J. Anat., Lond. (1962), **96**, 2, pp. 269–284
With 3 plates and 4 text-figures
Printed in Great Britain

AN EXPERIMENTAL STUDY OF THE PROJECTION OF THE COCHLEA

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INTRODUCTION

In a previous study of transneuronal cell degeneration in the auditory relay nuclei following destruction of the cochlea (Powell & Erulkar, 1962) one of the most significant findings was the occurrence of distinct changes in the cells of the lateral superior olive, the medial trapezoid and the so-called pre-olivary nuclei. These nuclei are commonly considered to be composed of third-order neurons (Barnes, Magoun & Ranson, 1943; Stotler, 1953). As was pointed out in the discussion of these findings, the severity and time-course of the degenerative changes so closely paralleled those in the ventral cochlear nucleus that two alternative interpretations of their cause had to be considered. The first possibility was that these changes were 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 could not definitely be excluded; such a connexion was suggested by Held (1893) and Cajal (1909) and experimental evidence for a projection to the medial trapezoid nucleus has been presented by Lewy & Kobrak (1936).

Another finding discussed in the previous paper 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 were considered in that paper, only the conflicting evidence for a direct projection of primary auditory nerve fibres to this nucleus is relevant to the present study. All the earlier workers (cf. Cajal, 1909) agree, from the study of both normal and experimental material, that the dorsal cochlear nucleus receives such primary fibres, and Lorente de Nó (1933*a*, *b*) and Lewy & Kobrak (1936) have confirmed this connexion. On the other hand, it has been questioned 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 & 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 has 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 study of the organization of this projection is outside the scope of the present study, but the finding of localized degeneration in all three cochlear nuclei after partial lesions of the cochlea indicates that this problem in amenable to study with the techniques used.

MATERIAL AND METHODS

In eleven cats and two rabbits lesions were made in the cochlea on one side through an opening in the tympanic bulla. After periods ranging from 12 hr. to 15 days the animals were anaesthetized with Nembutal and perfused through the ascending aorta with saline followed by 10% formol-saline. After further fixation in formol-saline the brains of the rabbits and all but two of the cats were embedded in paraffin wax and sections of 15 μ thickness were cut in the coronal plane. From each of these brains four 1 in 10 series through the pons and medulla were mounted and stained with thionin, the Nauta & Gygax (1951) method, the Marsland, Glees & Erickson method (1954) or the Bodian method (1936). The brains of the remaining two cats were sectioned on a freezing microtome at 25 μ and every tenth section stained by the Chambers, Liu & Liu (1956) modification of the Nauta & Gygax technique (1954) and every eleventh section with the Glees method (1946).

The brains of two cats from a previous series (Powell & Erulkar, 1962) were stained by the Bodian method. In one of these animals the cochlea had been destroyed 359 days before death, and in the other both the cochlea and cochlear nuclei had been injured for 60 days.

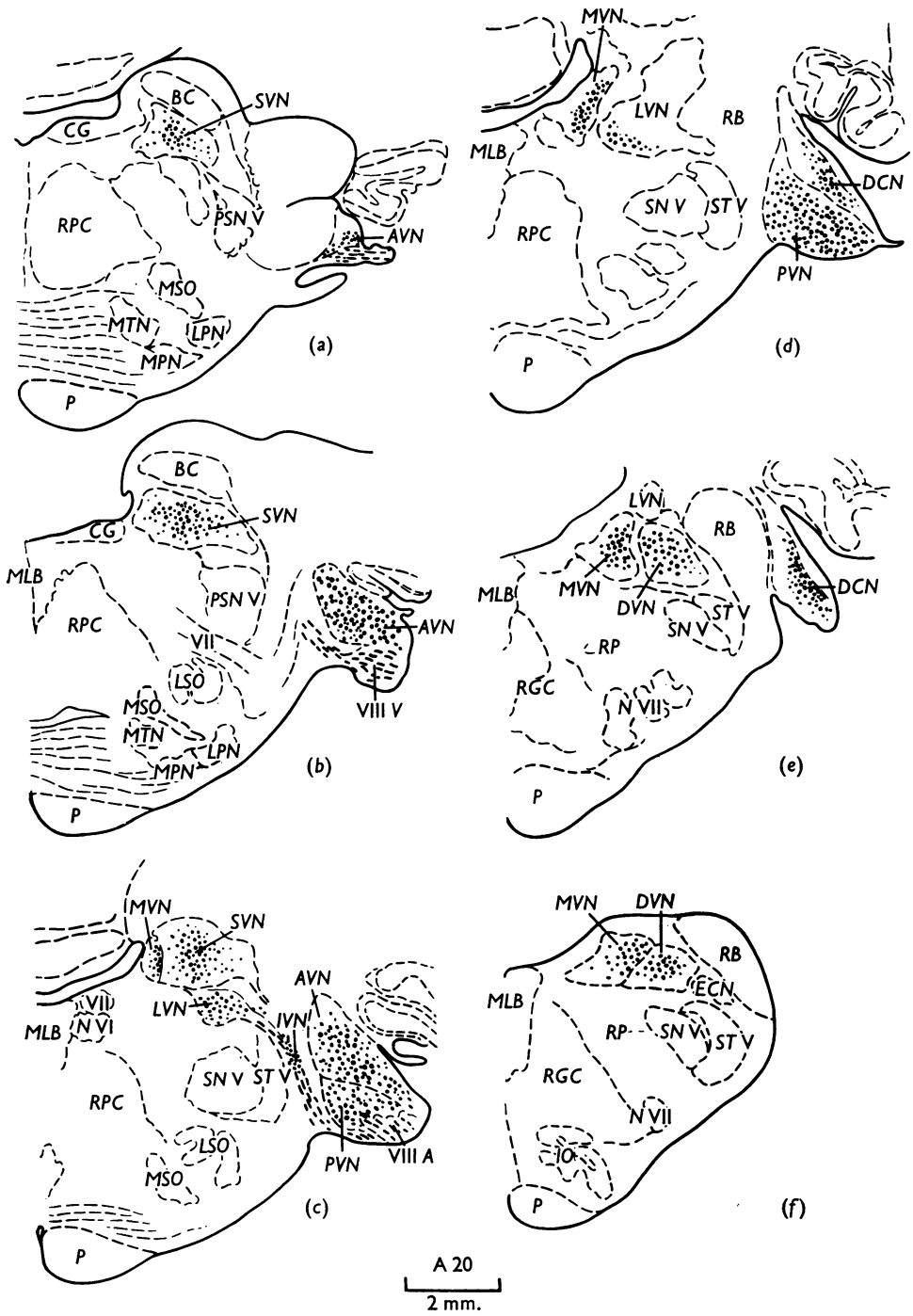
The temporal bones of some of the cats used in these experiments were decalcified and embedded in paraffin wax. The blocks were cut in the horizontal plane and series of sections were stained with haematoxylin and eosin.

