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Neuron Morphology and Synaptic Architecture in the Medial Superior Olivary Nucleus

Light- and Electron Microscope Studies in the Cat

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Summary. Dendritic arborization pattern, spatial and synaptic relations of various neuron types and the terminal distribution of afferent axons of various origin were studied in the medial superior olivary nucleus of the cat using Golgi, degeneration, electron microscope and horseradish peroxidase techniques. Three types of neurons clearly different in morphological features, distribution, neighbourhood relations, input and output characteristics were distinguished: (1) *fusiform cells* having specific dendritic orientations and arborization patterns and synaptic relations to various types of terminal axon arborizations (2) *multipolar neurons* with wavy dendrites bearing spine-like appendages, receiving relatively few synaptic contacts and having a locally arborizing axon, and (3) elongated *marginal cells,* largely restricted to the fibrous capsule of the nucleus. The fusiform and marginal neurons were identified by retrograde peroxidase labeling as the olivo-collicular projection cells.

Ultrastructural analysis of normal and experimental material revealed the presence of four distinct kinds of axon terminals differing in size, synaptic vesicles type, relation to postsynaptic targets and in origin: (i) large terminals with multiple extended asymmetric synaptic membrane specializations and containing round, clear vesicles arise from the spherical cells of the ipsilateral anteroventral cochlear nucleus, (ii) most of the small axon terminal profiles – engaged in asymmetric synaptic contacts – originated from the trapezoid nucleus, (iii) terminal boutons containing pleomorphic vesicles belong to fibers descending from the ipsilateral multipolar neurons in the central nucleus of the inferior colliculus and from the nuclei of the lateral lemniscus while (iv) boutons containing exclusively ovoid vesicles and remaining intact after complete deafferentation of the nucleus were considered to be of local origin.

Key words: Medial superior olive – Neuronal morphology – Golgi, electron microscope and horseradish peroxidase techniques

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Introduction

The position in the auditory system of the medial superior olivary nucleus (MSO) as a component of the superior olivary complex has been established by classical observations (LaVilla 1898; Cajal 1909). Its neuronal elements were reexamined by Clark (1969a, b), Perkins (1973), Scheibel and Scheibel (1974), Schwartz (1977) and Kiss and Majorossy (1977). The MSO has been shown by experimental degeneration studies in various laboratory animals (Stotler 1953; Warr 1966, 1972; Goldberg and Brown 1968; Osen 1969) to be the first level of convergence of ascending fibres from the left and the right cochlear nuclei. Its importance in binaural interaction (Galambos et al. 1959; Moushegian et al. 1964) and spatial sound localization (Erulkar 1972) has been pointed out, and the assumed structural substrate of the observed excitatory and inhibitory interactions was quantitatively analysed (Clark 1969a, b). Perkins (1973) and Lindsay (1975) provided ultrastructural evidence for the presence and relation of axon terminals arising from the anteroventral cochlear nucleus to postsynaptic structures and described two further varieties of unidentified origin. Ramón y Cajal's description was elaborated further by Scheibel and Scheibel (1974), with emphasis on the rostro-caudal dimension of the dendritic arbor of the fusiform cells. Schwartz (1980) presented data – in Golgi and EM material – about the differential distribution of terminal axon arbori-

zations destined for the various cell types of the MSO. The present study had as objective to provide a detailed description of the morphological features' spatial arrangement and synaptic relations of the several neuronal elements in the MSO, which might serve the purpose of their classification. Surgical interferences and electrolytic lesions were made in various nuclei of the auditory system to determine the origins of the several types of arborizations. The horseradish peroxidase (HRP) labeling technique was applied to identify the parent cells of input and output fibres localized in or projecting to the MSO.

Material and Methods

Thirty-two adult cats were used in this study and several dozens of complete series of rapid Golgi and perfusion Golgi-Kopsch preparations of adult cats and kittens available in our laboratory were consulted. Four intact cats were used for studying the normal ultrastructure of the MSO. The animals were transcardially perfused under deep Nembutal anaesthesia with Karnowsky's solution (1965). Small tissue blocks were excised, postfixed in osmium tetroxide, oriented and embedded in Durcupan (Fluka). An LKB III Ultratome was used for cutting thick and thin sections, the former were stained with toluidin blue, the latter with uranyl acetate and lead citrate. Thin sections were examined under a Tesla B 500 and JEOL 100B electron microscope.

In five groups of four animals partial or complete stereotaxic unilateral electrolytic lesions of the cochlear nuclei, nucleus of the trapezoid body, nuclei of the lateral lemniscus and inferior colliculus, respectively were performed.

Subtotal surgical isolation of the MSO was performed in the fifth group from a ventral paramedian approach.

The postoperative survival time ranged in all groups from 2–5 days. The experimental material was impregnated with the Fink and Heimer (1967) technique for light microscopy and samples were processed for routine electron microscopy. For studies of retrograde neuronal labeling two groups of four animals received unilateral stereotaxic injections of 0.1 and 0.3 µl HRP (33% in saline, Type VI, Sigma Chemical Co.) into the MSO and the inferior colliculus, respectively. Following 48 h survival the animals were perfused with 0.5% paraformaldehyde and 2.5% glutaraldehyde buffered solution. The brain was removed, cut, the sections developed in 3.3′-Diaminobenzidine-tetrahydrochloride and hydrogen peroxide and examined under the light microscope.

Results

I. Light Microscopy

A. Golgi Observations. On the basis of morphological characteristics and distribution within the MSO three types of neurons were distinguished.

1. Fusiform Neurons. The most characteristic and homogeneous group was constituted by typical spindle-shaped cells. Their cell bodies were slender, with the transverse diameter rarely exceeding 14 µm, tapering at both tips gradually into two main dendrites (Figs. 1 and 3). The main dendrites followed a straight course for distances of about 50–150 µm. Occasionally they bifurcated early but, as a rule, broke up quite abruptly into numerous (5–7) secondary branches. Initially these diverged at very small angles thus occupying narrow conical tissue spaces, their bases being directed towards the periphery of the nucleus (Figs. 6 and 7).

