DEGENERATION IN THE EFFERENT NERVE ENDINGS IN THE COCHLEA AFTER AXONAL SECTION

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ABSTRACT

Both roots of the olivo-cochlear nerve bundle to one ear were transected in the brain stems of 12 chinchillas. The animals were sacrificed at times ranging from 2 to 35 days after surgery. The normal olivo-cochlear terminals on the external hair cells in the cochleas of the control ears contained many mitochondria and small vesicles of constant size. The earliest evidence for degeneration was the presence of fine 100 A filaments in the proximal parts of the terminals. These were visible at 2 days. Animals sacrificed at later times showed a greater number of filaments and fewer vesicles, but few mitochondrial changes. After 1 week, disintegration of the terminals was more prominent. A few terminals showed mitochondrial swelling and lysis of the plasma membrane but few or no filaments within the first week. These latter terminals were interpreted as representing a more rapid process of disintegration than those terminals characterized by numerous filaments and seemingly unchanged mitochondria.

One of the first studies on the ultrastructural changes present in degenerating nerve fibers was that in 1956 by de Robertis (1), who found lysis of synaptic vesicles and mitochondrial alterations in the ventral acoustic ganglion within 22 to 48 hours after destruction of the cochlea. Reger (2), in 1959, likewise found an early decrease in the number of synaptic vesicles in the mouse motor end-plate. Birks, Katz, and Miledi (3) reported mitochondrial disintegration as well as lysis of vesicles in the frog motor end-plate. More recently Gray and Hamlyn (4), and Colonnier and Gray (5) found that degeneration in the avian tectum was characterized by the formation of many fine filaments, but that degenerating terminals in the mammalian cerebral cortex did not show filaments. About this time we were making a study on the course and terminals of the efferent nerves in the chinchilla cochlea and also noted that an early sign of degeneration in these nerve endings was the formation of filaments similar to those demonstrated by Colonnier and Gray (5), but that mitochondrial degeneration occurred later.

The olivo-cochlear terminals being large and easily located lend themselves readily to study, and it seemed useful to present our findings on the degenerative process occurring in these ears.

MATERIALS AND METHODS

Both the homolateral and contralateral components of the olivo-cochlear bundle to one ear were cut where the efferent fascicles traverse the dorsal pole of the nucleus and descending root of the trigeminal nerve. This was accomplished by means of a spade-shaped knife having a cutting edge of 3.3 mm oriented in a parasagittal plane. This knife was fitted into a stereotaxis instrument having a head holder designed for the chinchilla. It was found most convenient to predetermine the appropriate coordinates of approach to the target area by the use of a dissected formalin-fixed head held in the apparatus. Injury to any part of the cochlear nucleus was avoided; however, the knife in its passage ventral cut through the lateral cerebellar hemisphere, vestibular and the trigeminal roots and/or their nuclei. In one case the knife extended inadvertently through the homolateral nucleus of the trapezoid body. In
this case the degeneration of the crossed efferents to the opposite ear was anticipated.

Twenty-six chinchillas were prepared, but due to surgical difficulties and the severe vestibular reaction, only twelve were suitable for electron microscopic study. These twelve were sacrificed at 2, 3, 4, 6, 8, 11, 21, 32, and 35 days after surgery. There were two animals each in the 2, 3 and 32 day periods and one each for the other periods. Sections from the experimental and control ears of each animal were
examined. The control ear of one of the two day animals (Ch. 90) showed fixation artefact and was discarded.

The fixative (1 per cent Dalton's OsO₄) was perfused through the perilymphatic scalae of the ears of the anesthetized animals, as previously described (6). After decapitation of the animals, the temporal bones were removed, immersed in the fixative, and refrigerated for 1 to 1½ hours. They were then washed in cold Tyrode's solution and dehydrated in ethanol. Pieces of the membranous labyrinth were dissected free from the bone while in 70 per cent ethanol. The small pieces were embedded in Epon 812 according to Luft's (7) technique. The sections were cut on an LKB Ultratome and picked up on either Formvar-coated or uncoated grids. The sections were stained with either 10 per cent uranyl acetate or lead hydroxide (8), and examined in an RCA EMU3-F electron microscope.

The brain from each animal was removed and fixed by immersion in 10 per cent formalin. The pons medulla region was sectioned at 30 microns on a freezing microtome. All sections were processed with the myelin sheath Sudan black B stain. The site and extent of the lesion was easily determined in such preparations.

