

Anatomy and Projection Patterns of the Superior Olivary Complex in the Mexican Free-Tailed Bat, *Tadarida brasiliensis mexicana*

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ABSTRACT

The superior olivary complex (SOC) is the first station in the ascending auditory pathway that receives binaural projections. Two of the principal nuclei, the lateral superior olive (LSO) and the medial superior olive (MSO), are major sources of ascending projections to the inferior colliculus. Whereas almost all mammals have an LSO, it has traditionally been thought that only animals that hear low frequencies have an MSO. Recent reports, however, suggest that the medial part of the SOC in bats is highly variable and that at least some bats have a well-developed MSO. Thus, the main goal of this study was to evaluate the cytoarchitecture and connections of the principal superior olivary nuclei of the Mexican free-tailed bat, with specific attention directed at the MSO. Cell and fiber stained material revealed that the LSO and the medial nucleus of the trapezoid body (MNTB) are similar to those described for other mammals. There are two medial nuclei we refer to as dorsomedial periolivary nucleus (DMPO) and MSO. Tracer experiments exhibited that the DMPO receives bilateral projections from the cochlear nucleus, and additional projections from the ipsilateral MNTB. The DMPO sends a strong projection to the ipsilateral inferior colliculus. Positive staining for acetylcholinesterase indicates that the DMPO is a part of the olivocochlear system, as it is in other animals. The MSO in the free-tailed bat meets many of the criteria that traditionally define this nucleus. These include the presence of bipolar and multipolar principal cells, bilateral innervation from the cochlear nucleus, a strong projection from the ipsilateral MNTB, and the absence of cholinergic cells. The major difference from traditional MSO features is that it projects bilaterally to the inferior colliculus. Approximately 30% of its cells provide collateral projections to the colliculi on both sides. Functional implications of the MSO for the free-tailed bat are considered in the Discussion. © 1994 Wiley-Liss, Inc.

Key words: auditory pathways, medial superior olive, dorsomedial periolivary nucleus, periolivary nuclei

The superior olivary complex (SOC) is the first station in the ascending auditory pathway that receives converging inputs from both ears. It consists of three principal nuclei, the lateral superior olivary nucleus (LSO), the medial superior olivary nucleus (MSO) and the medial nucleus of the trapezoid body (MNTB). The three nuclei are distinguished by their position, by their principal cells, by their afferent inputs and by the targets of their projections (for review: Cant, 1991; Schwartz, 1992).

The main goal of this study was to evaluate the principal superior olivary nuclei of the Mexican free-tailed bat, with special attention directed at the MSO. The major question that we address is whether the Mexican free-tailed bat's

MSO is homologous to the MSO in other more common laboratory mammals. The question stems from the duplex theory of sound localization and ideas about the processing of binaural cues that emerged from comparative studies of the auditory system. The duplex theory holds that interaural phase disparities are the cues animals use for localizing low frequency sounds and interaural intensity disparities are the cues used for localizing high frequencies (Raleigh, 1907). Consistent with this theory are neurophysiological

Accepted December 1, 1993.

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studies, which showed that MSO neurons are sensitive to interaural phase disparities of low frequency sounds, whereas the LSO neurons process interaural intensity disparities of high frequency signals (Warr, 1966; Masterton and Diamond, 1967; Goldberg and Brown, 1968, 1969; Guinan et al., 1972a,b; Caird and Klinke, 1983; Yin and Chang, 1990).

Comparative neuroanatomical studies also led a number of authors to advance the idea that the MSO is well developed only in animals that hear low frequencies and have wide-set ears that generate interaural phase disparities that match closely with the range of timing differences that the auditory system can process, whereas the LSO is well developed only in animals that can hear high frequencies that produce large interaural intensity disparities (Harrison and Irving, 1966; Masterton and Diamond, 1967).

A number of recent reports on echolocating bats, which are small animals that hear predominantly high frequencies, showed that several species of bats have a well-developed MSO (Schweizer, 1981; Zook and Casseday, 1982a; Harnischfeger et al., 1985; Casseday et al., 1988; Vater and Feng, 1990). In one of these bats, the mustached bat, the putative MSO has been shown to have cell types, efferent projections and inputs similar, but not identical, to the MSO described in cats and other common mammals (Zook and Casseday, 1982b; Zook and Leake, 1989). The chief difference is that the ipsilateral inputs are greatly diminished in the mustached bat, and hence the majority of MSO neurons are monaural. However, these neurons utilize timing differences between the excitatory inputs from the cochlear nucleus and inhibitory inputs from the MNTB, in a manner similar to those proposed for the processing of interaural phase disparities. The difference is the absence of an input from the ipsilateral ear, thereby producing monaural response features of biological importance to the animal (Grothe, 1990, 1994; Covey et al., 1991; Grothe et al., 1992).

Other bats, however, appear to have a binaurally innervated MSO (Jen, 1978; Harnischfeger et al., 1985). For example, neurons in the putative MSO of *Molossus ater* are largely binaural. Those neurons express either EE (excitatory inputs from both ears) or EI (excitatory projections

from the ipsilateral and inhibitory projections from the contralateral ear) response properties, which are the same types found in the MSO of other mammals (Harnischfeger et al., 1985). Moreover, a closely related species, the Mexican free-tailed bat, also has a putative MSO.