Tertiary twigs were scarce. The dendrites tapered off towards their ends and appeared to terminate without any apparent characteristics. The surface of both the soma and dendrites was relatively smooth, occasional short wedge-shape protrusions could be observed, particularly in Golgi-Kopsch preparations (Fig. 6) where the terminal dendritic twigs appeared beaded. The axon might arise from the soma or a proximal dendrite, although the dendritic origin was more frequently observed (Fig. 3). The axon was directed usually towards the lateral border of the MSO and soon acquired a myelin sheet which generally prevented us tracing it over any longer distance. Initial axon collaterals were rarely encountered in this material. The orientation of fusiform cells was strictly perpendicular in the transversal plane to the long axis of the MSO. The neurons having the longest dendritic spans in both directions were shelved in register (Fig. 1) along the slightly curved long axis of the MSO, while two indistinct rows of neighbouring cells were shifted with approxi-

Figs. 1–7. Microphotographs of fusiform neurons in the MSO in the transversal plane. Scale: 100 µm

Fig. 1. Fusiform cells with polar dendrites. Parallel orientation of the bodies and dendrites of neurons. Golgi-Kopsch technique

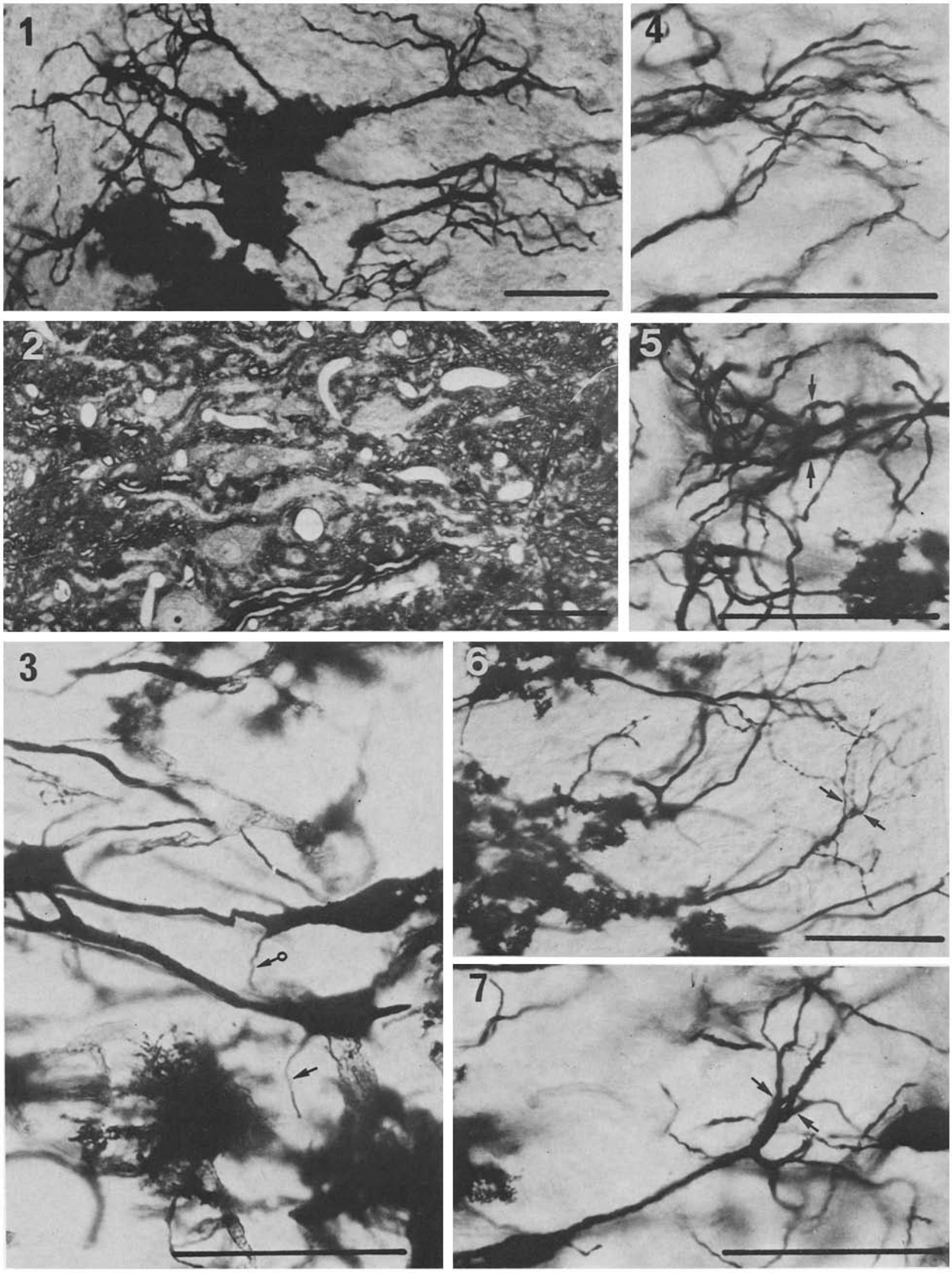
Fig. 2. Cell layers with partially shifted perikarya. One µm section. Toluidin blue stain

Fig. 3. Fusiform neurons. The axons arise from the perikaryon (*arrow*) or from the dendrite (*ringed arrow*)

Fig. 4. Fascicles of secondary dendrites

Fig. 5. Distended dendritic arborization cone (*arrows*)

Figs. 6–7. Conical branching pattern (*arrows*) of secondary dendrites. Golgi-Kopsch technique



Figs. 1–7

mately one perikaryon length towards the periphery in either medial or lateral direction alternatingly (Fig. 2). Neurons situated closer to the fibrous capsule of the nucleus had shorter dendrites, the arborizations of which fanned out towards the capsule (Fig. 5). This was more pronounced at the lateral concave border, where secondary dendrites took a course parallel to and partially intertwined with the axons of the capsule. Processes of the shortest cells situated most peripherally seldom extended beyond the midline of the nucleus and, thus, appeared to be entirely restricted to either the medial or the lateral half of the MSO. Towards the periphery individual dendritic arbors were gradually overlapping and also some fasciculation might occur (Figs. 1, 4 and 5). The dendritic packing density steadily increased with the distance from the cell body and reached its maximum in the peripheral zone. The foregoing description relates to Golgi preparations cut strictly perpendicular to the long axis of the brainstem. Sections cut in the sagittal and frontal planes revealed the occasional presence of ascending or cranial dendrites causing a certain distorsion of the strict order so far described but does not, though, alter the essential regularity of the dendritic architecture.

2. Multipolar Cells. Medium sized 16–20 μm cells with multiangular perikaryon were found in considerable numbers in the cellular core of the MSO. Their cell bodies gave rise to 5–6 thick principal dendrites projecting in various directions and usually following a wavy course (Fig. 8). Each might branch once or twice at irregular intervals and at various distances from the cell body. The secondary branches became appreciably thinner and the dendritic packing density remained rather low, in the essentially spheric dendritic arborization space in spite of the wavy or even recurving distal segments. Filiform and drumstick-shaped appendages appeared already in the proximal segment of the main dendrites (Figs. 8 and 9), their number steadily increased with the distance from perikaryon in the secondary branches. No obvious regularity could be detected in their general arrangement as far as size, shape, number, orientation or distribution were concerned. Few if any terminal multiple appendages, characteristic of local neurons found in other regions of the central nervous system were observed on these cells. The thin axon emerged generally from the cell body (Fig. 8) and arborized in its vicinity. The cells appeared to be distributed evenly without any particular preference to different regions of the MSO.