OBSERVATIONS
The structure of the nerve endings in the guinea pig's cochlea has been described previously by several investigators (9-11). However, no comparable description has been published for the chinchilla which has become a popular animal in the auditory research area. It is necessary to have a base value from which to determine alterations, and the observations on the normal will be useful for future studies. It is uncertain whether any of the olivo-cochlear fibers actually terminate on the inner hair cells (12), and only the terminals on the outer hair cells will be considered in the description.

Normal Terminals

Many small boutons (Figs. 1 and 2), approximately 0.9 micron in diameter at the synapse, are clustered about the base of the receptor cell, separated from it by a synaptic gap of approximately 180 A. These are the terminals of the cochlear nerve dendrites as determined by the degeneration experiment (12). They contain round or oval vesicles and mitochondria which sometimes have small, dark granules. The rounded vesicles have diameters ranging from approximately 300 to 800 A. Their interiors vary in density. Many of the largest vesicles contain a homogeneous dense substance. Others display a central dense granule surrounded by some material less dense.
detailed description of the synapse between olivo-cochlear terminal and external hair cell. Note the continuity of small structures, similar to the vesicles, with the synaptic membrane (arrows). ss, the subsynaptic cisterna. Pb(OH)$_2$ stain. × 75,000.

The terminals of the olivo-cochlear tract are much larger than the cochlear nerve endings, and usually only one or two are found on the outer hair cells (Fig. 1). We have not yet determined the precise distribution of such terminals in the chinchilla, but in no instance has any outer hair cell been found to be as richly supplied with efferents as the cells in the basal part of the guinea pig's cochlea (11). Their diameter at the synapse ranges from 1.8 to 3.5 microns. The synaptic gap is approximately 225 Å. Previous studies (11) showed that the synaptic gap adjacent to the large nerve endings in the guinea pig's cochlea was also about 40 Å wider than that adjacent to the small endings. The synaptic vesicles, approximately 375 Å in diameter, are of rather constant size and present in large numbers although they are not always as homogeneously distributed nor as numerous as those in Fig. 1.

Figure 4 Olivo-cochlear terminal from experimental ear of animal sacrificed 3 days after nerve section. Note filaments in preterminal axon, and in proximal half of the terminal (arrows). Uranyl acetate stain. × 30,000.
Sections of some terminals show only a moderate number of vesicles. A few larger vesicles may be present but these usually have a homogeneous dense interior and only rarely show the dense central core typical of those in the cochlear nerve endings. Sometimes, a few of the vesicles among the mitochondria appear to be attached to short filaments. Many mitochondria, some with occasional dense granules, are found distal to the synapse. The mean mitochondrial diameter is approximately 0.204 micron. The diameters of mitochondria measured in thirteen different endings had a range of 0.117 micron to 0.357 micron. Some other rounded, irregular, unidentified membranous structures and multivesicular bodies may be present (Fig. 1). The remainder of the neuroplasm in these terminals is also filled in with an ill-defined webby material. Some of this is occasionally resolved into short fibers but not often. Some has the appearance of vesicular ghosts (Fig. 3). In Fig. 3, small round processes (arrows) similar in size to the vesicles are visible at the synapse. These are continuous with the synaptic membrane, but are separated from the synaptic gap by what seems to be the outermost layer of the synaptic membrane. Examination of the large nerve ending in Fig. 1 reveals that the vesicles are randomly distributed throughout the more central part of the neuroplasm. Several small clusters of vesicles are close to the synaptic membrane at the hair cell synapse, and also on the right where another nerve fiber is partly embedded in the terminal. Contrarily, the remainder of the neural plasma membrane is rather free of vesicular clusters, but this could be a sectional coincidence. Occasionally some material of round or oval form is found in the synaptic gap (Figs. 1 and 3). Similar material is also visible in the extracellular space about the terminal in Fig. 1. Its source is indeterminate and the latter especially could be preparation artefact.

The subsynaptic cisterna, previously described in cat (15), guinea pig (11), and rat (16), is visible in the hair cell cytoplasm only adjacent to the olivo-cochlear terminals (Figs. 1, 3, and 4). Random cisternae of the endoplasmic reticulum may be found near the cochlear nerve synapses, but these are scattered and irregular. One other noteworthy observation is that the receptor cell
mitochondria near the synapse are about half the size of those closer to the nucleus.

**Degenerating Terminals**

All of the large nerve endings did not degenerate in all the animals, as previously noted (12). This could have been due to incomplete section of the tract in some animals or a delayed degeneration in some axons. The material described herein concerns only the altered terminals.

