SOME OBSERVATIONS ON TRANSNEURONAL CELL DEGENERATION IN THE OLFACTORY BULB OF THE RABBIT

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INTRODUCTION

The degeneration, or atrophy, which may ensue in neurons deprived of their afferent connexions was first convincingly described in the lateral geniculate nucleus, after interruption of optic nerve fibres (Minkowski, 1913), and for many years this remained the only site in which it had been demonstrated unequivocally. It seemed to depend upon the completeness of the deafferentation. For example, Cook, Walker & Barr (1951) failed to find transneuronal degeneration in the spinal cord after section either of dorsal roots or of cortico-spinal fibres, although it had been claimed to occur by earlier workers (Barron, 1933); and Glees & Le Gros Clark (1941) noted that it did not occur in the ventral posterior nucleus of the thalamus following section of the medial fillet.

These and other studies were based upon the qualitative examination of Nissl-stained sections. An important contribution was made by the careful quantitative study of Cook *et. al* (1951) of the cytological changes produced by transneuronal atrophy in the lateral geniculate nuclei of the cat and rabbit. They showed the changes in these species to be rather slow and slight, though reasonably consistent. Following this work, closely similar transneuronal degeneration has been shown to occur both in the superior cervical ganglion of the rabbit (Hamlyn, 1945) and in the cochlear nuclei of the cat (Powell & Erulkar, unpublished observations). In each of these regions it is possible to achieve more or less complete destruction of afferents. The populations of neurons are relatively homogenous. They are therefore suitable for study by quantitative methods, which are a valuable aid to the confirmation of slight changes.

Transneuronal degeneration has now, however, been found to occur in situations in which the destruction of afferents is by no means complete, and this positive finding also may be attributed to the choice of technique or of preparation. The experiments already mentioned were made upon mature animals. Torvik (1956), using very young kittens, found rapid and severe cell degeneration in the pontine and olivary nuclei following unilateral lesions in the cortex or brain-stem which he considered could not have destroyed more than half the afferent connexions. Le Gros Clark (1957) has recorded that, as seen in sections prepared by the protargol technique, the principal dendrites of mitral cells in the olfactory bulb of the rabbit degenerate following destruction of the olfactory mucosa. This phenomenon seems of particular interest, since the structure of the mammalian olfactory bulb has been worked out in considerable detail by Cajal (1911) and the principal dendrite is known to be the only part of the mitral cell which is directly related to the incoming olfactory nerve

fibres, while the accessory dendrites and cell body are exposed to other sources of afferent stimulation. A fuller study has therefore been made of the changes in the olfactory bulb of the rabbit after extensive lesions of the olfactory mucosa, and the results are reported in this paper.

MATERIALS AND METHODS

The observations were made upon thirty-two 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 & Warwick, 1946). Pentobarbitone anaesthesia (without supplementary ether) was used for all animals.

In eighteen 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 to 200 days, and were then perfused, under pentobarbitone anaesthesia, through the ascending aorta with normal saline followed by 10 % formol-saline. In fifteen of these animals the brain was then removed from the cranial cavity and immersed in formol-saline; in the remaining three 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 fifteen animals had two disadvantages. First, 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 ten 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 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 deafferentation 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 anaesthetized 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-sectional 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 five 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 arbitrary units.

A complication arising in relation to survival periods of 2 months or more was that seven 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 twenty-one brains of those animals in which this condition did not occur; six of the 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.

RESULTS

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 (Pl. 1, fig. 1).

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 mid-way 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

Table 1. *Measurements of area of cross-section of various layers of the olfactory bulbs (mid-point of bulb) and of the olfactory tracts (five corresponding levels, first* 6 *mm. behind bulb) in one rabbit,* O14, 130 *days after unilateral destruction of olfactory epithelium*

Region measured	Area of cross-section (approximate)	
	Unoperated side	Side of operation
	arbitrary units	
Glomerular layer	664	367
Outer plexiform layer	950	536
Granule cell layer / Internal plexiform layer	1368	872
Periventricular layer	317	321
Olfactory ventricle	86	76
Olfactory tract	μ^2	
Level 1 (nearest bulb)	595	568
Level 2	570	533
Level 3	461	426
Level 4	427	360
Level 5	414	280

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 dendrites of mitral and tufted cells and by processes of the numerous periglomerular 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 peri-glomerular 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 (Pl. 1, figs. 2, 3).