Although there is no information on the response properties of putative MSO cells in Mexican free-tailed bats, a physiological study of its inferior colliculus showed that many binaural neurons have sensitivities for interaural time disparities similar to those seen in MSO neurons in other animals (Pollak, 1988). This raises the possibility that neurons in the putative MSO of Mexican free-tailed bats have functional properties similar to those of MSO neurons in animals that hear low frequencies. Since there is no information about the connections of the principal nuclei of the superior olivary complex in molossid bats, a family to which both *Molossus ater* and Mexican free-tailed bats belong, we evaluated the anatomical organization of the superior olive in the Mexican free-tailed bat. Here we show that the major features of the free-tailed bat's MSO are homologous with the MSO of other mammals, and present some ideas that address the functional implications of the MSO for the free-tailed bat. We also show that other major nuclei of the superior olivary complex, the LSO, MNTB and dorsomedial periolivary nucleus (DMPO), are similar to those of other mammals.

MATERIALS AND METHODS

Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, captured in Austin, Texas were used in this study. Three series of experiments were conducted. In the first experimental series, frozen sections from three brains were stained for cells and fibers. This material was used to distinguish the various nuclei of the superior olivary complex on the basis of location, cytoarchitecture and distinctiveness within the fiber plexus of the brainstem. Frozen sections from two other brains were processed for acetylcholinesterase (ACHE) to show efferent neurons and efferent nuclei. In the second series of experiments, horseradish peroxidase (HRP) was iontophoretically injected in one of four nuclei: inferior colliculus (two animals), medial nucleus of the trapezoid body (MNTB; one animal), anteroventral cochlear nucleus (AVCN; seven animals) and posteroventral cochlear nucleus (PVCN; two animals). Anterograde and retrograde transport of HRP was evaluated in these studies to determine both the targets of the projections from each nucleus and some of the sources of their afferent inputs. In the third experimental series, in which five bats were used, fluorescent latex microspheres were injected into the inferior colliculus. These experiments complemented the HRP injections in the inferior colliculus. In addition, different latex tracers were injected in the two colliculi of one bat, thereby allowing us to determine which neurons send collaterals to both colliculi.

General procedures for Nissl, fiber and ACHE stained material

Bats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and perfused transcardially with saline followed by 4% paraformaldehyde (Nissl and fiber stain) or 1% paraformaldehyde + 1.25% glutaraldehyde in phosphate buffer (ACHE reaction). The brains were refrigerated overnight sequentially in solutions of 10%, 20%, and 30% sucrose for cryoprotection. The brains were embedded

Abbreviations

ACHE	acetylcholinesterase
AI	primary auditory cortex
AVCN	anteroventral cochlear nucleus
CN	cochlear nucleus
DAB	diaminobenzidine
DCN	dorsal cochlear nucleus
DMPO	dorsomedial periolivary nucleus
DNLL	dorsal nucleus of the lateral lemniscus
DPO	dorsal periolivary nucleus
HRP	horseradish peroxidase
IC	inferior colliculus
INLL	intermediate nucleus of the lateral lemniscus
LNTB	lateral nucleus of the trapezoid body
LSO	lateral superior olivary nucleus
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olivary nucleus
PVCN	posteroventral cochlear nucleus
SOC	superior olivary complex
SPN	superior paraolivary nucleus
TMB	tetramethylbenzidine
VMPO	ventromedial periolivary nucleus
VNLL	ventral nucleus of the lateral lemniscus
VNTB	ventral nucleus of the trapezoid body
VPO	ventral periolivary nucleus

in egg yolk, blocked and mounted in a standard plane for sectioning. Transverse sections were cut at 36 μm in a cryostat. Sections were stained alternately with cresyl violet for cells and with the Gallyas silver method for fiber stain (Gallyas, 1979). Two brains were reacted for ACHE and counterstained with neutral red (Hardy et al., 1976).

Procedures for HRP Injections

Animals were anesthetized with Metofane, and the head secured in a head holder with a bite bar. The muscles and skin overlying the skull were reflected, and a topical anesthetic was applied to all open wounds. The surface of the skull was cleared of tissue and a nail was attached to the skull with cyanoacrylate adhesive and dental cement. A small hole was made in the skull either directly above the inferior colliculus (for injections into the inferior colliculus) or above the cerebellum (for injections in the MNTB, the AVCN or the PVCN). The animal was fixed in a stereotaxic recording apparatus. Earphones were positioned within the funnel of both pinnae, and pure tone stimuli of 10 ms duration with a fall and rise time of 1 ms were presented. Micropipettes filled with 3 M KCl or 0.2 M sodium acetate, with tips broken to a diameter of 5 μm , were used for multiunit recordings to localize sites in the inferior colliculus, cochlear nucleus (CN) or MNTB. The electrodes were advanced with a Burleigh microdrive and the best frequencies as well as the monaural or binaural properties of the unit clusters were determined every 50 μm . After the target nucleus was reached and its appropriate response properties confirmed, the recording electrode was withdrawn and replaced with an HRP electrode (10% HRP, Sigma type VI, in 0.2 M sodium acetate, pH 7.4). The tips of these electrodes were broken under microscopic observation to a diameter of 5 to 8 μm (3–6 MOhm). The electrodes were lowered into the brain and the best frequencies of units were again monitored every 50 μm . After reaching the appropriate site, HRP was deposited iontophoretically (0.7 μA constant current for 5–7 minutes). In two cases (both were injections into the cochlear nucleus) the duration of the injection was 13 and 15 minutes.