The earliest evidence for change in the chinchilla ears in the present series is the presence of fine filaments, which first appear distal to the synapse, and a decrease in the amount of vesicles. These are found in all four animals sacrificed 2 days (53½ hours) and 3 days after the axons were cut. One of the 3-day animals (Ch. 90), in which it was estimated nevertheless that the entire tract had been cut in the brain stem, showed fewer altered terminals than the other three animals. Fig. 4 shows one nerve ending from the middle cochlear coil three days after section. Many of the endings in the 2-day animals are similar in appearance. Fine filaments approximately 100 Å in diameter are visible in the preterminal axon and can be followed up into the proximal half of the ending. Filaments rather than vesicles are found among the mitochondria, and no other background material is present. In contrast, the region above is occupied by well defined vesicles and the usual webby
material. Some other endings in this same ear exhibit an even greater distribution of filaments and a more marked reduction in the number of vesicles (Fig. 5). In Fig. 6, which shows two endings from a 4-day postoperative animal, one sees thick dense strands and small dense granules in the neuroplasm among the mitochondria. The strands and granules are intermingled with the filaments and some are apparently attached to the latter. Later (Figs. 7 and 8), the filaments may pervade the entire terminal replacing the webby material, and the remaining vesicles become indistinct and clumped.

Most of the mitochondria in these degenerating terminals possessing numerous filaments are essentially normal in appearance even up to 8 days after nerve section. They are no longer restricted to the proximal part of the endings for as the vesicles decrease in number they tend to be scattered throughout. Otherwise they seem unaltered. The mean mitochondrial diameter measured in seventeen endings is 0.202 micron, very similar to that in the normal endings. The mitochondria in the terminal in Fig. 7 are the largest of any measured, and range from 0.22 to 0.57 micron in diameter. It could be that this ending, from a 6-day animal, represents the last stage of a degenerating but still intact ending. Some of the terminals contain dense osmiophilic granules, membranes, and membrane-bounded bodies, such as are visible in Figs. 4, 5, and 7. Such bodies are seen rather often in the terminals from one of the 2 day animals (Ch. 92), where most have the appearance of multivesicular bodies, and in the neighborhood of the clumped vesicles in the later animals such as illustrated in Fig. 7. Some terminals, especially in the 8-day animal (Ch. 71), show massed vesicles and membrane bodies close.
FIGURE 8  Olivo-cochlear terminal from experimental ear of animal sacrificed 8 days after nerve section. A partial retraction of both synaptic membranes is visible (arrows); the subsynaptic cisterna (ss) appears intact. A normal appearing cochlear nerve terminal (c) is at left. Uranyl acetate stain. X ~8,000.

to the synapse, whereas the proximal half of the ending is occupied by filaments and normal appearing mitochondria. It is possible that some of these membrane-bounded structures are degenerated mitochondria; nevertheless the presence of many normal appearing mitochondria in the terminals from the seven animals sacrificed within the first 8 days does not favor a concept of gross mitochondrial loss within this period.

Some irregularity in the synaptic gap may be found within the first few days, and about 1 week after section a retraction of the synaptic membranes is sometimes visible. The terminal in Fig. 8, 8 days postoperative, shows a rather wide cavity between the synaptic membranes with some interposed material, probably presynaptic in origin. The synaptic membrane of the receptor cell is indented, but does not appear to be broken. Other endings, however, show no separation at the synapse, and often the synaptic membranes remain attached even after the remainder of the terminal has disintegrated. Fig. 9 shows the site of one of the large terminals at the eleventh postoperative day. The presynaptic membrane with attached debris, although poorly defined and incomplete, is still recognizable and an irregular synaptic gap is maintained. The postsynaptic membrane appears intact, and the adjacent subsynaptic cisterna is clearly evident.

FIGURE 9  External hair cell (ehc) from experimental ear of animal sacrificed 11 days after nerve section. The sub-synaptic cisterna (ss) gives evidence that this was the site of an olivo-cochlear terminal. Part of the presynaptic membrane (arrows) with attached debris is still present. Uranyl acetate stain. X ~84,000.

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Apparently, gross disintegration begins at about 1 week after nerve section. Some of the terminals in the 8-day animal are still intact and rather similar in appearance to those in Figs. 5 and 7; others are partially disintegrated, similar to that in Fig. 10; a few have completely disappeared leaving only the subsynaptic cisterna to indicate their former location. No intact olivo-cochlear terminals are to be found in the material from the 11-day animal. The sites of at least eleven terminals could be identified by means of the remaining subsynaptic cisterna in samples taken from several locations along the basilar membrane.