RESULTS

No account of the normal morphology of the auditory relay nuclei will be given as they have been briefly described in the previous paper (Powell & Erulkar, 1962). The position of these nuclei in successive transverse sections of the brain stem is illustrated in Text-fig. 1.

Projection of the primary auditory fibres. The first experiment to be described, A21, serves to demonstrate virtually the total projection of the cochlea upon the brain stem. This animal was allowed to survive for 7 days after extensive 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.

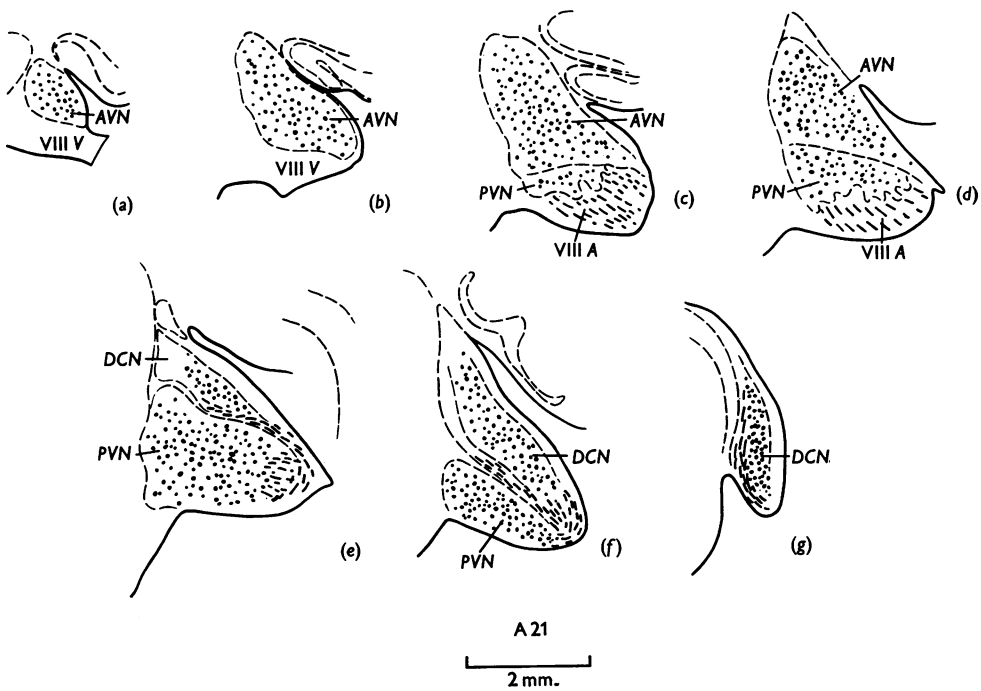
The Nauta preparations of the brain show that, at its level of entry into the brain stem, the cochlear division of the VIIIth nerve has almost completely degenerated. Against a clear background the fibres are seen to be severely fragmented and in regular rows of intensely stained droplets. Only an occasional 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



Text-fig. 1. The distribution of degenerating fibres (indicated by dashes) and terminal degeneration (dots) in outlines of successive rostrocaudal sections of the brain stem in cat A 20, in which both the cochlear and vestibular divisions of the VIIIth cranial nerve were destroyed.

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.

In the antero-ventral cochlear nucleus severe preterminal degeneration is seen in the form of fine droplets arranged in clusters around the cells. The most rostral and medial parts of the nucleus contain less degeneration, probably due to the fact that the cochlea was not completely destroyed. The appearance of the postero-ventral nucleus is essentially the same, apart from the somewhat greater density of



Text-fig. 2. 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 (Cat A 21, 7-day survival, Nauta technique on frozen sections).

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 postero-ventral 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 (Pl. 3, figs. 22, 23).

Careful examination of serial sections from this brain has shown that after an almost complete destruction of the cochlea, fibre and preterminal degeneration is confined to the cochlear nuclei. In particular it should be emphasized that no degenerating fibres are seen passing to the medial trapezoid nucleus or the superior olivary complex.

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 antero-ventral nucleus of the normal side (cf. Pl. 3, figs. 20, 21) 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 in the neuropil of the cochlear nuclei at this early stage, so that in the affected areas the dendrites of the cells stand out conspicuously.