3. Marginal Cells. Medium sized, elongated, but often triangular neurons embedded in or lined up along the fibrous capsule were frequently encountered in the MSO. Their somata as well as their essentially polar dendritic stems were parallel to and intermingling with the dense axonal plexus of the capsule (Fig. 11). The stem dendrites and brush-like secondary branches were straight or gently curved, depending on their actual site along the fibrous

Figs. 8–9. Microphotographs of multipolar neurons in the MSO. Golgi-Kopsch technique. Scale: 100 μm

Fig. 8. Multipolar cell bodies, spinous dendrites and an axon (*arrow*). The afferent axons follow a straight course (*ringed arrow*)

Fig. 9. Long fusiform (*arrow*) and drumstick-shaped (*ringed arrow*) appendages on secondary dendrites

Figs. 10–12. Microphotographs of marginal neurons in the MSO. Golgi-Kopsch technique. Scale: 100 μm

Fig. 10. Dendrites (*arrow*) entering the cellular core of the MSO

Fig. 11. Marginal cell (*arrow*) embedded in the nuclear capsule

Fig. 12. Dendrites (*arrow*) projecting to the periolivary nuclei

Figs. 13–16. Microphotographs of afferent axons in the MSO. Golgi-Kopsch technique. Scale: 100 μm

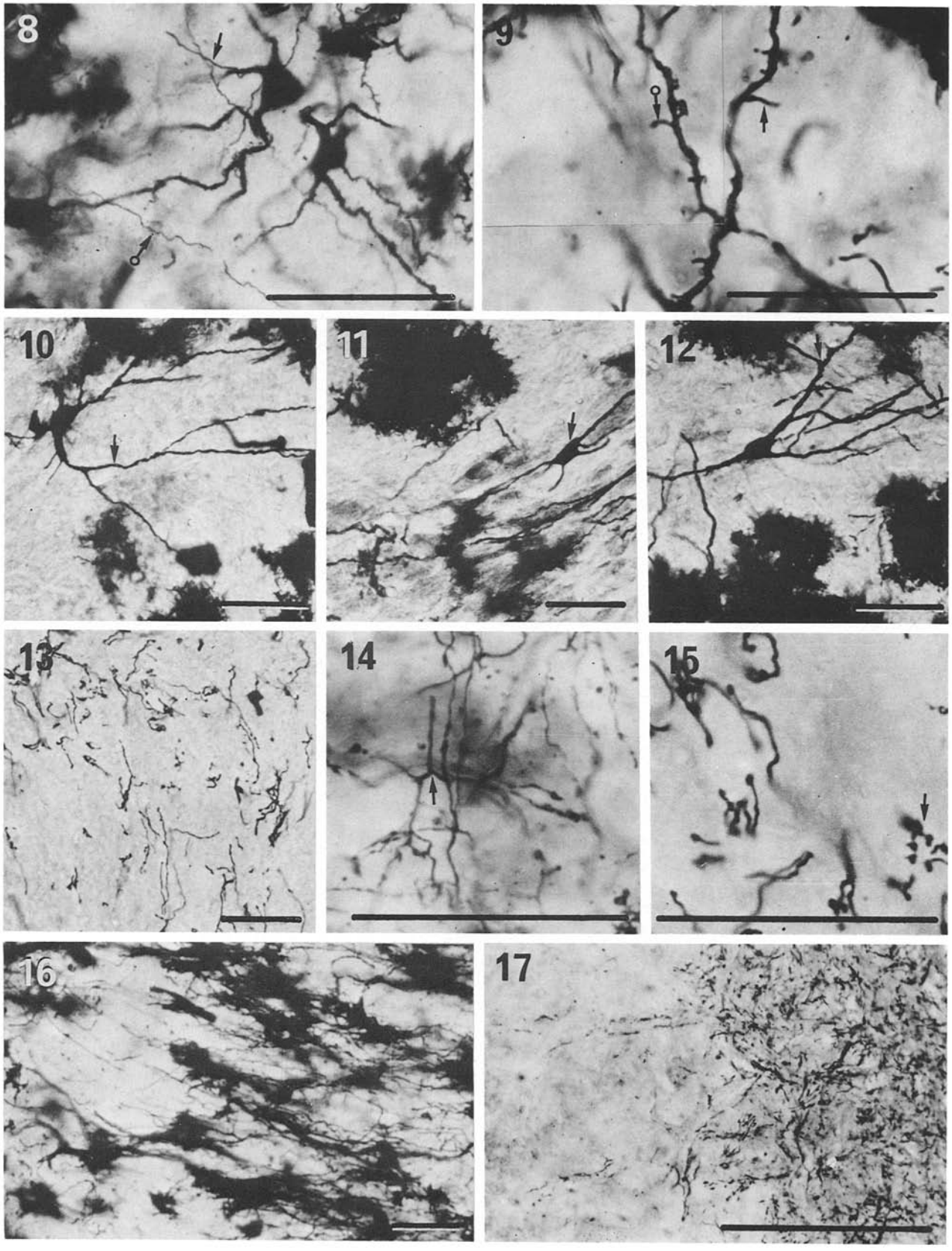
Fig. 13. Large axons entering the nucleus

Fig. 14. Dense plexus (*arrow*) of preterminals

Fig. 15. Clusters of terminal boutons (*arrow*)

Fig. 16. Bundles of fibres entering the nucleus and running up to the midline of the nucleus

Fig. 17. Degenerated fibres and terminals restricted to the lateral half of the MSO after ipsilateral lesion of the cochlear nucleus. Fink-Heimer technique. Scale: 100 μm



Figs. 8–17

perimeter of the MSO and possessed few short spines or appendages. One or two large specific dendrites, however, transgressed the boundaries of the capsule, entering the territory of the MSO (Fig. 10) and/or projecting to the adjacent periolivary nuclei (Fig. 12). Those entering the MSO scarcely branched and followed a long, straight course parallel and closely attached (although separated from) the dendrites of the fusiform cells. The axon of the marginal cells invariably turned away from the MSO, projecting to extraneous destinations and gave off very few collaterals within the capsule.

4. Afferent Axons. Large axons entered the lateral and medial borders of the MSO (Fig. 13), usually in bundles (Fig. 16). They ran mainly parallel with the dendrites and perikarya of the transversely oriented fusiform cells, broke up into dense plexuses of numerous (7–8) preterminal collaterals (Fig. 14) matching the branching pattern of the dendrites and gave rise to individual endings as well as to clusters of terminal boutons (Fig. 15) with short preterminal segments. The intimate relation and repeated apposition over considerable distances of the afferent preterminals and terminals to dendritic and somatic surfaces of fusiform cells were indicative of specific synaptic contacts. Individual collaterals entered the dendritic field of spinous multipolar cells approaching their secondary dendrites predominantly at right angles (Fig. 8). The individual large axons, however, were restricted to one half of the MSO only (Fig. 16). Degeneration studies indicated the origin of the large axons and confirmed their arrangement. Following unilateral lesions of the cochlear nuclei, coarse degeneration granules densely filled the lateral half of the MSO but failed to extend beyond the midline of the nucleus (Fig. 17). In Golgi sections axons of finer, albeit varying caliber invaded the MSO radially from different directions and distributed randomly to fusiform, spinous multipolar and marginal cells.