At 1 month after nerve section one can find cells very similar in appearance to that in Fig. 9; i.e., with a variable part of the presynaptic membrane still attached to the receptor cell and no marked change in postsynaptic membrane, or subsynaptic cisterna. However, other receptor cells exhibit weakening of the plasma membrane as indicated by indentations and wrinkles at the site of a lost terminal. If the terminals were more centrally located within the nerve cluster, other endings and the supporting cells finally encroach upon the space vacated by the degenerated neural tissue. On the other hand, even a month after section some shadowy remnants of the plasma membrane may still retain in place indistinct and degenerated organelles (Fig. 10).

An occasional terminal can be found within the first 8 days that exhibits changes quite different from those described above. The mitochondria are swollen and sometimes ruptured. The mitochondria are so irregular in shape that it is difficult to give a meaningful value for their diameter. The vesicles show no apparent decrease in number, but they are enlarged and form dense clumps in between the mitochondria. Large osmiophilic bodies may be present. Sometimes a few filaments are visible. Even as early as 3 days postoperatively the neural membrane seems to be beginning to disintegrate. Fig. 11 shows one of these endings from an animal sacrificed 6 days postoperatively. This ending exhibits all of the above characteristics and contains many vacuoles of variable size.

Cellular debris can still be seen at the site of

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**Figure 10** Degenerated olivo-cochlear terminal (oc) from experimental ear of animal sacrificed 35 days after nerve section. A normal appearing cochlear nerve terminal (c) is visible at left. Uranyl acetate stain. × 42,000.

**Figure 11** Olivo-cochlear terminal from experimental ear of animal sacrificed 6 days after nerve section. The mitochondria show marked distension. Irregular cisternae are present; one cisterna above occupies about one-third of the sectioned neural area. The plasma membrane is absent at arrows. No filaments are evident in this micrograph. Pb(OH)₄ stain. × 39,000.
 DISCUSSION

The majority of the degenerating terminals in all the chinchillas sacrificed within the 1st week show three major features. They demonstrate a variable number of fine filaments, a decreased number of vesicles, but mitochondria which are in normal in appearance. It is possible that some mitochondria degenerate early and that the membranous bodies are remnants of these. Chinchilla 92, sacrificed at 2 days, would appear to confirm this, for it shows more membranous bodies than the other animals. One might then expect Ch. 90, which demonstrates what we interpret as a delayed response at 3 days, to show active mitochondrial degeneration. Neither it nor the other 2- and 3-day animals give any definite evidence for such a process. Obviously, experimental animals sacrificed prior to 48 hours would be necessary to supply the answer. Nevertheless the filamentous terminals from all the animals sacrificed up to 8 days postoperatively contain so many normal appearing mitochondria that any early, gross mitochondrial degeneration seems altogether unlikely. It is interesting that those few terminals which do have clearly recognizable swollen mitochondria have only a few filaments at best, and that the only structures which might be degenerated mitochondria, the membrane structures, are associated with vesicles rather than filaments in the later animals. It would appear from the evidence in these animals that the degenerative change which leads to an early decrease in number of vesicles and formation of filaments in these nerve endings is not also conducive to mitochondrial degeneration.

The filaments described here are similar in size and dimension to those found in normal neural tissue (14, 17). The cochlear nerve dendrites from the control ears exhibit both fine and thick (300 A) filaments, and although the filaments persist into the cochlear nerve endings on the inner hair cells, they do not extend into these endings on the outer hair cells (18). Neither are they usually present in the normal olivo-cochlear terminals on the outer hair cells of the chinchilla. However, recent observations on the squirrel monkey (19) have revealed filaments in the effenter terminals of that animal's cochlea. Some normal terminals in other organs also exhibit fine filaments, although infrequently. Gray and Guillery (20) found rings of filaments in spinal cord terminals from cats and rats. Birks, Huxley, and Katz (21) found them to be present in the axon terminals of the frog motor end-plate. Andersson-Cedergren (22), on the other hand, could find no filaments of any kind in mouse motor end-plate.

The filaments are not always found in degenerating terminals. They were first described by Colonnier and Gray (5) and by Gray and Hamlyn (4) in the avian tectum following section of the optic tract. However, Colonnier and Gray (5) and Colonnier (23) were not able to find them in degenerating nerve endings in the cerebral cortex. Walberg (24) in recent studies of degeneration in the medial accessory segment of the inferior olive found that mitochondrial changes seemed most prominent and that no filaments were present. It is possible that different technical procedures employed by the various investigators may have influenced some of these observations, but it seems apparent that all neural tissues are not precisely the same.