After a survival period of 24–28 hours, the bats were anesthetized with sodium pentobarbital and perfused transcardially with 0.9% sodium chloride for 5 minutes followed by 1% paraformaldehyde + 1.25% glutaraldehyde in 0.05 M phosphate buffer pH 7.4 for 30 minutes and by 10% sucrose in the same buffer for 10 minutes. The brains were removed and stored overnight in 20% followed by 30% sucrose in phosphate buffer for cryoprotection.

The following day, 48 μm transverse sections were cut in a cryostat. Three alternate sets of sections were processed with diaminobenzidine (DAB; Adams 1977) or with tetramethylbenzidine (TMB; Mesulam 1978) and mounted on gelatinized slides. Two sets were counterstained with cresyl violet (DAB) or neutral red (TMB). In most cases, one set of sections (TMB) was not counterstained.

The sections were evaluated under the microscope with brightfield or polarizing filters. If HRP labeling was ambiguous, only the DAB series was used to confirm positive HRP reaction. In order to evaluate the number and density of retrograde labeled cells within the SOC nuclei following injections of HRP in the inferior colliculus, labeled cells were drawn from the uncounterstained TMB series. These drawings were matched with drawings from the corresponding counterstained TMB sections to associate labeled cells with different SOC nuclei.

Procedures for injection of latex microspheres

Surgical procedures were the same as described above for HRP injections. Fluorescent latex microspheres (diameter 30 nm; Interfacial Dynamics Corp.) conjugated to fluorescein, rhodamine or Texas red were pressure-injected into the inferior colliculus of 5 bats. In 4 animals, only one tracer was injected unilaterally into the inferior colliculus. In one animal, fluorescein was injected into the right and rhodamine into the left inferior colliculus in order to show bilaterally projecting neurons in the SOC. The animals were anesthetized with ketanest (10 mg/ml) plus 2% rompun (doses 1 ml/100 g body weight). The tracers were in a 10% aqueous suspension (400–500 nl) and were injected by pressure through broken glass microelectrodes (tip diameter 40 μm). The injections were made deep into the inferior colliculus (about 1,200 μm) and spread throughout much of the nucleus. After 72 hours, the animals were perfused with a 4% phosphate-buffered solution of paraformaldehyde (pH 7.4). The brains were placed sequentially in solutions of 10%, 20% and 30% sucrose in phosphate buffer for 12 to 20 hours. The next day they were cut in a cryostat in 36 μm transverse sections. The sections were mounted on gelatinized slides and analyzed with a Leitz microscope equipped with a Ploemopak fluorescence device (filter blocks: I $\frac{3}{8}$ for fluorescein and N2 for rhodamine and Texas red). Since it is not possible to visualize the cytoarchitecture of the nuclei and the staining with fluorescent microspheres, the following procedure was used to evaluate number and location of filled cells. The microscope stage was equipped with two linear encoders of 1 micron resolution. The encoders were nulled on an arbitrary but significant point within each section and all drawings were related to this reference point. This allowed us to determine precisely the individual coordinates of each labeled cell. After recording the labeled cells, the sections were counterstained with cresyl violet in order to visualize the SOC nuclei. Using the individual coordinates, the labeled cells could then be counted and related precisely to the different SOC nuclei.

RESULTS

The superior olivary complex of the Mexican free-tailed bat has four principal nuclei (Fig. 1). They are the lateral superior olive (LSO), the medial nucleus of the trapezoid body (MNTB), and two nuclei that lie just medial to the LSO that we shall refer to as the dorsomedial periolivary nucleus (DMPO) and the medial superior olive (MSO). In this report we consider all four nuclei, but devote particular attention to the DMPO and MSO. Our goal is to illustrate their distinguishing features and thereby establish criteria for discussing whether these nuclei are homologous to the MSO and DMPO of other animals. The features we compare are relative location, neuronal architecture, presence or absence of cholinergic cells, and sources of afferent and targets of efferent innervation. In the section below we first present a brief description of each nucleus from Nissl- and fiber-stained material. We then present the results obtained with ACHE staining so that we can identify those nuclei that provide feedback to the cochlea or cochlear nucleus from those that do not. Finally, we turn to the patterns of afferent and efferent connections based on results of injections of HRP and fluorescent latex microspheres into the inferior colliculus, and of HRP into the cochlear nuclei and medial nucleus of the trapezoid body.