Two further examples of almost complete destruction of the cochlea without involvement of the vestibular division of the VIIIth nerve are cat A 25 and rabbit R 98. The findings in cat A 25 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 & Gygax (1951) method as applied to paraffin sections, the fibre degeneration has proceeded to distinct droplet formation and the endings of Held in the antero-ventral nucleus are distinctly swollen and fragmented. In the postero-ventral 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 distribution of the degeneration in rabbit R 98 is precisely the same and it need only be emphasized that there is no bundle of degenerating fibres passing to the medial trapezoid nucleus comparable to that described by Lewy & Kobrak (1936).

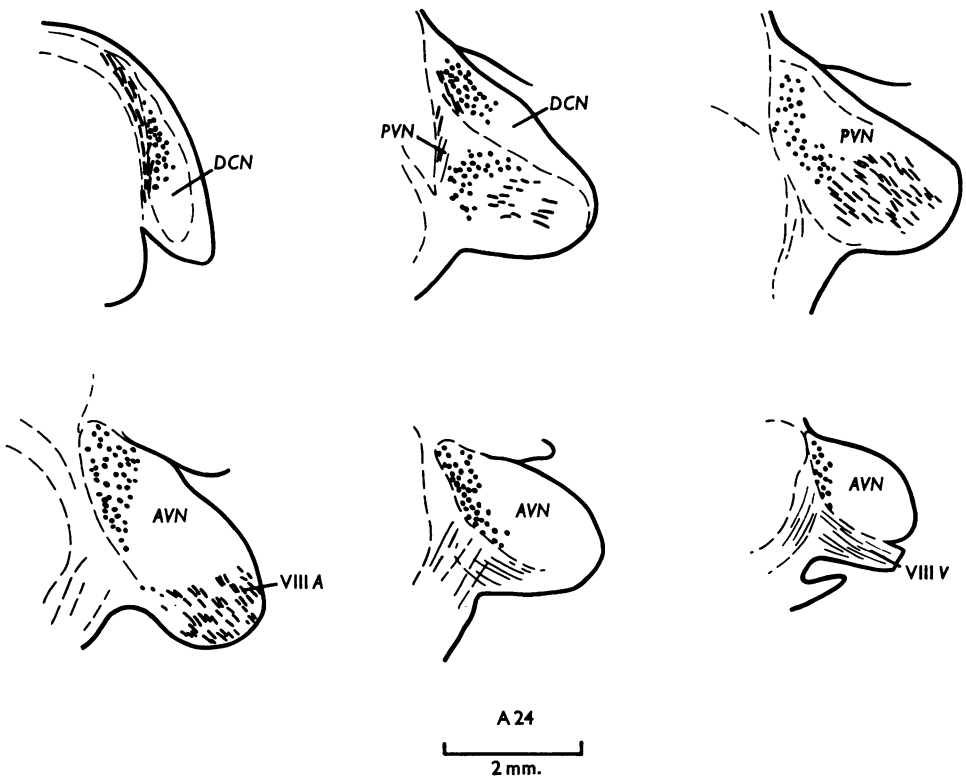
The findings in the present material from experiments with short survival periods, are confirmed by a study of Bodian preparations of the brain of a cat which had been allowed to survive for 359 days after destruction of the cochlea. The cellular changes in this brain have been described in the previous paper (Powell & Erulkar, 1962). 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 antero-ventral nucleus and there is a marked decrease in the number of boutons in the postero-ventral division. Secondly, the dendritic processes of the cells, particularly in the postero-ventral 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 (Powell & Erulkar, 1962), but there is no apparent reduction of the afferent fibres or of the intrinsic fibre plexus. Although it is difficult to 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 cat 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-fig. 3. 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 postero-ventral 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 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 mid-line 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 pre-olivary 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



Text-fig. 3. The distribution of fibre and terminal degeneration in experiment cat A 24 in which there was a partial lesion of the cochlea involving only the basal coil.

from the trapezoid body to terminate on the cells of this nucleus. None of the characteristic endings of Held (Pl. 3, fig. 19) are preserved on this side (Pl. 3, figs. 17, 18). In the medial superior olive changes are seen on both sides: on the operated side the 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 superior olive the medial half of the nucleus shows equivalent changes following the deafferentation of the medially directed dendrites. No appreciable change is seen in the lateral lemniscal nuclei of the two sides or in the inferior colliculi.

In one cat, A 20, with a survival period of 5 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 & 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 (Pl. 2, figs. 9–14). The degeneration in the vestibular nerve can be clearly traced dorso-medially 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 (Pl. 2, figs. 15, 16). The distribution of the terminal degeneration in the vestibular nuclei is shown in Text-fig. 1 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 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 paper (Powell & Erulkar, 1962), 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. However, this observation should not be interpreted as being incompatible with Rasmussen's description of the origin of this bundle.

A study of the appearance of the terminal degeneration after relatively short survival periods was considered essential after examination of Marsland *et al.* (1954) and Bodian (1936) 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 Marsland *et al.* 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 6-week-old kittens were operated upon and allowed to survive for 12, 24, 48 and 90 hr., respectively. At 12 and 24 hr. no changes were seen in either the auditory nerve or the cochlear nuclei with any of the techniques used. At 48 hr., 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, cat 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 may be 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 (Pl. 1, figs. 5, 6). 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 antero-ventral nucleus are swollen and irregular while the boutons in the postero-ventral 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 Marsland *et al.* preparations, but the appearance of the coarse auditory nerve fibres is unusual and it has not (as far as we are aware) been recognized in the more recent literature on fibre degeneration. At its entry into the brain stem advanced fibre degeneration is seen amongst the incoming auditory nerve fibres in the form of large, ring-like structures about the size of small neurons, arranged in regular rows between the normal fibres from uninjured parts of the cochlea (Pl. 1, figs. 1, 2). 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 intensely 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 auditory nerve fibres present an essentially similar but less striking appearance, many more of the rings having a solid structure; the appearance of the preterminal degeneration in these preparations is shown in Pl. 1, figs. 7 and 8. 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 Pl. 1, figs. 3, 4).