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.

Table 2. *Mean areas of cell body for group of 50 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)				Shrinkage (%)	Significance by *t*-test (%)
			Normal	S.E.	Degenerate	S.E.		
O8	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
O14	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

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 (Pl. 1, figs. 2, 3). The tufted cells, gathered mostly in the outer part of the layer (but some are placed more deeply within it and others just outside it among the periglomerular cells), are smaller than normal and have rather paler cytoplasm, with their dendrites much less often filled with Nissl substance, 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 decreased 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 (Pl. 2, figs. 6, 7). Its texture is lighter than normal, and the constituent fibres, including the main dendritic stems, tend to be finer. The main dendrites of the mitral cells, which cross the layer to reach the glomeruli, do not obviously pursue a more tortuous 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.

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 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 (Pl. 1, figs. 2, 3). 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. This 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 periglomerular cells, and in the nuclei of the granule cells.

The olfactory tract. This 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 (Pl. 2, figs. 8, 9). 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 & Meyer, 1957); 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 no longer be recognized: 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 (Pl. 1, figs. 4, 5). 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 five other experiments in which a long survival period was used (96–200 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 be separately 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 these 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 sufficiently 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 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 periglomerular cells are to be seen, with their dendrites and intraglomerular arborizations (Pl. 3, figs. 10, 11). 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 neither intraglomerular arborizations are to be seen, nor any of the neurons which form them (Pl. 3, figs. 10, 12). 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 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.

DISCUSSION

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 neurons (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 & 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; secondly, the peripheral processes of granule cells, the basal dendrites of which are thought to receive commissural and centrifugal impulses; thirdly, any centrifugal or commissural fibres which may pass directly into the outer plexiform layer (Le Gros Clark & Meyer, 1947; Allison, 1953);

fourthly, and perhaps important, the electrical fields produced by activity in other dendrites. Some of these influences might be inhibitory (cf. Kerr & 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 600 μ 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 commissural and centrifugal fibres. The changes in the granule cells were of later onset than those in the 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 difficult 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 the 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 & 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.

SUMMARY

1. A study has been made of transneuronal cell degeneration in the main and accessory olfactory bulbs of the rabbit at periods varying from 6 to 200 days after destruction of the olfactory mucosa.

2. All layers of the bulb, except the periventricular layer, show severe shrinkage, and the periglomerular, tufted mitral and granule cells undergo transneuronal atrophy.

3. Sections stained with Bodian's protargol method show that the dendrites of the mitral and tufted cells atrophy and that there is loss of fine fibres in the outer part of the molecular layer subjacent to the lateral olfactory tract.

4. All these changes begin before the end of one month after denervation.

5. Golgi–Cox preparations show that periglomerular, tufted and mitral cells which are undergoing transneuronal degeneration are more resistant to impregnation by this method.

6. The epithelium of the vomero-nasal organ has been found not to be involved by atrophic rhinitis.

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LIST OF ABBREVIATIONS

D	Dendritic arborization within glomerulus.
G	Glomerulus.
Gr	Granule cell layer.
M	Mitral cell layer.
Mo	Molecular layer of pyriform cortex; (*a*) outer half, (*b*) inner half.
N	Neuroglial cells.
O	Olfactory nerve layer of olfactory bulb.
OP	Outer plexiform layer of olfactory bulb.
OPed	Lateral olfactory tract.
P	Periglomerular cells.
T	Tufted cells.
VN	Vomero-nasal fibre layer of accessory olfactory bulb.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Photomicrograph showing the degree of shrinkage of the olfactory bulb 130 days after removal of the olfactory mucosa. Stained with thionin. ×18.

Figs. 2, 3. Photomicrographs to show shrinkage of the periglomerular, tufted, mitral and granule cells 130 days after removal of the olfactory mucosa (fig. 2) as compared with the normal side (fig. 3). Stained with thionin. ×75.