Morphologically, three sources of origin of the filaments are indicated. They may infiltrate from the axon; they may be formed from the disintegrating vesicles; they may be produced from the webby neuropil. Some fine filaments may normally be present in the olivo-cochlear fibers, although these are few in number. If they actually migrate into the terminal from the axon, they would first have to be produced in quantities far in excess of normal. It seems more likely they are laid down in situ. It may be assumed that alterations take place first closest to the cut, then proceed outward, and that the process simply occurs in the preterminal fiber before it does in the terminal bulb. The other two sources are difficult to separate, because both the vesicles and the webby material disappear when the filaments appear. Those terminals which retain their vesicles demonstrate only a few filaments at best, and the neuropil is so crowded that it is impossible to distinguish any background material. It is probable that normally at least some of the webby material is a residue of vesicles which have discharged their contents. After nerve section the more rapid vesicle disintegration may give rise to...
the filaments. It was suggested by Gray and Hamlyn (4), in regard to the filaments in degenerating chick tectum, that the neuroplasm was altered so that a soluble protein was then precipitated in filamentous form. In the olivo-cochlear terminals, some webby material is already present and may act as a framework. Davison and Taylor (25) have recently shown that filaments about 60 to 80 A were present in extruded squid axoplasm and that under certain conditions such as increased concentration or pH alteration the fibrous protein became “disassociated” into smaller fragments. These experimental results fit in quite well with what is seen in degenerating terminals. The early cytoplasmic alteration in the isolated olivo-cochlear terminals is apparently not drastic because the major visible sign is a replacement of vesicles and webby material with long filaments. Indeed, within the first few days the altered terminals look rather similar to the normal cochlear nerve fibers. It is only later, when the isolated nerve processes are apparently unable to maintain themselves, that real degeneration takes place.

There is some variability in the apparent stage of degeneration in the terminals from all the earlier animals. Such a variability might well be expected, since it is well known that the distal stump fibers and endings of cut nerves do not all degenerate simultaneously (26, 27). Secondly, the terminals are large, and the filaments often localized. A single section, or even several, may not constitute a representative sampling, and it was not always possible to examine a larger portion. If each terminal were examined in its entirety, perhaps there would be a greater similarity than is apparent. Thirdly, there are regional differences in the exposure of the terminals to the extracellular fluid. For example, the terminals on the medial side of the first row of hair cells are exposed to large fluid spaces of Nuel, whereas those on the other rows are more protected by the Deiters’ cells. This last factor may help explain the prolonged persistence of some terminals. Because of the variability present it is not possible to determine whether there is a steady continuous progression of events in the first week. As no animals were sacrificed prior to 53½ hours, it is not known precisely how early the filaments appear. However, their relative paucity in one of the animals sacrificed 3 days postoperatively indicates that the time of their appearance may vary. It may be that there is a slow but continuous increase in the number of filaments and a decrease in the number of vesicles within the first week. On the other hand, the filament formation and the decrease in the number of vesicles may take place quickly, with or without an initial delay, with a consequent static period for several days, followed by rapid disintegration.

Whatever the time interval of events may be, the entire process in the filamentous endings takes place rather slowly. On the other hand, those terminals with swollen mitochondria and massed vesicles are characterized by disintegration of their plasma membranes. Undoubtedly the swollen organelles create a strain on the terminal resulting in early rupture. Probably this latter kind of degeneration is responsible for the early loss of a few terminals. Two kinds of degenerative process thus seem to be present. One, which predominates, is characterized by filaments, fewer vesicles, and normal appearing mitochondria, and a second, very minor, is characterized by massed vesicles, swollen mitochondria, and rapid breakdown of the plasma membrane. Gray and Hamlyn (4) also found in the avian tectum a few terminals with massed vesicles rather than filaments, and therefore these features are probably not peculiar to the cochlea.

It was originally hoped that a study of the degenerating terminals would give us information which might help identify degeneration in nerve endings wherever it occurs. And we believed that the filaments might be a characteristic sign. However, now it seems that the alterations are characteristic for specific axons, and that even terminals of the same bundle may present different degenerative characteristics. It is uncertain whether the filament production is characteristic of the olivo-cochlear terminals of all animals or only of the chinchilla. Three other reports have been made on degeneration of the olivo-cochlear bundle, i.e. in guinea pig (28), rat (29), and cat (30), but these were concerned mainly with identification of the terminals and have made no note on the presence of filaments. It may be that the precursor of the transmitter substance or the substance itself present in the terminals could influence the degenerative pattern. If it is present in the vesicles, rapid lysis of the vesicular membranes would release the enclosed material and perhaps alter the neuroplasm sufficiently to result in a protein denaturation detectable morphologically. According to our best evidence these termi-
nals are inhibitory in nature (31), but the trans-
mitter substance has not yet been identified (32).

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