DISCUSSION

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 of the cell bodies were seen in the characteristic spindle-cell layer of this nucleus in the previous study (Powell & Erulkar, 1962), 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 & 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) of 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 (Powell & Erulkar, 1962). 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 Lorente de Nó (1933*a*, *b*) but the distribution of the terminal degeneration after partial and complete lesions of the cochlea clearly indicates that each part of the cochlea is represented in all three components of the cochlear nuclear complex (i.e. the dorsal, antero-ventral and postero-ventral 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 antero-ventral nucleus 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 fibre to the dorsal and ventral cochlear nuclei. The fibres to both divisions of the ventral cochlear nucleus are relatively coarse while the portion of the descending branch which passes to the dorsal cochlear nucleus is distinctly finer.

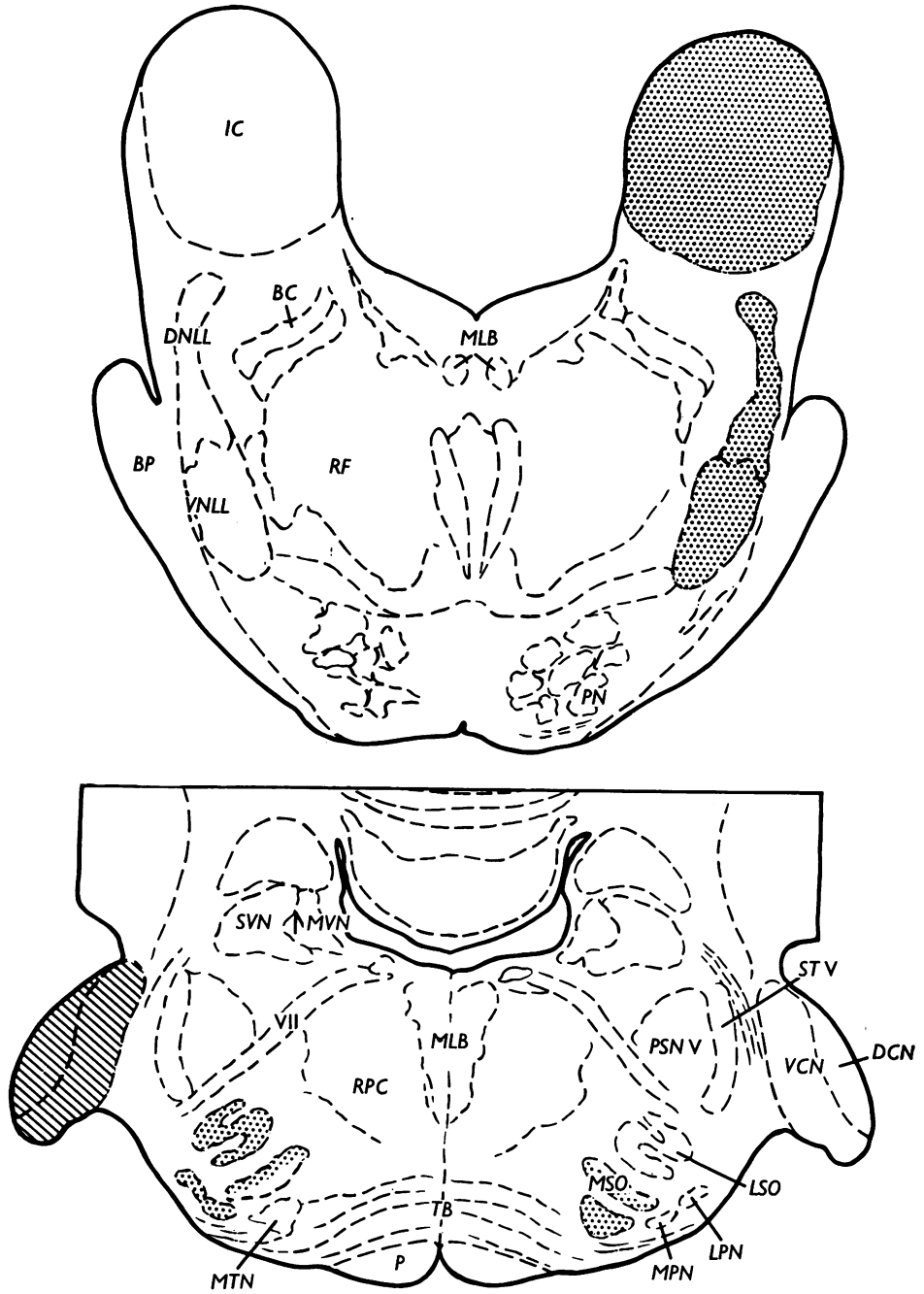
Although a systematic study of the organization of the projection of the cochlea upon the three nuclei of the primary relay group has not been made the findings in experiment A 24 illustrate two points. First, they confirm the description of Lewy &

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 method were used on the same material). An experimental study of this type would provide an anatomical basis for the tonotopic localization which has been demonstrated by Rose *et al.* (1959) in their single unit study of the cochlear nuclei.

The findings in the Bodian sections of the animal in which the cochlear nuclei had been involved as well as the cochlea itself, fully confirm Stotler's description (1953) of the projection of the second-order neurons as far as the nucleus of the lateral lemniscus. However, the material is not suitable for the study of the projection to higher levels of the auditory pathway because the loss of a small proportion of fibres in a composite tract like the lateral lemniscus, or in a site such as the inferior colliculus where afferents from many sources converge, cannot be determined with this method. The Bodian and Nissl sections together show that the cochlear nuclei project to the two pre-olivary 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. In addition to these projections from the cochlear nuclei Woollard & Harpman (1940) and Barnes *et al.* (1943) have shown that fibres pass to the inferior colliculi.