B. HRP Labeling. Following unilateral stereotaxic injection of HRP into the inferior colliculus (Fig. 18) a large number of neurons was labelled in the ipsilateral MSO. The reaction product filled the cell bodies and proximal segments of dendrites densely, revealing the characteristic fusiform shape of the neurons (Figs. 19 and 22). Less profusely labelled areas confirmed the characteristic spatial arrangement of the fusiform projection cells observed in the Golgi material. The fusiform neurons were arranged in columns with the adjacent cells partially shifted peripherally by approximately the length of one neuron soma (Fig. 19) and having every third or fourth cell in register. Few tri- or even multiangular somata, characteristic of marginal neurons and located in or near to the capsule, were also heavily labelled in this experiment (Figs. 19 and 20). No multipolar cells were labelled with peroxidase tracer in the cellular core of the MSO. After unilateral injection of the tracer into the MSO (Fig. 23) densely labelled neurons appeared ipsilaterally in the central nucleus of the inferior colliculus (Fig. 25) and the nuclei of the lateral lemniscus (Fig. 24). The number

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Fig. 18. Injection of HRP in the inferior colliculus. Scale: 1 mm

Figs. 19, 20 and 22 are from cases with HRP injections in the inferior colliculus

Fig. 19. Labelled fusiform neurons (*arrows*) in characteristic arrangement in the MSO and labelled marginal cell (*ringed arrow*)

Fig. 20. Labelled marginal cell (*arrow*) in the MSO. Darkfield illumination. HRP technique. Scale: 100 μm

Fig. 21. Fusiform neurons (*arrows*) in the MSO densely covered with axon terminals. One μm sections. Toluidin blue stain. Scale: 10 μm

Fig. 22. Labelled fusiform cells in the MSO. Darkfield illumination. HRP technique. Scale: 10 μm

Fig. 23. HRP injection deposit in the MSO (*arrow*). Scale: 100 μm

Figs. 24–28 are from cases after HRP injections in the MSO

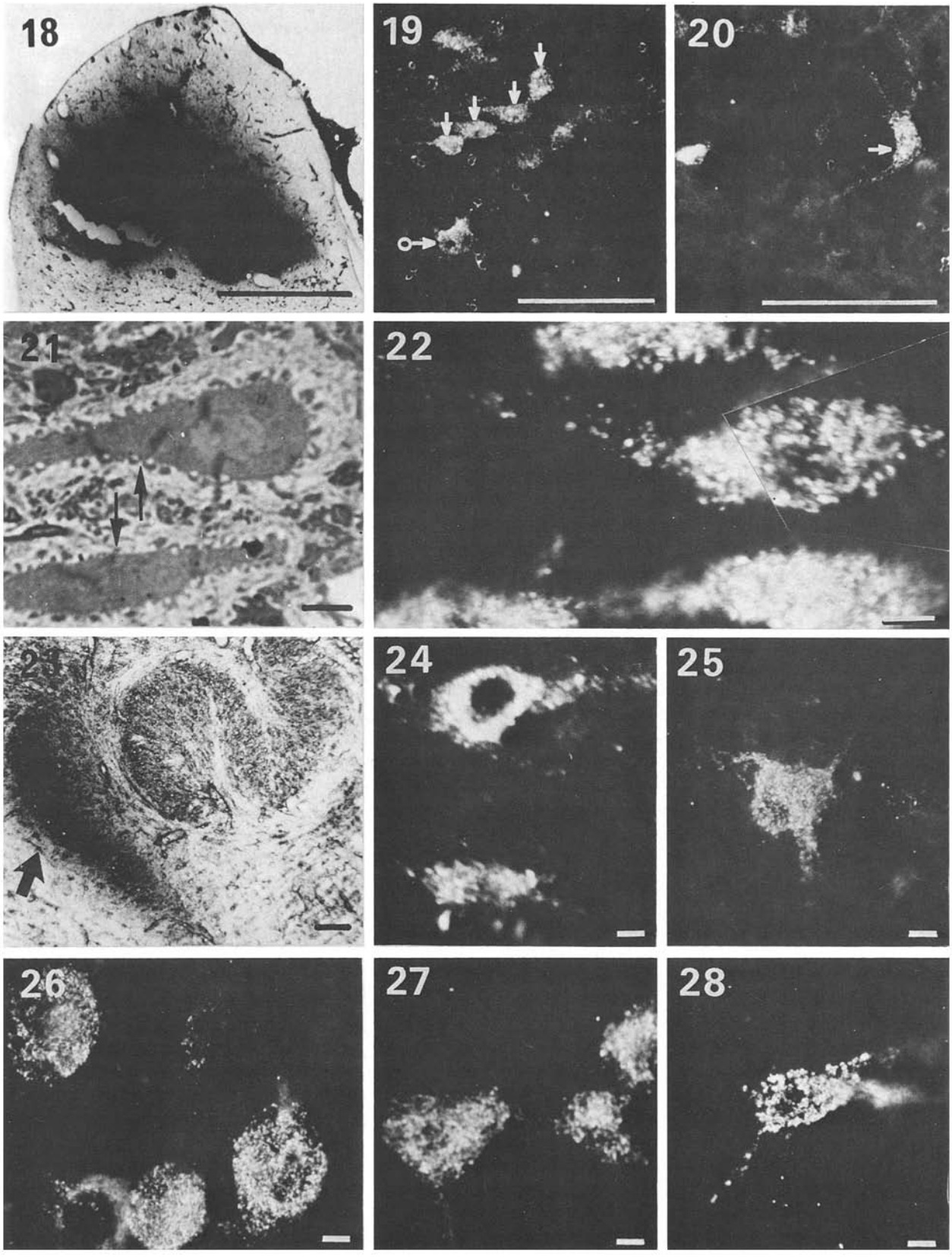
Fig. 24. Labelled multipolar neurons in the ventral nucleus of the lateral lemniscus

Fig. 25. Labelled multipolar neuron in the central nucleus of the inferior colliculus

Fig. 26. Labelled spherical cells in the anteroventral cochlear nucleus

Fig. 27. Labelled multipolar cells in the posteroventral cochlear nucleus

Fig. 28. Labelled pyramidal cell in the dorsal cochlear nucleus. Darkfield illumination. HRP technique. Scale: 10 μm