Figs. 4, 5. Photomicrographs of sagittal sections of the normal (fig. 4) and atrophied (fig. 5) accessory olfactory bulbs 96 days after destruction of the vomero-nasal nerve. Stained with thionin. ×42.

Plate 2

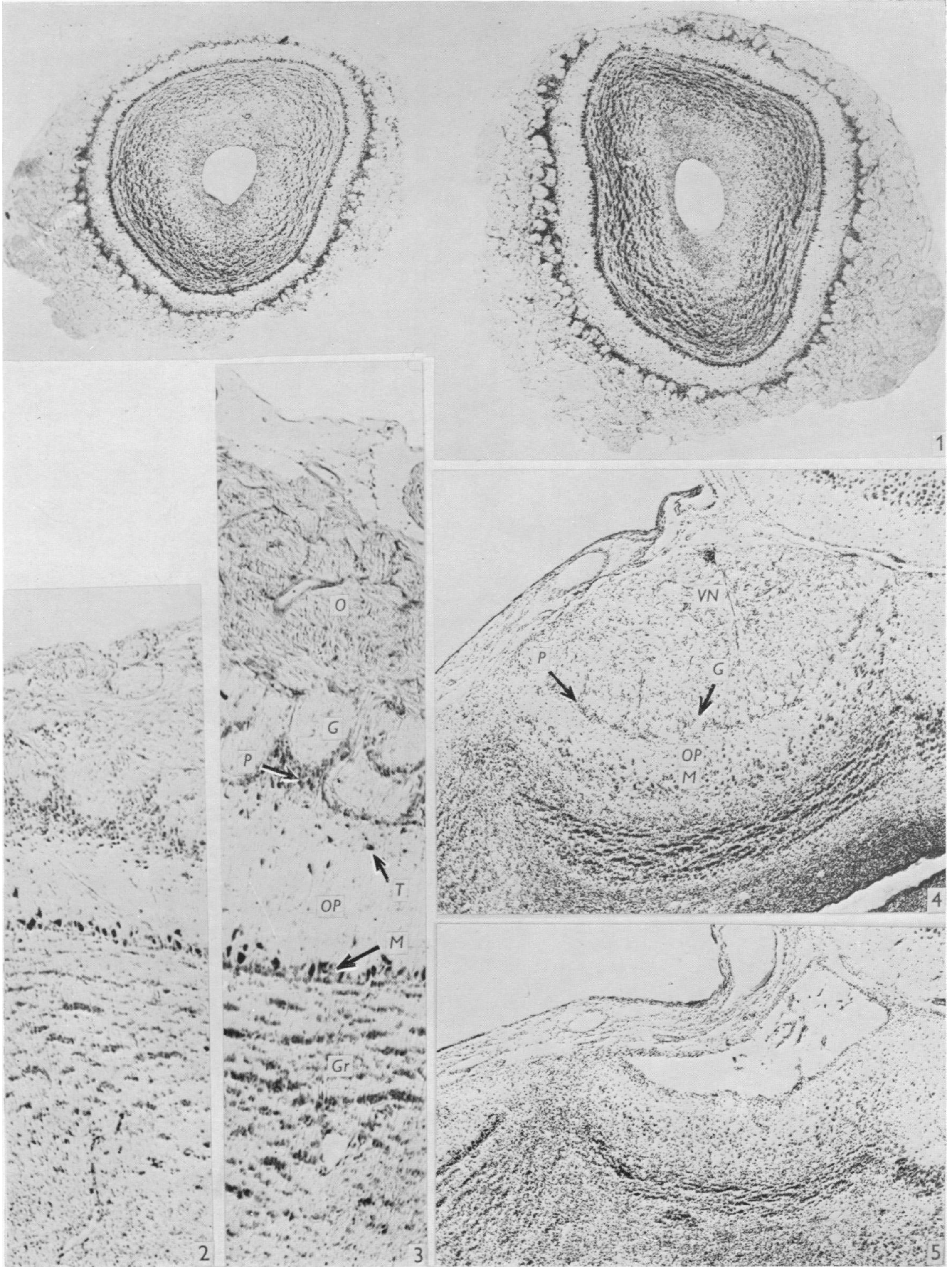
Figs. 6, 7. Photomicrographs to show the atrophy of the dendrites of the mitral and tufted cells in the glomeruli and outer plexiform layer 152 days after destruction of the olfactory mucosa (fig. 6) as compared with the normal side (fig. 7). Bodian's protargol method. ×185.