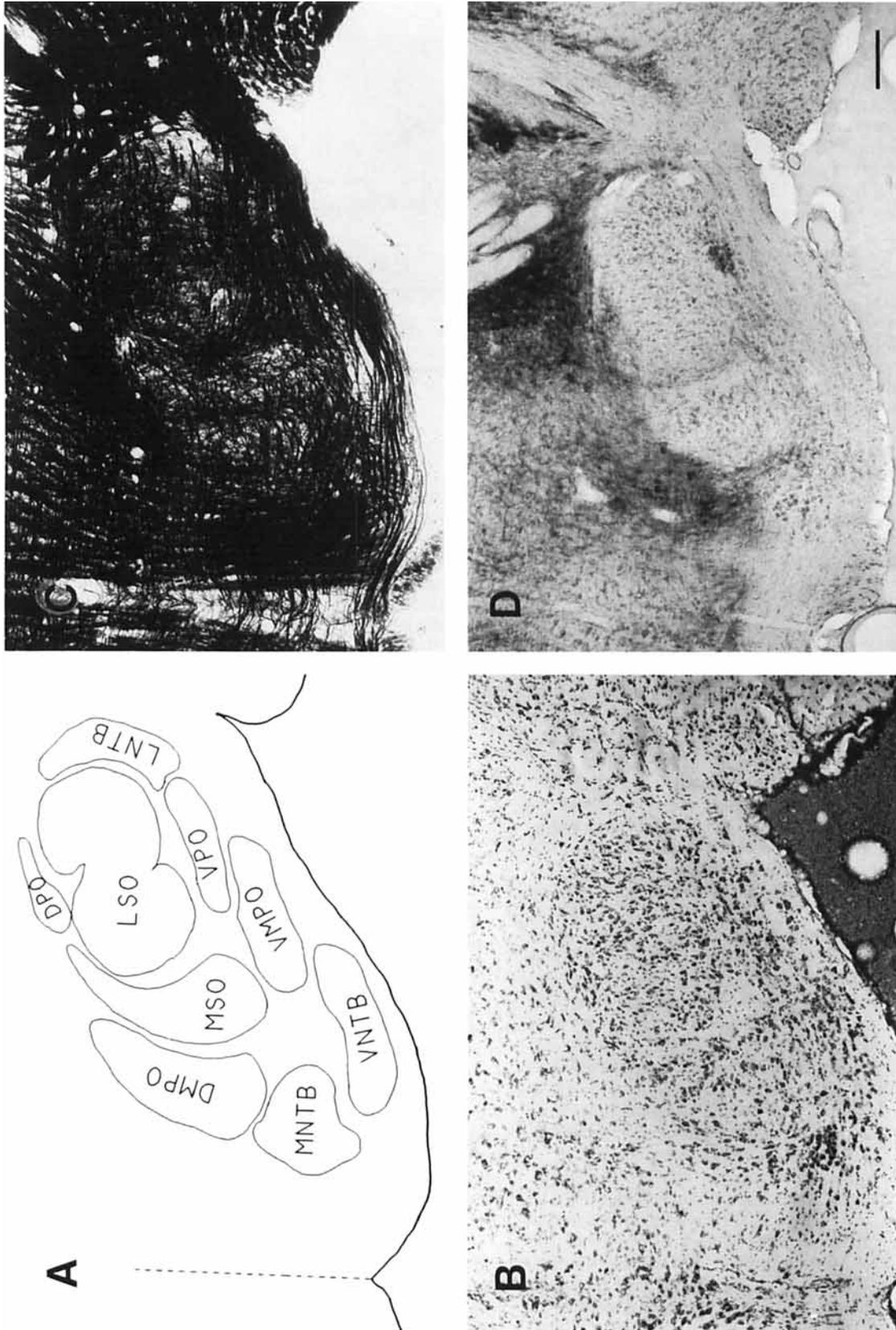


Fig. 1. The superior olivary complex in the free-tailed bat *Tadarida brasiliensis mexicana*. A: Parcellation of the SOC; schematic drawing from Nissl-stained material (B). B: Nissl-stained section. C: Fiber-stained section revealing LSO, MSO and DMPO due to the much lower density of fibers within these nuclei. D: ACHE staining, neutral red counterstained. There is no ACHE staining in the MSO and MNTB. Note the stained cell bodies in the DMPO and ventral to the LSO, as well as the prominent staining in the neuropil of DMPO and DPO. Calibration bar = 200 μ m.

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The four principal nuclei of the superior olivary complex

The relative positions of the four principal nuclei of the superior olivary complex were clearly revealed in fiber-stained material (Fig. 1C). The principal cell types and their orientation in each nucleus could also be discerned both from Nissl-stained material (Fig. 1B) and from cells that were backfilled from HRP injections made in the inferior colliculus. We present first the cytoarchitectonic features of the most laterally situated nucleus, the lateral superior olive, and then the features of the medial nuclei, MNTB, MSO, and DMPO.

The LSO principal cells were fusiform in shape in Nissl-stained sections and had primary dendrites that were oriented perpendicular to the major axis of the nucleus. These features were confirmed in sections where cells were filled with HRP following injections in the inferior colliculus. Those cells had a Golgi-like appearance, and clearly revealed that the orientation of their primary dendrites was orthogonal to the curvature of the nucleus.

The second nucleus, the MNTB, was located ventromedially. In Nissl material, its principal cells were large and oval-shaped with no apparent major dendrites. The cells were arranged in horizontal rows between the crossing fibers of the trapezoid body. One of the distinguishing features that was apparent following HRP injections in the cochlear nucleus was that large calyces of Held embraced the somata of MNTB principal cells, which were similar to those described in the MNTB of other mammals.

The third nucleus is the MSO, which lay just medial to the LSO and dorsolateral to the MNTB. The MSO was characterized both by its shape and position in the superior olivary complex. In addition, most of the principal cells were fusiform-shaped, although multipolar cells were also common. The majority of fusiform cells had dendrites that were elongated and arranged orthogonal to the major axis of the nucleus. This was particularly apparent in HRP-filled principal cells whose primary dendrites extended across the width of the nucleus (Fig. 2). However, the cells were not as tightly packed nor the arrangement as orderly as in the MSO of other mammals, such as cat and gerbil.