Figs. 18–28

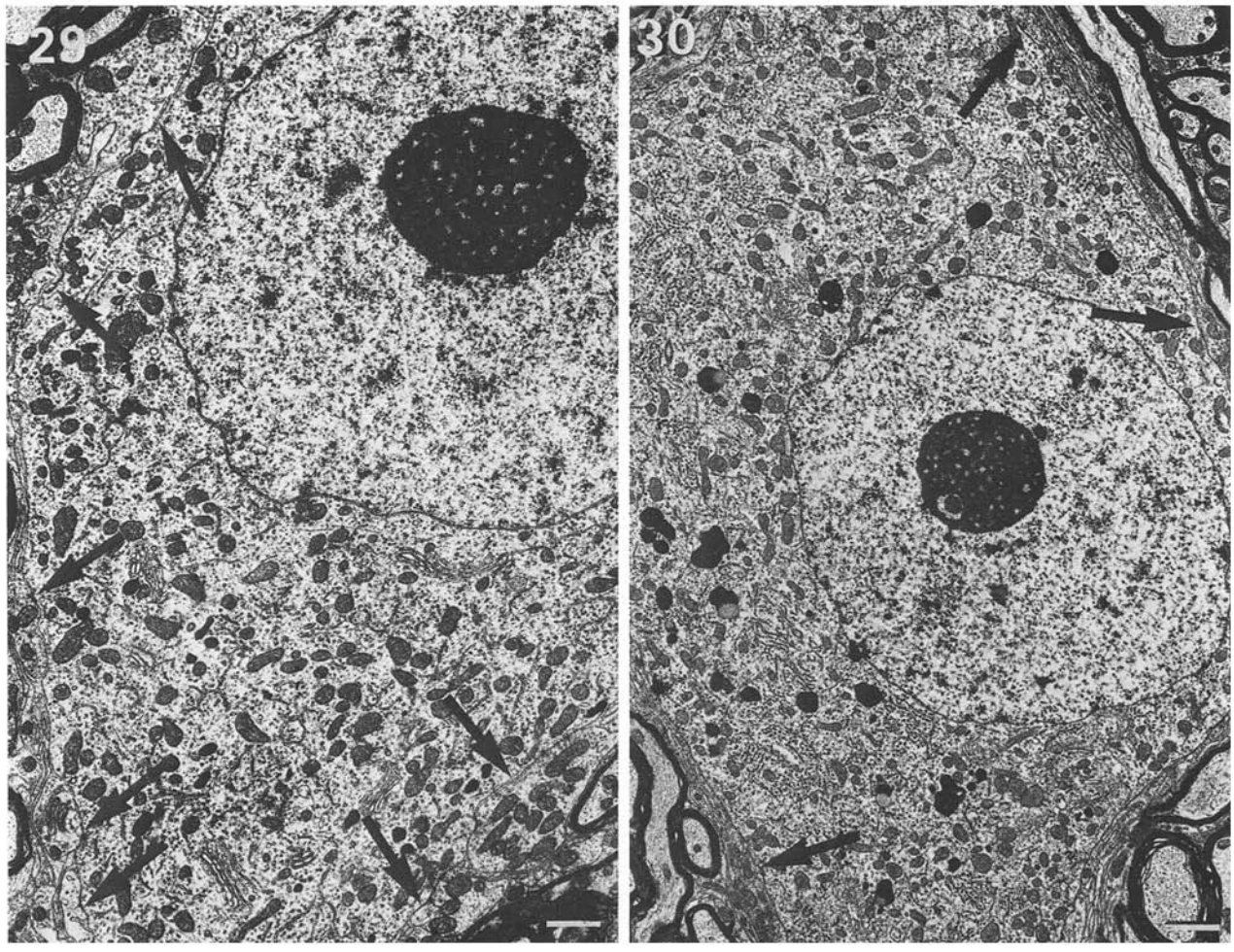


Fig. 29. Electronmicrograph of a fusiform perikaryon in synaptic articulation with numerous (*arrows*) terminals. MSO. Scale: 1 μm

Fig. 30. Electronmicrograph of a multipolar perikaryon with a few contacting terminals (*arrows*). MSO. Scale: 1 μm

of labelled neurons was even larger in the ipsilateral cochlear nuclei. As HRP granules filled the cell bodies densely, labelled neurons could be identified, with reasonable certainty, as spherical cells in the anteroventral cochlear nucleus (Fig. 26), as multipolar cells in the posteroventral cochlear nucleus (Fig. 27) and as pyramidal cells in the dorsal cochlear nucleus (Fig. 28).

II. Electronmicroscopy

A. Normal Material. With the electron microscope the conventional cytological criteria used in classifying neuronal somata e.g. size, shape, cytoplasmic organelles etc. appeared rather equivocal when sections were oriented randomly. In carefully oriented preparations, sliced with the aid of a stereotaxic instrument perpendicular to the axis of the brainstem, thin sections revealed elongated perikarya when cut across the nucleolus. The smoothly contoured, rounded nucleus was surrounded by a thin rim of cytoplasm which became abundant only in polar positions and tapered gradually towards the principal dendrites. Cysterns of the endoplasmic reticulum, Golgi membrane complexes, mitochondria, and ribosomes were most numerous in the thickened cytoplasmic portions of the ovoid somata. The dendritic profiles had smooth contours, an electron-dense cytoplasm and numerous organelles. The most decisive criteria for identification of fusiform cells were the neighbourhood relations of the cell and the type and number of presynaptic elements engaged in synaptic contact with the cell body (Figs. 21 and 29).

The perikaryon of these cells was densely covered by axon terminals of various vesicular content,