Figs. 8, 9. Photomicrographs to show the olfactory tract and the subjacent molecular layer on the normal (fig. 9) and the operated side (fig. 8) 49 days after destruction of the olfactory mucosa. The olfactory tracts (above) show no change, but in the outer part of the molecular layer on the operated side a marked loss of fine fibres has occurred. Bodian's protargol method. × 124.

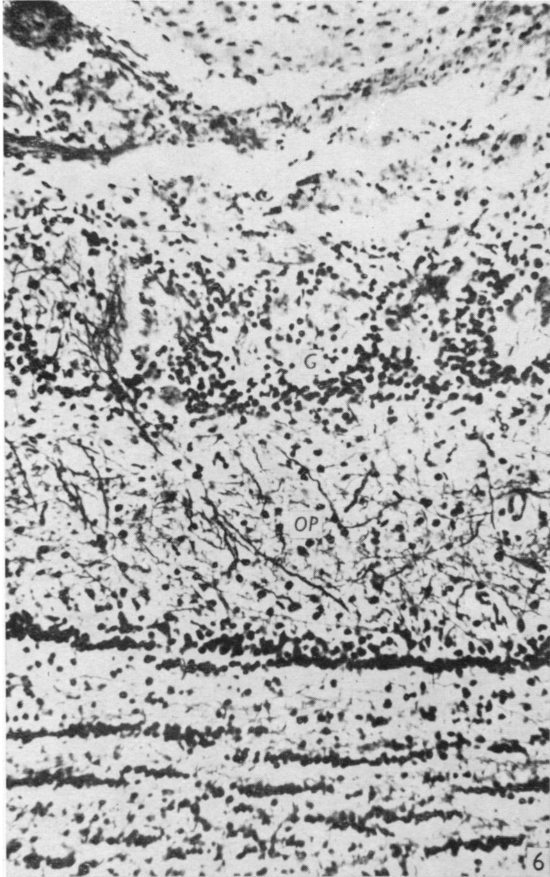
PLATE 3

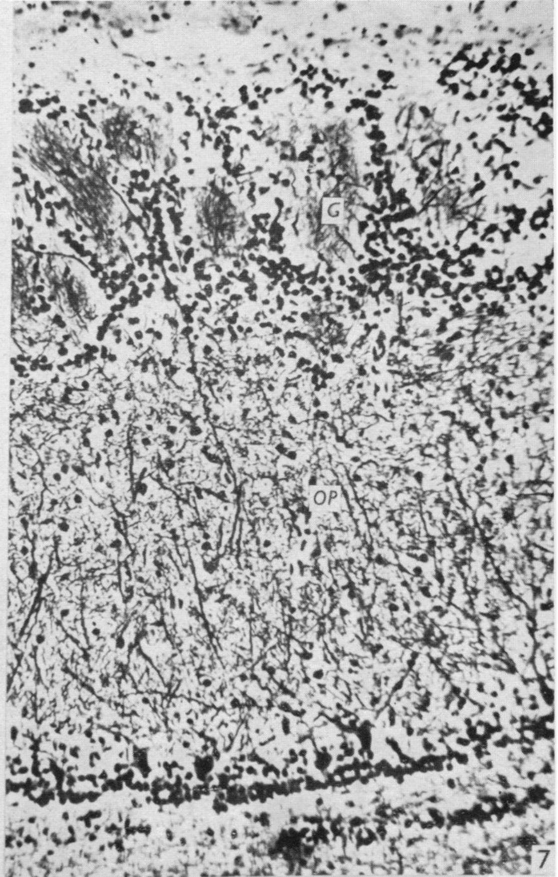
Fig. 10. Photomicrographs showing the normal and atrophied bulbs 96 days after destruction of the olfactory mucosa. Golgi–Cox method. × 16.