A fourth nucleus, the DMPO, lay medial to the MSO and dorsolateral to the MNTB. This nucleus was distinguished clearly in fiber-stained sections, and a variety of multipolar cells were observed in Nissl-stained material. We refer to this nucleus as the DMPO because of its relative location and the prevalence of large multipolar and stellate cells, which have been described in the DMPO of other mammals (Morest, 1968a). In addition, some of the cells in this nucleus stained intensely for acetylcholinesterase (ACHE; Fig. 1D and Fig. 3), a feature we consider in greater detail below. The positive staining for ACHE suggests that some of the cells comprise part of the descending olivocochlear system, as do DMPO cells in other mammals.

These four nuclei, the LSO, MNTB, MSO, and DMPO, were surrounded by a number of periolivary nuclei (Fig. 1), named according to the nomenclature of Zook and Casseday (1982a). The most prominent periolivary nucleus was the lateral nucleus of the trapezoid body (LNTB). Other periolivary nuclei include the ventral nucleus of the trapezoid body (VNTB), the ventromedial periolivary nucleus (VMPO), the ventral periolivary nucleus (VPO), and the dorsal periolivary nucleus (DPO).

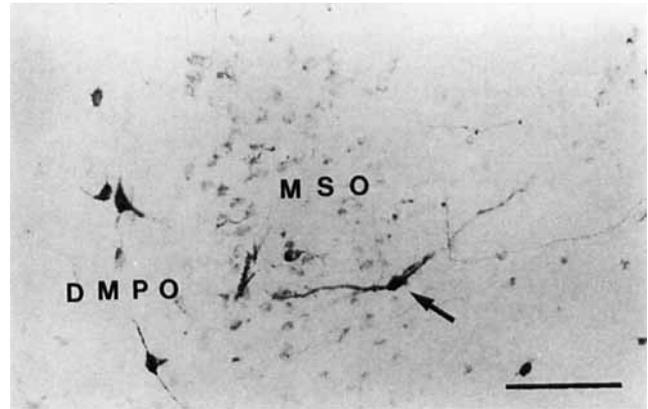


Fig. 2. Typical bipolar MSO neuron filled with HRP after an injection in the ipsilateral inferior colliculus (arrow). Note the mediolateral orientation of the two principal dendrites. Calibration bar = 100 μ m.

Distribution of ACHE

To allow us to make additional distinctions among the four principal nuclei, sections obtained from two animals were reacted for ACHE. The nature of the ACHE staining made it difficult to distinguish whether somata, dendrites or fibers were stained. Since it is well established that both the cells of the nucleus of the facial nerve and its fibers stain positively for ACHE (Brown and Howlett, 1968), we considered cells and fibers to be positively stained if the staining intensity was comparable to that of facial nerve fibers and cells in that nucleus.

The neuropil of the entire DMPO stained intensely for ACHE. Additionally, some clearly ACHE-positive somata were present, mainly located in the dorsal half of the nucleus (Fig. 1D and Fig. 3). In contrast, the neuropil of the MSO was completely free of ACHE staining and no ACHE-positive cells were found. Likewise, the MNTB and large parts of the LSO contained no ACHE-positive cells or fibers. However, some prominent cholinergic cells were found in the body of the LSO scattered within the medial half of the nucleus and close to the ventral hilus (Fig. 3).

The LSO was surrounded by several periolivary nuclei that contained numerous positive cells. Ventrally, a prominent group of cholinergic cells was in the VPO, and these cells were concentrated mainly at the ventral hilus of the LSO. Additionally, there was a prominent group of ACHE-positive cells ventromedial to MSO and ventral to DMPO that we could not unambiguously associate with one of the periolivary nuclei. Some positive cells were found in the VMPO. Dorsally, the DPO had many positive cells. The cholinergic cells in the DPO extended from the dorsal margin of the LSO ventromedially and formed a dorsoventral band between LSO and MSO. Finally, in the rostral part of the SOC the neuropil of the LNTB stained heavily for ACHE, but no positive cells could be detected.

HRP injections in the inferior colliculus

To evaluate the projections from the principal nuclei of the superior olivary complex to the inferior colliculus, we made injections of HRP in the inferior colliculus of two bats. In one bat, we made four small injections, each in a different frequency contour, whereas in the other bat, we