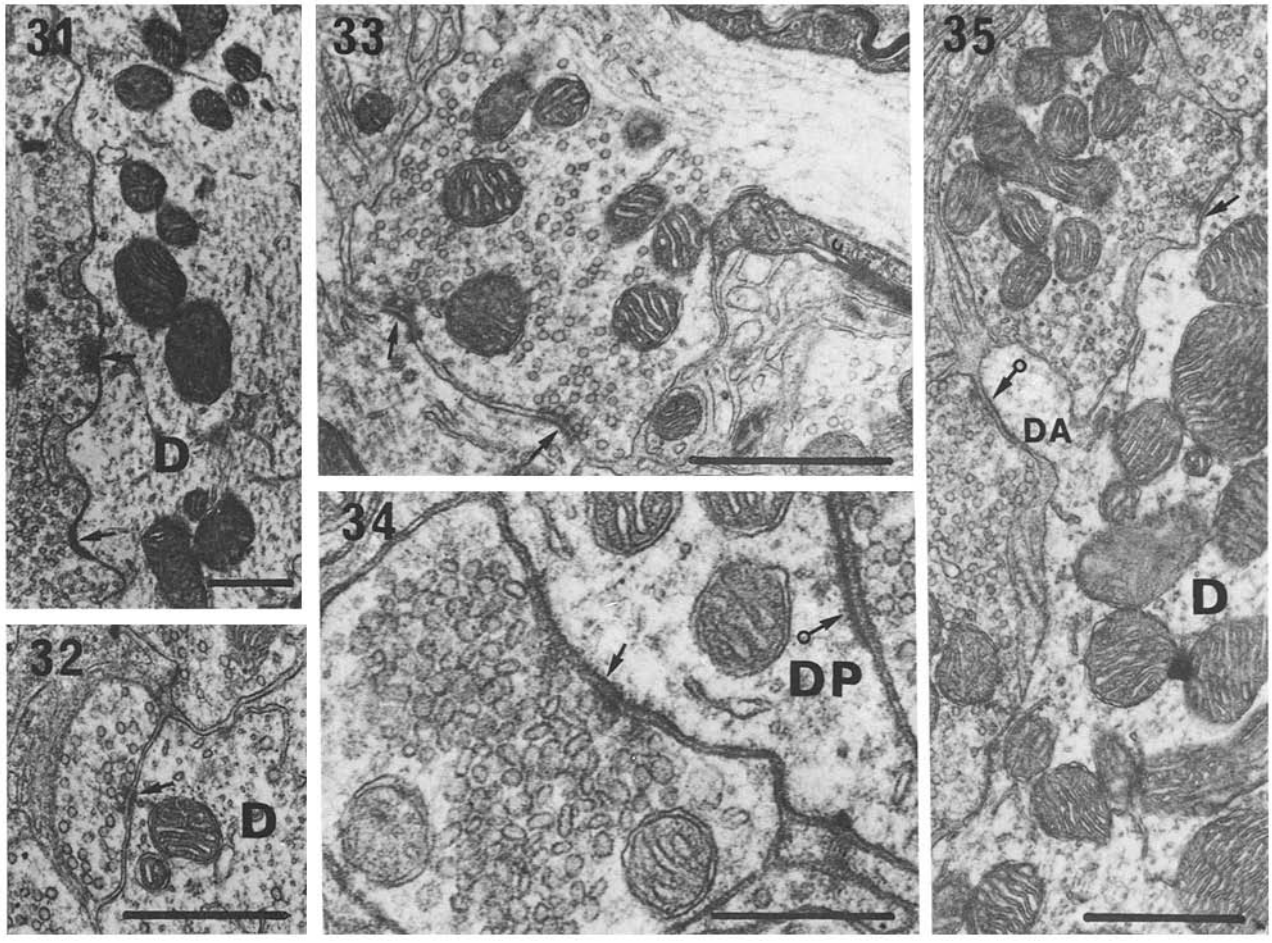


Fig. 31. Electronmicrograph of a terminal of round vesicle content, irregular surface and multiple asymmetric membrane specializations (*arrows*) presynaptic to a dendrite (*D*)) of a fusiform neuron. MSO. Scale: 1 μm

Fig. 32. Small terminal containing round synaptic vesicle articulates (*arrow*) with a dendrite (*D*) of a fusiform neuron. MSO. Scale: 1 μm

Fig. 33. Large terminal establishing multiple asymmetric contacts (*arrows*) and containing round synaptic vesicles. The myelin sheath of the preterminal is characteristically extending close to the postsynaptic profile. MSO. Scale: 1 μm

Fig. 34. Two terminals establishing synaptic contacts on a dendritic protrusion (*DP*). One contains pleomorphic vesicles and forms symmetrical contact (*arrow*), while the other contains round vesicles and forms asymmetrical contact (*ringed arrow*). Scale: 0.5 μm

Fig. 35. Dendrite (*D*) and dendritic appendage (*DA*) of a multipolar cell in synaptic position to a terminal with ovoid vesicles (*arrow*) and to another with round synaptic vesicles (*ringed arrow*). MSO. Scale: 1 μm

establishing synaptic contacts. Large myelinated axons were lined up for considerable distances along dendrites and somata. They retained their myelin sheaths until close to the synapse (Fig. 33) and the large terminals established multiple synaptic contacts with dendritic and somatic membrane specializations (Fig. 31). The axon terminal approached the dendrite without exceptions from distal forward central. These articulations were of the asymmetrical type, and contained round, clear synaptic vesicles of 40–60 nm in diameter. Small axon profiles with round vesicles and asymmetric contacts were frequently observed on the dendrites and perikarya without any particular strategic position (Fig. 32). Medium-sized terminals, with an ovoid synaptic vesicle population establishing symmetrical synaptic contacts, were less frequent although consistently present (Fig. 35), similar to synaptic profiles with pleomorphic vesicles (Fig. 34).

Neurons of similar ultrastructural characteristics and presynaptic axon arrangements, but not of the fusiform or elongated type, were found in isolation

embedded in dense myelinated fibrous neuropil of the capsule and were on the basis of their position considered as marginal cells.

A smaller fraction of perikarya appeared to be irregularly multiangular in shape, somewhat smaller in size and tapering into several main dendrites. The nucleus was usually infolded. Cytoplasmic organelles were found in large number in the somata and proximal dendrites. On the surface of the perikaryon characteristically few terminals were found and repeated synaptic contacts of large terminals were practically absent (Fig. 30). In addition to minor morphological differences these features were considered as the most important mark in identifying ultrastructurally the neurons distinguished as fusiform and multipolar cells in Golgi preparations. The intermediate and distal dendritic portions exhibited long filiform and medium drumstick-shaped, spine-like appendages and had an uneven surface (Fig. 35). The dendritic shafts were in contact with a relatively small number of presynaptic terminals of different vesicular content and membrane specialization, and no specific pattern was detected in their mode of articulation. The spine-like appendages, however, were synaptically engaged with two types of axons, one containing spherical vesicles and forming asymmetrical contacts and the other containing flattened or pleomorphic vesicles along with symmetrical attachment (Fig. 34). No single or complex terminal dendritic appendages characteristic of local neurons in other areas of the central nervous system were observed on the dendrites of the multiangular neurons; presynaptic dendrites or cell body sites of presynaptic structure were not found in this material. Desmosome-like symmetrical membrane attachment plaques connecting dendrites or somata of the same type were observed but rarely.