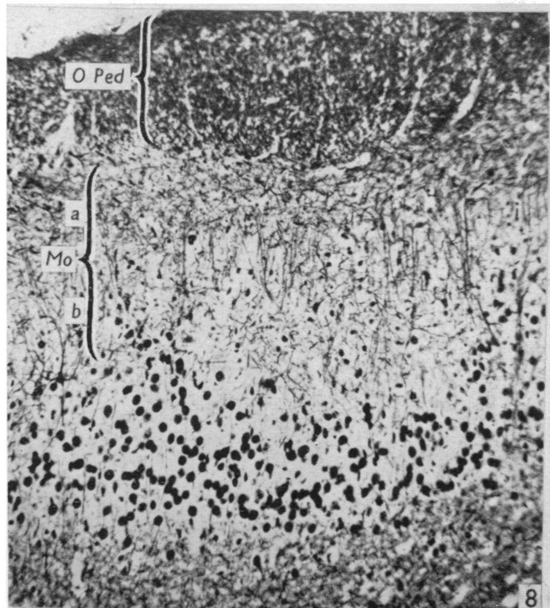
Figs. 11, 12. Photomicrograph to show the impregnation of the mitral, tufted and periglomerular cells on the normal side (fig. 11), and the corresponding area of the degenerate bulb (fig. 12). Golgi–Cox method. × 66.