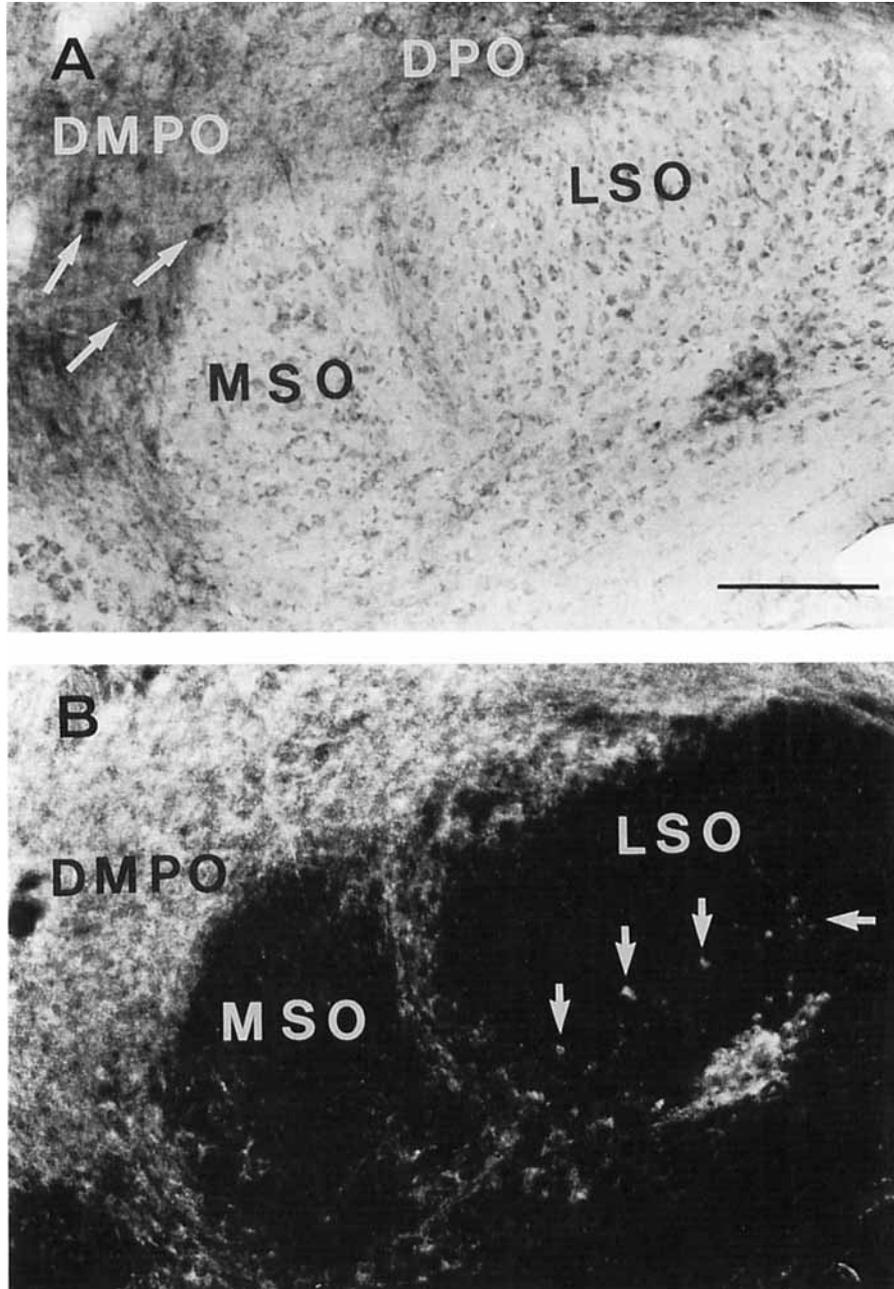


Fig. 3. ACHE activity in the SOC. **A:** Brightfield photograph of a neutral red counterstained section. Note the darkly stained cells in the DMPO (arrows), and near the ventral hilus of the LSO in VPO. **B:** Darkfield illumination of the same section as shown in A. In contrast to

the DMPO there is no ACHE activity in MSO and LSO except some ACHE-positive cells in the ventral part of the LSO (arrows). Calibration bar = 100 μ m.

made a small injection in only one frequency contour. Table 1 summarizes the results of the two experiments.

We first consider the case in which four injections in different frequency contours were made. The best frequencies of the unit clusters at the four injection sites were 24 kHz, 25 kHz, 30 kHz, and 37 kHz. These injections resulted in large numbers of labeled cells throughout most of the lower auditory nuclei. Of special interest is the labeling in the MSO and DMPO. The labeling in the MSO was somewhat surprising. In most, but not all other mammals,

there is only an ipsilateral projection from MSO to inferior colliculus. In the free-tailed bat, however, labeled cells were found bilaterally in the MSO. The majority of cells were in the ipsilateral MSO and a lesser number in the contralateral MSO. Labeled cells were found throughout the nucleus.

In DMPO, labeled cells were found mainly on the ipsilateral side and were scattered throughout the nucleus, although a few cells were found contralateral to the injection site. Labeled cells within the DMPO were mostly large multipolar cells.

TABLE 1. Labeled Neurons in the Auditory Nuclei After HRP Injections in the IC¹

	Ipsi	Contra
DCN	-	++
PVCN	-	++
AVCN	-	++
LSO	++	++
MSO	++	++
MNTB	-	-
DMPO	++	(+)
VNTB	++	-
VMPO	+	-
VPO	+	(+)
LNTB	++	++
DPO	-	-
DNLL	++	++
INLL	++	-
VNLL	++	-
IC	++	++
AI	(+)	-

¹++ = heavy labeling; + = weak labeling; (+) = few cells labeled; - = no labeling.

There was also heavy labeling in LSO bilaterally, with about equal numbers of labeled cells in the nucleus on each side (Fig. 4). Labeled cells were found throughout the rostrocaudal extent of the nucleus, but heavily labeled cells were especially numerous in the central region of the nucleus.

Other nuclei that had labeled cells include the three divisions of the contralateral cochlear nucleus, several of the periolivary nuclei, and the three divisions of the nuclei of the lateral lemniscus. Labeling was found only in the ipsilateral ventral and intermediate nuclei of the lateral lemniscus, while bilateral labeling was found in the dorsal nucleus of the lateral lemniscus. The patterns of labeling were similar in both DAB and TMB reacted material, although the number of labeled cells was far greater in the TMB material.

