	Lateral preolivary nucleus
LSO	Lateral superior olive
LVN	Lateral vestibular nucleus
MLB	Medial longitudinal bundle
MPN	Medial preolivary nucleus
MSO	Medial superior olive
MTN	Medial trapezoid nucleus
MVN	Medial vestibular nucleus
NL	Nucleus of lateral lemniscus
NR	Nucleus reticularis tegmenti pontis
NVI	Nucleus of VIth cranial nerve
NVII	Nucleus of VIIth cranial nerve
P	Pyramidal tract

PN	Pontine nuclei
PSNV	Principal sensory nucleus of the Vth cranial nerve
PVN	Posteroventral cochlear nucleus
RB	Restiform body
RF	Reticular formation
RGC	Nucleus reticularis gigantocellularis
RP	Nucleus reticularis parvocellularis
RPC	Nucleus reticularis pontis caudalis
SNV	Spinal nucleus of Vth cranial nerve
STV	Spinal tract of Vth cranial nerve
SVN	Superior vestibular nucleus
TB	Trapezoid body
V	Vth cranial nerve
VAL	Latero-ventral cochlear nucleus
VAM	Medio-ventral cochlear nucleus
VM	Motor nucleus of Vth cranial nerve
VMC	Mesencephalic nucleus of Vth ranial nerve
VNLL	Ventral nucleus of lateral lemniscus
VII	VIIth cranial nerve
VIIIA	Cochlear division of VIIIth cranial nerve
VIIIV	Vestibular division of VIIIth cranial nerve