Summing up: four types of axons clearly different in ultrastructural characteristics, localization and relation to the soma or dendrites of the three categories of neurons were distinguished in the MSO. Most prominent and numerous among them were the large terminals of usually elongated shape and irregular surface, establishing mutliple synaptic contacts with asymmetric membrane specialization over large areas of neuronal somata and proximal dendrites (Figs. 31 and 33). They contained round, clear synaptic vesicles of 50–60 nm diameter. Their preterminal filammentous axon stalk was very short because they lost, as a rule, their myelin sheaths only in the immediate vicinity of their endings (Fig. 33). The curvature of the serially arranged synaptic specializations appeared to be on both the proximal dendrites and somata concave presynaptically. They were far less frequently found on the multiangular than on fusiform neurons but their distribution between dendrites and somata of the same type of cells was approximately identical. They were followed in frequency of occurrence by small axon terminals of round synaptic vesicle content and asymmetric membrane specialization (Figs. 32 and 34). They were evenly distributed to all target areas except for the dendrites of the medium sized multiangular cells where their number was lower. When present, however, they established axon-spine type synapses, and shared the same spine or protrusion with a terminal of ovoid or pleomorphic vesicles (Fig. 34). The third group of axons was represented by terminals containing round and flattened vesicles in approximately equal number and also transitional forms (Fig. 34). These terminals of pleomorphic vesicle content occurred somewhat more frequently on medium sized multipolar cells, but there was no difference in their distribution between dendrites and somata. They tended to occupy, however, more peripheral positions on the dendritic arbors. The distribution of terminals of homogenously ovoid vesicle content (Fig. 35) was similar to the latter, except for a clear preference for localization on the most proximal dendritic segments and somata. Axo-axonic contacts were not observed in this material.

B. Experimental Material. Following stereotaxic lesions of the ipsilateral ventral cochlear nucleus, the large axon terminals, with repeated asymmetrical membrane specialization and round synaptic vesicles, underwent secondary degeneration of the electron-dense type (Fig. 36). The large myelinated axon stems also displayed degenerative changes. By choosing an appropriate postoperative survival period the degenerated terminals could still be seen attached to the postsynaptic surface and the membrane specializations remained intact. Encroaching glia processes might, however, partially surround the altered terminals, some of which showed advanced signs of phagocytosis by glia. The postsynaptic relations of the large degenerated terminals were identical with those observed in normal material and their distribution within the medial olive corresponded to that of the degeneration fragments observed under the light microscope following similar interferences. When the electrolytic lesion was placed into the inferior colliculus of the same side, the medium sized terminals containing pleomorphic synaptic vesicles and participating in symmetrical synaptic articulations degenerated (Fig. 38). Following lesion of the nucleus of the trapezoid body, most of the small terminal boutons with round vesicles and asymmetric membrane specialization displayed dark degeneration changes (Fig. 37). Among these some may be

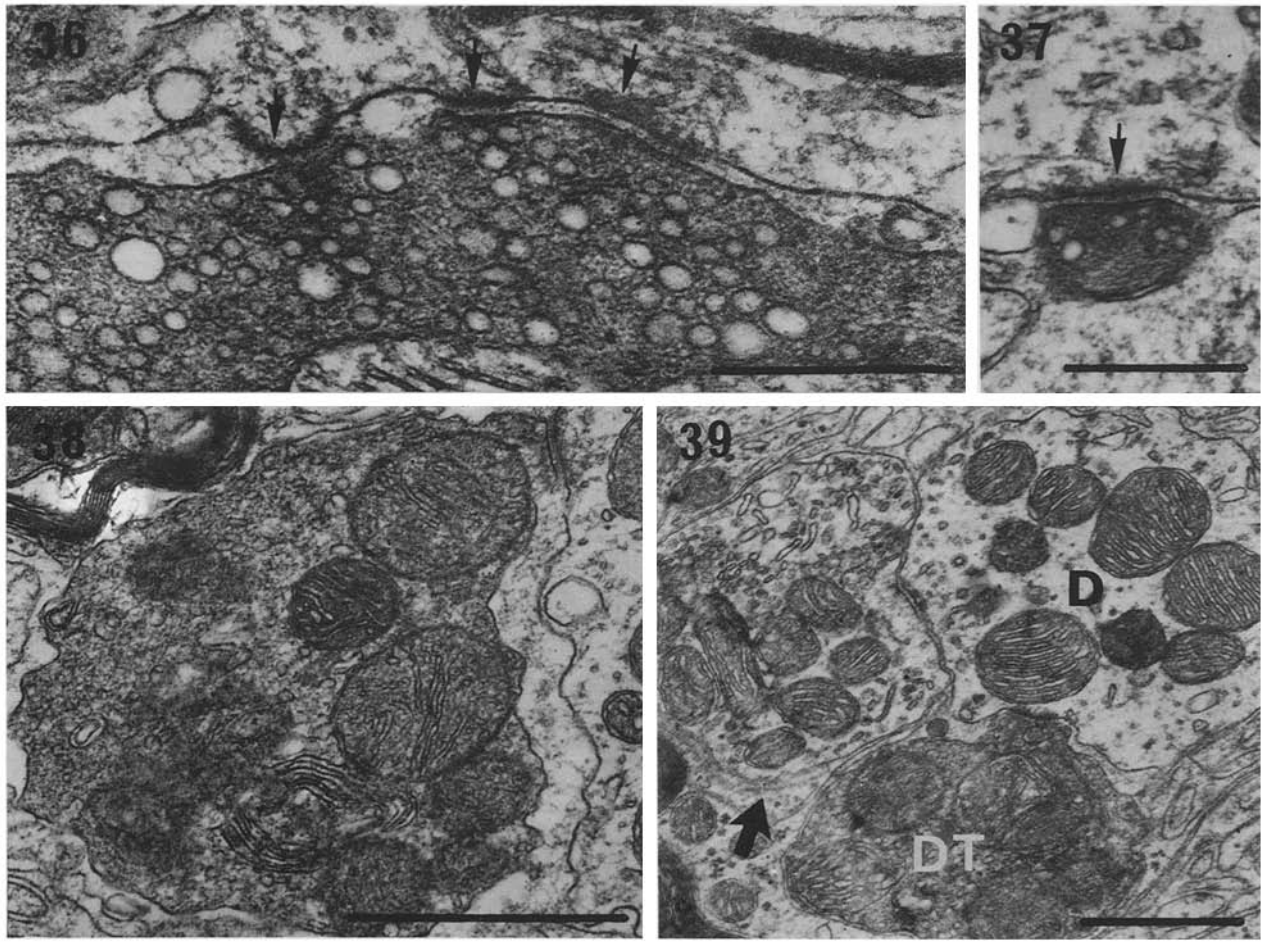


Fig. 36. Degeneration of a large terminal with round synaptic vesicles and multiple asymmetric contacts (*arrows*) in the MSO following destruction of the ipsilateral anteroventral cochlear nucleus. Scale: 0.5 μm

Fig. 37. Small terminal containing round vesicles and establishing asymmetric contact (*arrow*) degenerates in the MSO after the lesion of the nucleus of the trapezoid body. Scale: 0.5 μm