The sites of projection of the first- and second-order neurons of the auditory pathway are summarized in Text-fig. 4. This is constructed from the results of the present study, from those of the previous investigation on transneuronal degeneration, and from those of Barnes *et al.* (1943) and Stotler (1953). In view of the absence of any evidence on the different pathways and sites of termination of fibres arising in the three primary relay nuclei (dorsal cochlear nucleus, antero-ventral and postero-ventral nuclei), and because of the numerous discrepancies in the literature regarding the composition of the striae, the precise pathway of the fibres from the three nuclei cannot be reconstructed. From the evidence on the organization of the projection of the cochlea upon the primary nuclei, and the different synaptic arrangement in the primary and secondary relay nuclei, it is clear that further knowledge of the details of the organization of the afferent fibres to the secondary (and subsequent) relay nuclei would be of considerable interest.

It is not generally recognized that the original Nauta & Gygax (1951) method can be used successfully on paraffin material. In the discussion of their original paper these authors stated that their technique yielded unsatisfactory results when used on paraffin sections, but in a recent paper Guillery, Shirra & Webster (1961) presented a modification of this technique which in their hands has worked well on paraffin-embedded material. As it seemed unlikely that the minor modifications introduced in this latter technique were responsible for the successful impregnation, and since we had found in the avian visual pathway that the original Nauta & Gygax (1951) method gave essentially similar results to sections stained according to their method (Cowan, Adamson & Powell, 1961) in this study, we have compared the two methods in the mammalian brain stem. In the cochlear nuclei adjacent



Text-fig. 4. A diagrammatic representation of the primary and secondary auditory relay nuclei based on the findings of the present study and those of Barnes *et al.* (1943), and of Stotler (1953). The primary relay nuclei for fibres from the spiral ganglion of the left cochlea are indicated by cross-hatching; the secondary relay nuclei by coarse stippling.

sections stained according to the two methods have consistently given indistinguishable results. The apparent discrepancy between the findings of Nauta & Gygax (1951) on the one hand, and Guillery *et al.* (1961) and ourselves on the other, may be resolved, by further experience on an extended series of observations of the use of these techniques in paraffin-embedded material.

The principal advantage of this technique over other 'on-the-slide' methods is that the degenerating fibres are more conspicuous as they are seen against a more lightly impregnated background, but in the brain stem the degree of suppression is not at all comparable with that seen in the cerebral hemisphere or with frozen sections stained by the Nauta method. Like the Glees and Bodian preparations, however, it has one important advantage over the frozen Nauta method in that it shows degeneration not only in the preterminal axons but also in the terminal endings and boutons.

The appearance of profound degeneration in the fibres of the auditory nerve of the cat after only 48 hr. was an unexpected feature of this study, for we have not seen in any other material or in recent reports degenerative changes of this type. At first sight the grossly swollen, ring-like structures found in regular rows in the nerve appear reminiscent of fat-engorged microglia, but in the absence of any evidence of an early gliosis in the Nissl-stained preparations this possibility may be excluded. The most likely explanation is that these represent enlarged axonal bulbs comparable to those described by Cajal (1928) after lesions in the central nervous system and so clearly depicted in figure 272 of his monograph. It should, however, be pointed out that we have not been able to identify short segments of axons attached to the rings as Cajal describes after shorter survival periods, but despite this the identification of these structures as 'terminal spheres' is hardly questionable.

SUMMARY

1. The projection of the cochlea upon the nuclei of the brain stem of the cat and rabbit has been investigated using the Nauta, Glees and Bodian methods on frozen and paraffin sections.

2. The primary auditory nerve fibres have been found to terminate in the antero-ventral, postero-ventral and dorsal cochlear nuclei. No fibres were seen ending in the superior olivary or medial trapezoid nuclei.

3. Evidence is presented to illustrate the topical organization which is present in this projection; the basal coil of the cochlea is related to the medial parts of all three cochlear nuclei.

4. In one experiment, in which the cochlear nuclei were destroyed, it was found that these nuclei project to the two pre-olivary nuclei and 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.

This work was supported by a grant from the Medical Research Council.

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ABBREVIATIONS

AVN	Antero-ventral cochlear nucleus	*P*	Pyramidal tract
BC	Brachium conjunctivum	*PN*	Pontine nuclei
BP	Brachium pontis	*PSN* V	Principal sensory nucleus of Vth cranial nerve
CG	Central gray		
DCN	Dorsal cochlear nucleus	*PVN*	Postero-ventral cochlear nucleus
DNLL	Dorsal nucleus of lateral leminiscus	*RB*	Restiform body
DVN	Descending vestibular nucleus	*RF*	Reticular formation
ECN	External cuneate nucleus	*RGC*	Nucleus reticularis gigantocellularis
IC	Inferior colliculus	*RP*	Nucleus reticularis parvocellularis
IO	Inferior olive	*RPC*	Nucleus reticularis pontis caudalis
IVN	Interstitial nucleus of the vestibular nerve	*SN* V	Spinal nucleus of Vth cranial nerve
		ST V	Spinal tract of Vth cranial nerve
LPN	Lateral pre-olivary nucleus	*SVN*	Superior vestibular nucleus
LSO	Lateral superior olive	*TB*	Trapezoid body
LVN	Lateral vestibular nucleus	*VCN*	Ventral cochlear nucleus
MLB	Medial longitudinal bundle	*VNLL*	Ventral nucleus of lateral lemniscus
MPN	Medial pre-olivary nucleus	VII	VIIth cranial nerve
MSO	Medial superior olive	VIII A	Cochlear division of VIIIth cranial nerve
MTN	Medial trapezoid nucleus		
MVN	Medial vestibular nucleus	VIII *V*	Vestibular division of VIIIth cranial nerve
N VI	Nucleus of VIth cranial nerve		
N VII	Nucleus of VIIth cranial nerve		

EXPLANATION OF PLATES

(All the photomicrographs have been prepared from the cat material.)