Labeling in the periolivary nuclei was diffuse and not very prominent. Labeled cells were found ipsilaterally in VNTB, VPO, VMPO, and LNTB, contralaterally in LNTB and in VPO. The highest density of HRP-labeled cells among the periolivary nuclei was found in the LNTB bilaterally.

Additionally, there were stained cells in the contralateral inferior colliculus and few cells in the ipsilateral auditory cortex. Many labeled fibers and terminals were found within the ipsilateral medial geniculate body.

In the second bat, only one injection was made in the inferior colliculus and it was confined to the 23 kHz frequency contour. The pattern of labeling was similar to that observed in the previous case. The major difference was that fewer cells were labeled due to the smaller injection, and the labeled cells were restricted to regions of each nucleus that presumably represented 23 kHz. Labeled cells in the MSO, for example, were also found bilaterally and were confined to the dorsal portion of the nucleus. In LSO, labeled cells were found bilaterally but only in the lateral limb. Labeling in the other periolivary nuclei, as well as the nuclei of the lateral lemniscus, was similar to that seen in the first case.

Injections of fluospheres in the inferior colliculus

In order to confirm and extend the results of the HRP injections described above, we injected fluospheres with different fluorochromes into the inferior colliculus in five animals. In four of the animals, only one fluorochrome was

injected into the colliculus. In one animal, however, fluorescein was injected into one colliculus and rhodamine into the other colliculus. By noting which cells were labeled with both fluorochromes, we determined whether cells in nuclei that project bilaterally to the inferior colliculus send branches to both colliculi or whether they project only to one colliculus. The injections were large, encompassing about 30 to 60% of the inferior colliculus, which resulted in a substantial amount of retrograde labeling. The large number of labeled cells allowed us to quantify the projections with some confidence.

The results obtained when one fluorochrome was injected in the inferior colliculus were consistent in all four experiments, and were similar to the pattern of labeling obtained with HRP injections. Labeled neurons were found bilaterally in MSO, DMPO, LSO, VPO, VMPO, and LNTB. The proportion of ipsi- or contralaterally labeled neurons differed among nuclei (Fig. 5).

Turning first to the MSO, the majority of labeled cells were found in the ipsilateral MSO (about 60% ipsilateral and 40% contralateral). The predominance of the ipsilateral labeling was even greater in the DMPO (about 89% of the labeled cells were in the ipsilateral DMPO). The labeling in the LSO, in contrast, was about equally divided between the two LSOs. The labeling in the VMPO was almost largely ipsilateral and comprised about 78% of the labeled cells. In contrast, about 61% of labeled VPO and about 57% of labeled LNTB neurons were contralateral to the injection side.

In the double labeling experiment, where fluorescein was injected in the right and rhodamine in the left inferior colliculus, about one-third (31%) of MSO neurons that were labeled had both fluorescein and rhodamine fluorochromes (Fig. 6). This suggests that about 30% of MSO neurons project to both the ipsilateral and contralateral inferior colliculi. The double labeling in the other nuclei of the superior olive was markedly different. Only a negligibly small percentage of labeled cells in DMPO, LSO, VMPO, VPO, and LNTB were double labeled. Since each of these nuclei provides a substantial bilateral projection to the inferior colliculus, it would appear that separate cell populations project to the ipsilateral and contralateral colliculus.

HRP injections in the MNTB

We made HRP injections in the MNTB of one bat to evaluate both the source of its inputs and the targets of its axonal projections (Fig. 7). One of the injections was made in the rostral MNTB, where the best frequency was 63 kHz, and the other more caudally where the best frequency was 42 kHz. Because of the short distances among the nuclei in the superior olivary complex, we evaluated anterograde transport from the MNTB only in DAB material. For retrograde transport, we investigated both DAB and TMB reacted material.

We turn first to the cells projecting to the MNTB. The majority of labeled cells were in the caudal part of the contralateral AVCN. Most of the cells were most likely globular bushy cells since they had only one dendrite with profuse branching that was evident in cells filled with HRP. There were also cells labeled in the dorsal part of the contralateral PVCN, although the number of cells in PVCN was smaller than in AVCN. The labeled cells in PVCN were large multipolar cells with a spherical or globular cell body. One of the labeled cells was similar to octopus cells described in other mammals.