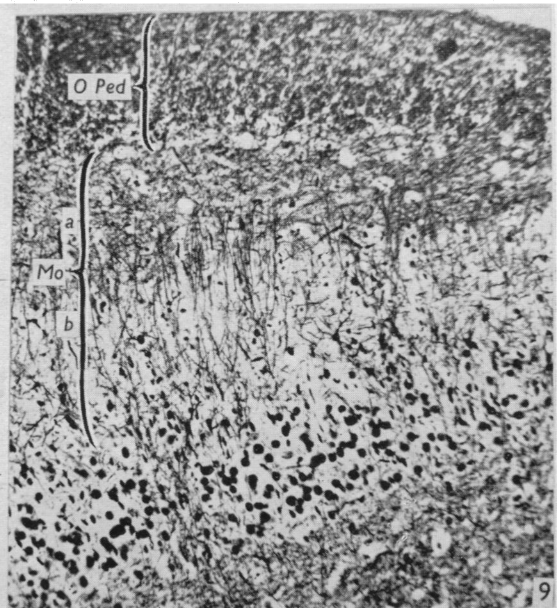


MATTHEWS AND POWELL—TRANSNEURONAL CELL DEGENERATION IN OLFACTORY BULB OF RABBIT









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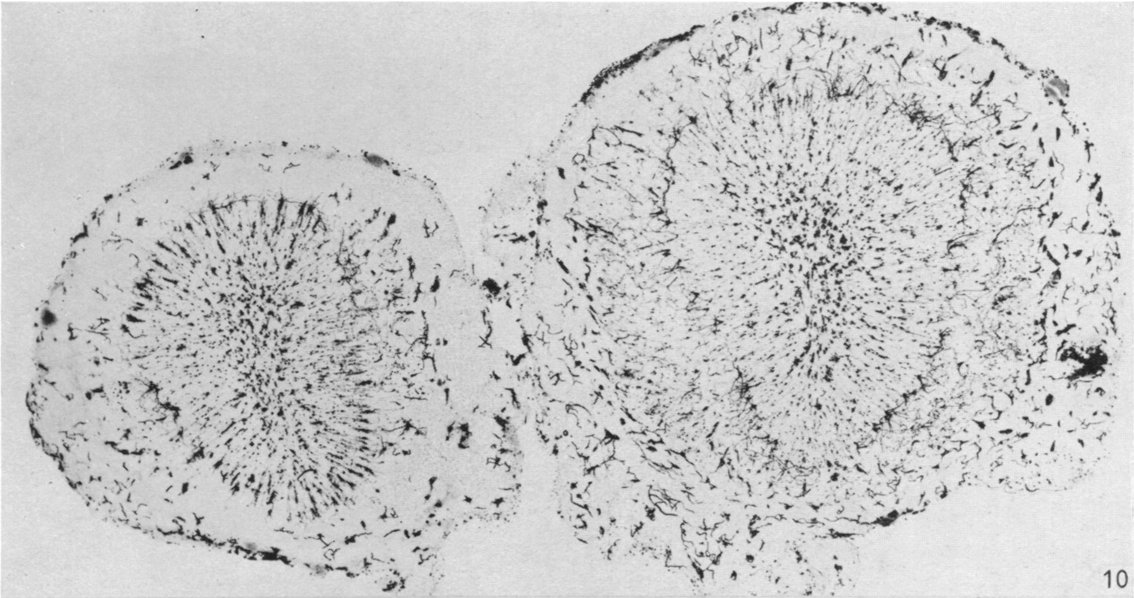
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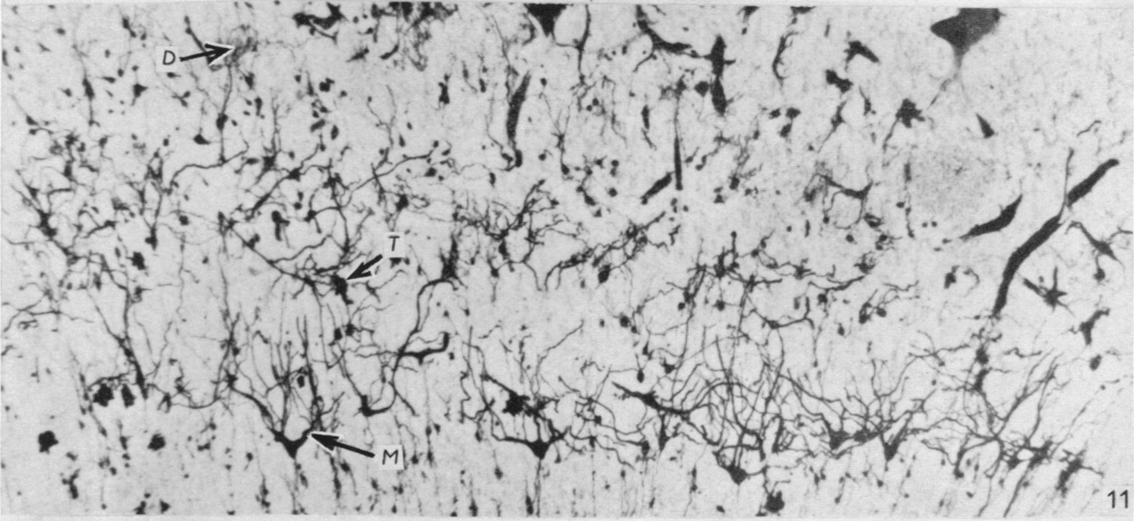
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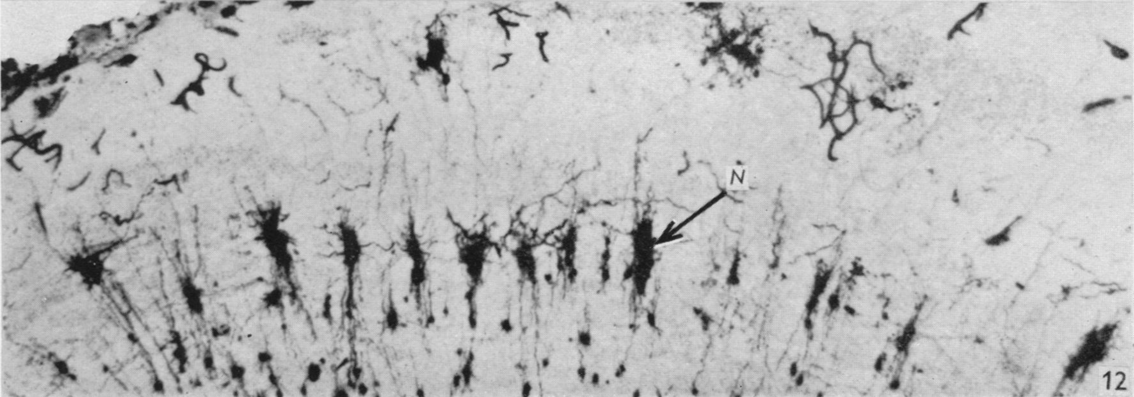
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