PLATE 1

Fig. 1. Normal fibres in the cochlear division of the VIIIth cranial nerve as seen in a paraffin section stained according to the Marsland *et al.* technique. ×490.

Fig. 2. Appearance of degenerating axons in the VIIIth cranial nerve 48 hr. after destruction of the cochlea, experiment A 29; Marsland *et al.* method. ×490.

Figs. 3, 4. Nauta preparations of the posterior part of the ventral cochlear nucleus on the normal side (Fig. 3) and on the side of the cochlear lesion in experiment A 30 (2-day survival; frozen sections). ×490.

Fig. 5. The normal fibre plexus in the ventral cochlear nucleus (posterior part) as seen in a Bodian preparation. ×490.

Fig. 6. Photomicrograph to show the severe break-up of the fibre plexus in the postero-ventral cochlear nucleus 2 days after a lesion of the cochlea (experiment A 29). Bodian preparation from the same level as Fig. 5. ×490.

Fig. 7. The fibre plexus of the ventral cochlear nucleus in a paraffin section stained according to the Nauta & Gygax (1951) method. ×490.

Fig. 8. Fine preterminal degeneration around the cells of the postero-ventral cochlear nucleus 48 hr. after destruction of the cochlea; Nauta paraffin preparation. ×490.

PLATE 2

Figs. 9, 11, 13. Photomicrographs showing the appearance of the normal fibre plexus in the antero-ventral nucleus (Fig. 9), the postero-ventral nucleus (Fig. 11) and the deep polymorph layer of the dorsal cochlear nucleus (Fig. 13) in experiment A 20. Paraffin sections stained with the Nauta & Gygax (1951) method. ×490.

Figs. 10, 12, 14. To show the break-up of fibres in the cochlear nuclei in regions corresponding to those shown in Figs. 9, 11 and 13 on the side of the cochlear lesion. Experiment A 20, 5-day survival.

Figs. 15, 16. The appearance of the fibre plexus in the interstitial nucleus of the vestibular nerve of the normal (Fig. 15) and operated sides (Fig. 16) in experiment A 20; 5-day survival. Paraffin Nauta preparation. ×490.

PLATE 3

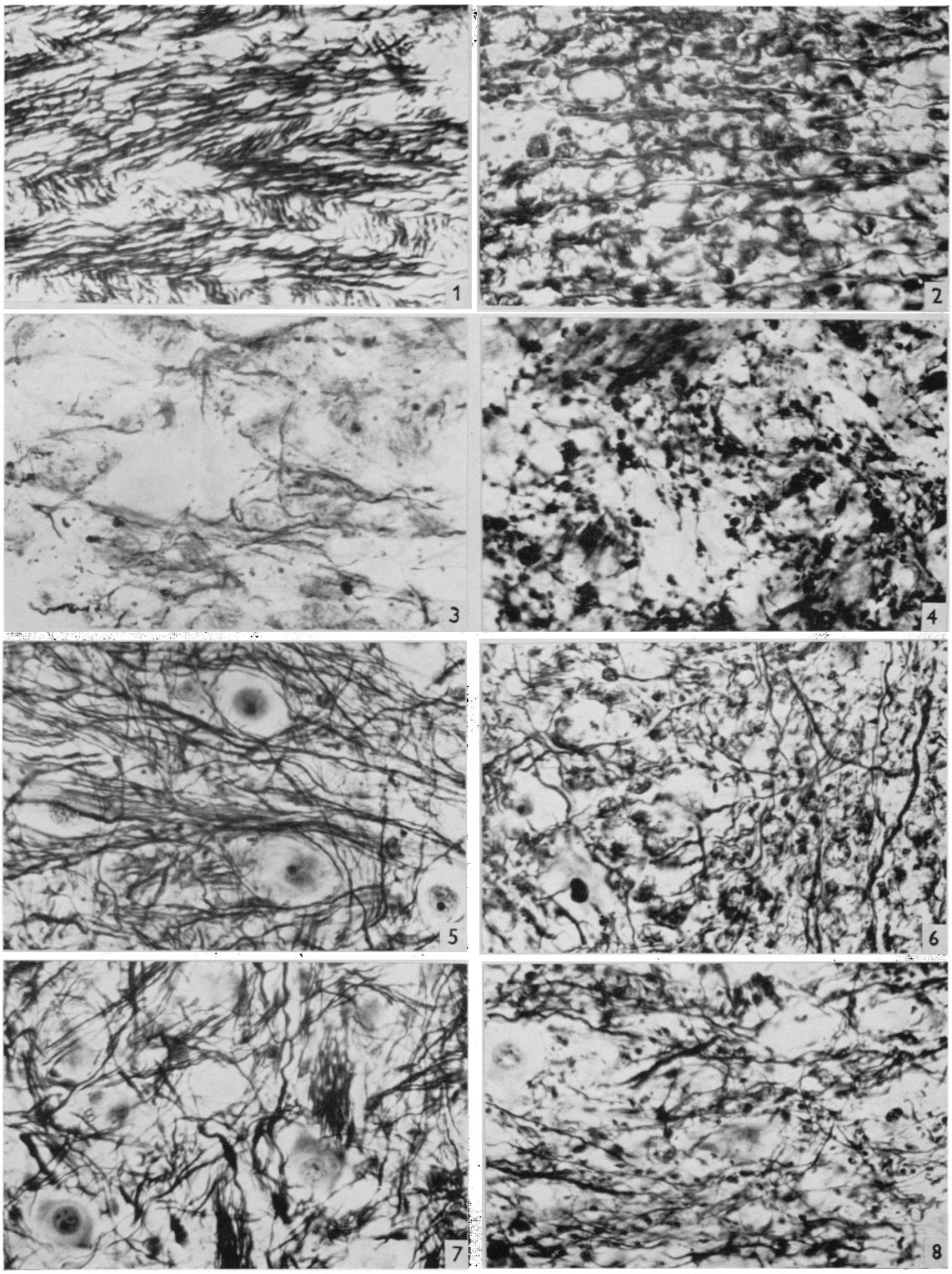
Fig. 17. Terminal calyces of Held in the medial trapezoid nucleus. Bodian preparation. ×490.