Fig. 38. Terminal of pleomorphic vesicles and symmetrical articulation degenerates in the MSO following the lesion of the ipsilateral inferior colliculus. Scale: 1 μm

Fig. 39. Intact terminal (*arrow*) containing ovoid vesicles seen in the MSO after surgical deafferentation of the nucleus. Degenerated terminal (*DT*). Dendrite (*D*). Scale: 1 μm

due to the unintentional lesion of other periolivary fibers. The occasional degeneration of large terminals establishing asymmetric contacts and containing round synaptic vesicles might be due to severed cochleo-olivary fibres crossing the midline. Following virtually complete surgical deafferentation of the MSO, the only type of axon which persisted in reasonable number contained flattened, ovoid synaptic vesicles and the synapses were symmetrical (Fig. 39). Axon terminals with a round synaptic vesicle population were, however, observed on a few occasions even in experiments where the surgery and postoperative reactive changes virtually destroyed the periolivary cell groups as well. Other types of axons identified in the previous experimental groups were found degenerated in this case, although at various stages of the process. The changes in the neuropil were indicative of extensive reactive gliosis (Fig. 39).

Discussion

This study confirms earlier observations and provides a light and EM description of ultrastructural characteristics and spatial relations of neuronal elements in

the MSO. Three types of neurons were recognized, differing in morphological features, distribution, neighbourhood relations, input and output connexions. The fusiform cells, which appear ubiquitous in the common laboratory animals e.g. mouse (Ramón y Cajal 1909), rat (Harrison and Irving 1966), cat (Stotler 1953), dog (Goldberg and Brown 1968), rhesus monkey (Strominger and Strominger 1971) and chinchilla (Perkins 1973) and were first described by LaVilla (1898), appear to be the most numerous. They were strictly oriented and successively shifted in medial and lateral directions from the axial neuronal row of the essentially crescent shaped MSO. A shortening of the dendritic span, if caused by the excentric position of the cell body, was mainly at the expense of the initial segment and was also due to the large spread of the secondary dendritic tufts. This widening reached its extreme along the concave edge of the MSO where the secondary dendrites run parallel with the circling axonal plexuses of the capsule. This is the zone where the fusiform cells may potentially be exposed to a wider range of input than the corresponding segments of the opposite dendrites or even the rest of the dendritic tree. The progressively peripheral shifting of the neurons with consequently more and more unequal contribution of the dendritic arbor to the medial and lateral half of the nucleus, which means changed input opportunities, may contribute to the differences in responsiveness of MSO neurons to stimuli (Goldberg and Brown 1968).

Although the fusiform neurons were described in previous light and EM studies, their histological separation from the multipolar and marginal cells only became possible in this study. Both the light microscopic evidence of dendritic branching pattern, lack of spines, spatial distribution, projective nature, ultrastructural characteristics and predominantly the wealth of synaptic articulations of various type characterized them unequivocally. We were able to confirm most of the numerical parameters indicated by Clark (1969a, b) and Perkins (1973) and contrasted them to the paucity of terminals, particularly of cochlear nucleus origin, available on spinous multipolar cells.

The quantitative difference amounted to 30% of the total somatic and dendritic surface covered by axon terminals if fusiform and spinous multipolar cells were compared (Kiss et al. 1982). In experiments with HRP we demonstrated the fusiform cells as projective neurons to the inferior colliculus in agreement with Elverland (1978), although the multipolar neurons labelled in our material were marginal cells only. Due to their strategic position the marginal cells appear to have opportunity to monitor the widest range of information.

Spinous multipolar cells, with an essentially spherical dendritic arbor distributed randomly, but restricted largely to the cellular core, were recognized in this study as local elements of the MSO. Both their somata and dendrites have substantially less synaptic contacts with markedly different proportions of the various presynaptic afferents. HRP labeling provided a strong although indirect evidence of their local neuron character indicated already by the Golgi material. Neurons with similar light- and electron microscopic characteristics were considered as local interneurons in several areas of the central nervous system (Szentágothai 1963; Morest 1964; Tömböl 1967; Ralston and Herman 1969). Their functional importance can be envisaged in various complex inhibitory processes (Clark and Dunlop 1968) as an alternative to the postulated presynaptic inhibition (Clark 1969a).

Four types of axons were described in this study, based on differences in size, shape, vesicle content and type of synaptic contact. Their origin was determined in degeneration experiments. Predominant among them were the large elongated terminals with multiple synaptic contacts, which were found in agreement with Perkins (1973) to degenerate following lesions of the anteroventral cochlear nucleus. HRP tracing revealed that their cells of origin were the spherical cells of the anteroventral cochlear nucleus and, to a lesser extent, the multipolar cells of the posteroventral cochlear nucleus and the pyramidal cells of the dorsal cochlear nucleus. The possibility of some uptake of HRP by fibers running through the injected locus cannot be ruled out entirely. The differences in the cells of origin might be reflected in the minute variations of the spherical vesicle diameter observed in previous studies (Clark 1969a, b). The distribution of the terminals appears to follow the gross pattern observed at the light microscope level i.e. they were found in the lateral half of the ipsilateral and in the medial half of the opposite MSO. They were more frequently found on fusiform cells but evenly distributed on the soma and proximal dendrites. Their multiple synapses at strategic loci of the projection cells are likely to provide favourable conditions for a preferential transmission of impulses. The small axon terminals containing round synaptic vesicles arise mainly from the nucleus of the trapezoid body. As already mentioned the lesion of other, mainly contralateral periolivary fibers is also a possibility which, however, could not change the picture of massive degeneration. Their most characteristic localizations were on the spines or protrusions of multipolar cell bodies and on the most peripheral

dendrites, but may occur on any surface area of all types of cells. The round vesicle population of these terminals appeared homogeneous. Axons descending from the inferior colliculus terminated with symmetrical synapses containing pleomorphic vesicles on interneurons and projection cells. The quantitative preference to the former, however, is indicative of a stronger inhibitory influence on the locally distributing interneuron than on the projection cells. The terminals that survive complete deafferentation arise obviously from local cells. As the projection cell axons are known to end in the inferior colliculus with excitatory synapses, the only possible candidate that may give rise to terminals with symmetrical membrane specialization and flattened synaptic vesicles – criteria that are thought to characterize inhibitory terminals (Uchizono 1965) – is the spinous multipolar interneuron. The inhibitory nature of interneurons on projection cells is suggested by the relatively high number of inhibitory terminals on projection cells (Kiss et al. 1982). The absence of axo-axonic synapses in this material lends additional significance to the interneurons, to descending projections from collicular level and also to their combined and/or opposite action in various inhibitory processes by feedforward and recurrent action. There were no complex or glomerular synapses with presynaptic dendrites found in this material, but the structure of the main neuronal chains and their internal arrangements are sufficiently involved to warrant a large variety of modes of transmission through the MSO.

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