Fig. 18. Absence of coarse fibres and endings of Held in the medial trapezoid nucleus of experiment A 8, 2 months after destruction of the contralateral dorsal and ventral cochlear nuclei. Bodian preparation. ×490.

Figs. 19, 20, 21. Photomicrographs showing normal endings of Held in the medial trapezoid nucleus (Fig. 19) and the anterior part of the ventral cochlear nucleus (Figs. 20, 21). Bodian preparations. Fig. 19, ×1120; Figs. 20, 21, ×490.

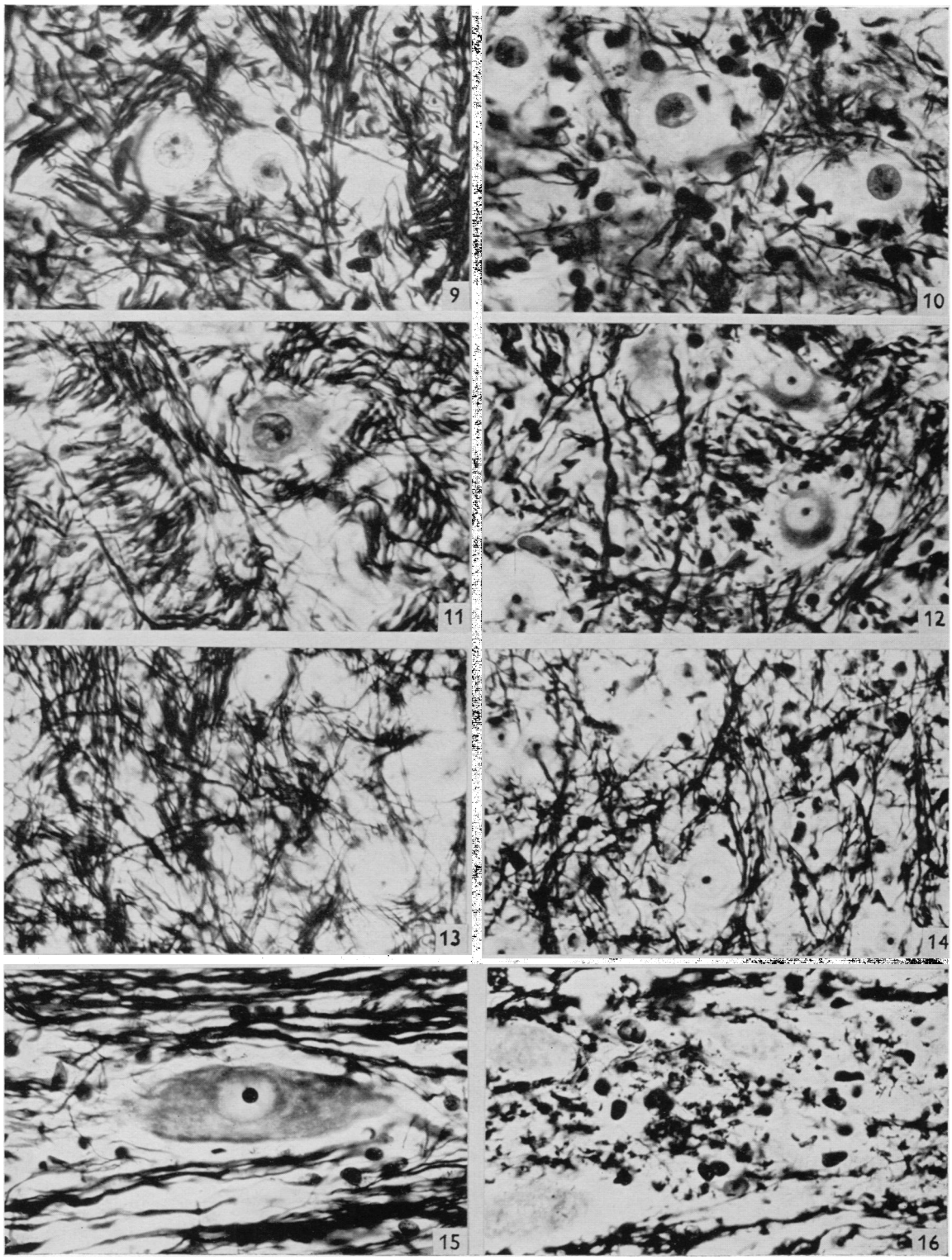
Fig. 22. Photomicrograph of a frozen section stained by the Nauta & Gygax (1954) technique showing degeneration extending dorsally from the fibrous lamina between the dorsal and ventral cochlear nuclei into the deep polymorph layer of the dorsal cochlear nucleus. Observe the abrupt ending of this degeneration at the level of the characteristic spindle-cell layer (indicated by arrows). Experiment A 21; 7-day survival. ×120.

Fig. 23. Photomicrograph at higher magnification of the same section as in the previous figure to show the degeneration around the deep dendrites and bodies of the spindle cells. In this preparation the degree of suppression is such that only a faint outline of the cells is seen. ×490.



POWELL AND COWAN—AN EXPERIMENTAL STUDY OF THE PROJECTION OF THE COCHLEA

(*Facing p.* 284)



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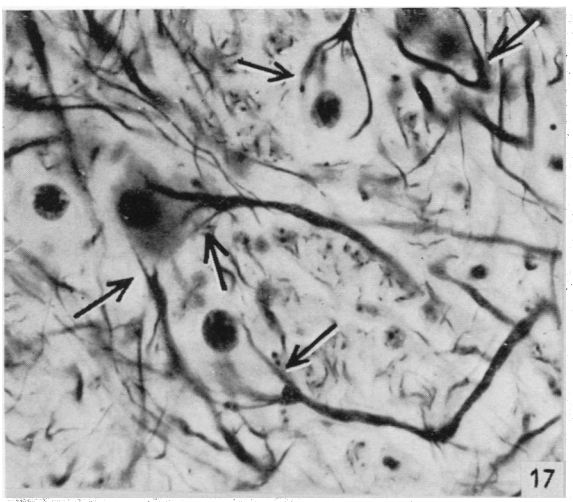
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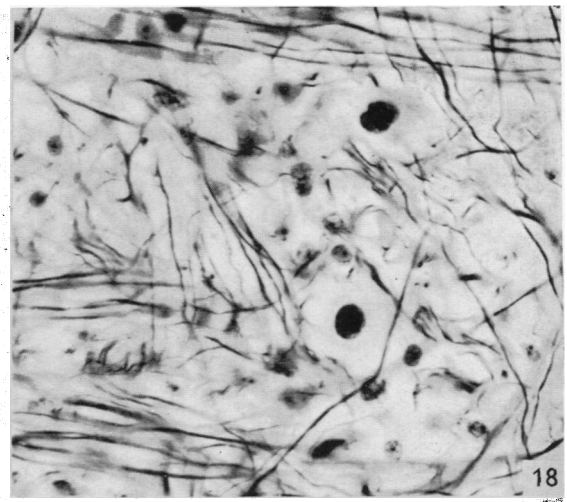
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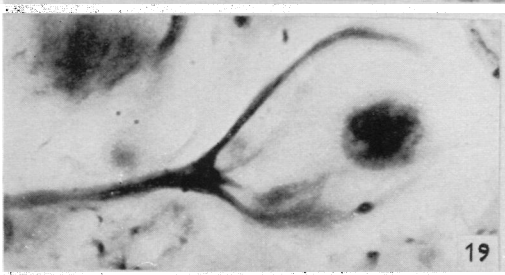
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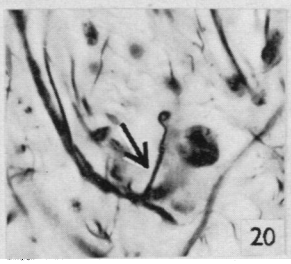
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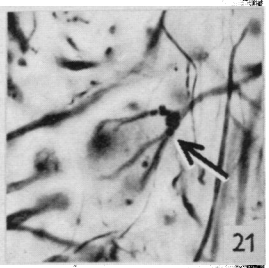
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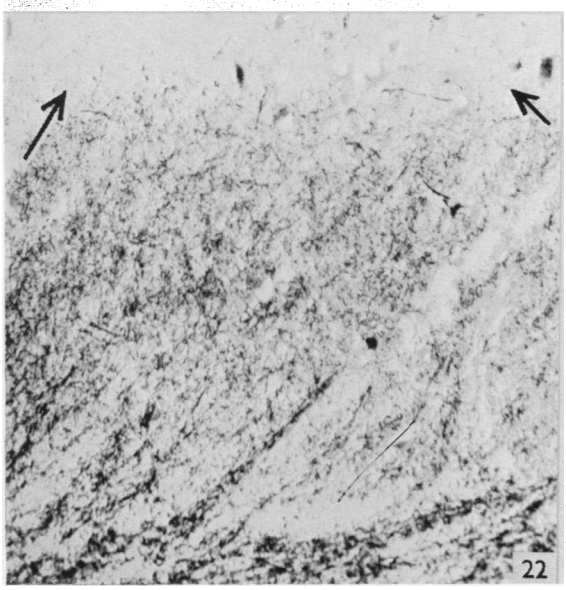
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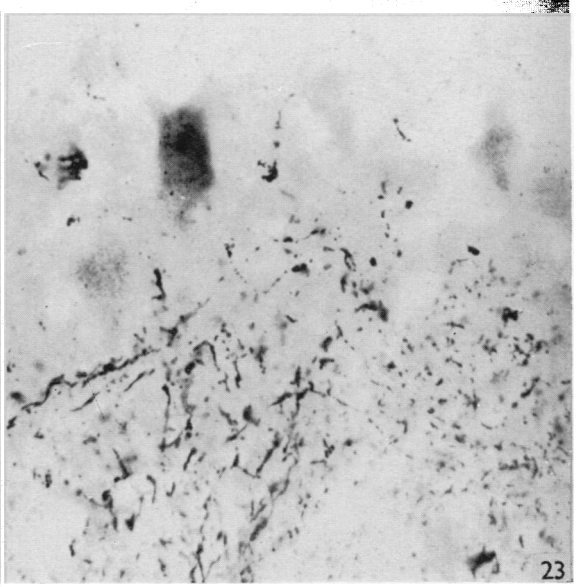
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