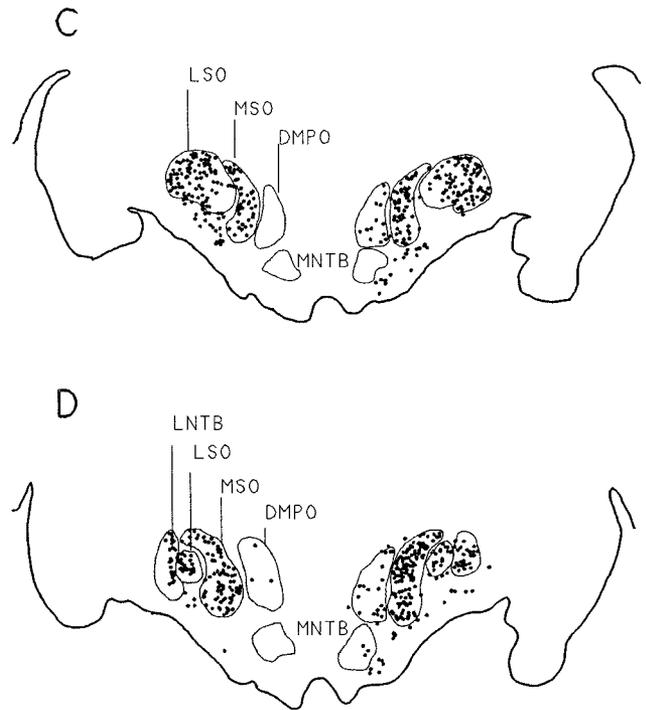
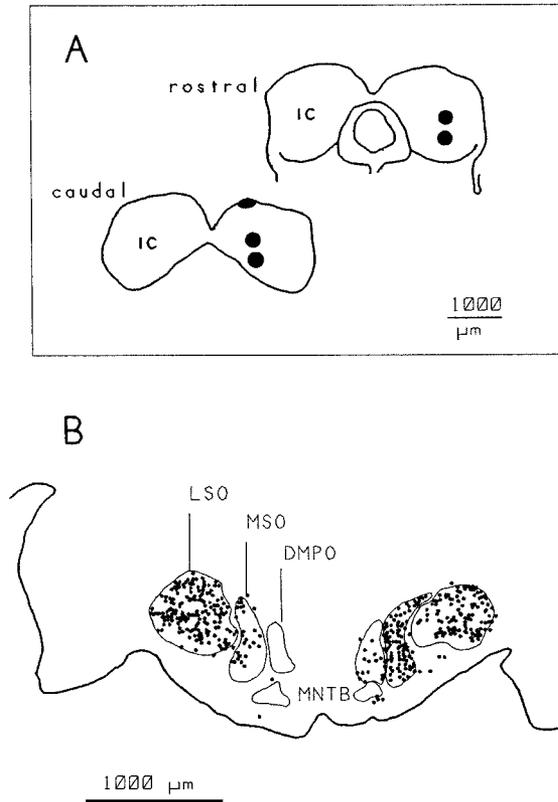


Fig. 4. Labeling pattern in the SOC after injections of HRP in the central nucleus of the inferior colliculus. **A:** Injection sites in the inferior colliculus (two injections are made more caudal, and two more rostral in the same inferior colliculus). **B–D:** Drawings of labeled

neurons within the SOC from TMB reacted material (B is caudal, D more rostral). Note the heavy bilateral labeling in LSO and MSO and that labeled neurons in DMPO are mainly ipsilateral.

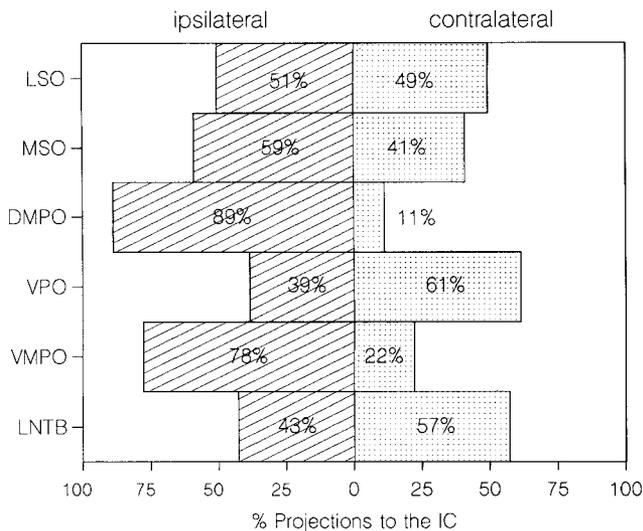


Fig. 5. Percentage of ipsilaterally and contralaterally projecting neurons in the nuclei of the SOC after injection of rhodamine into the inferior colliculus. Note that a high percentage of MSO neurons project contralaterally, whereas most DMPO neurons project ipsilaterally.

Filled fibers from the MNTB were followed into MSO, DMPO, LSO as well as some of the periolivary nuclei and to the ventral nucleus of the lateral lemniscus (VLL) and

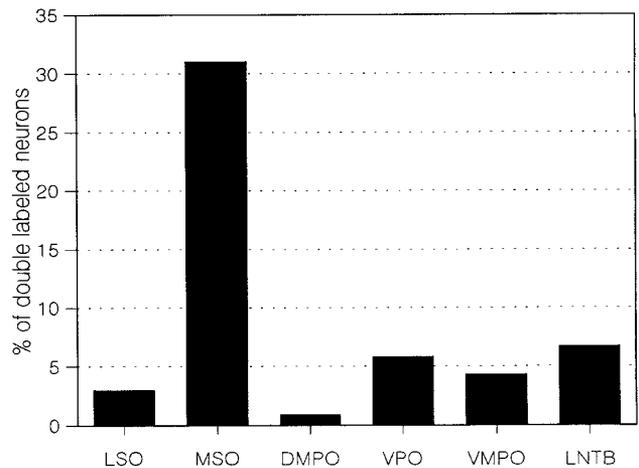


Fig. 6. Neurons projecting bilaterally to the inferior colliculus: percentage of double labeled neurons in the nuclei of the SOC after injections of fluorochromes in the ICs of both sides.

intermediate nucleus of the lateral lemniscus (INLL). The projections to each of these nuclei were not equally strong. The projections to MSO were substantial, although not as heavy as those to the LSO, which is described below. Projections were confined to the ipsilateral MSO. The fibers entered the MSO medially, and terminated predomi-

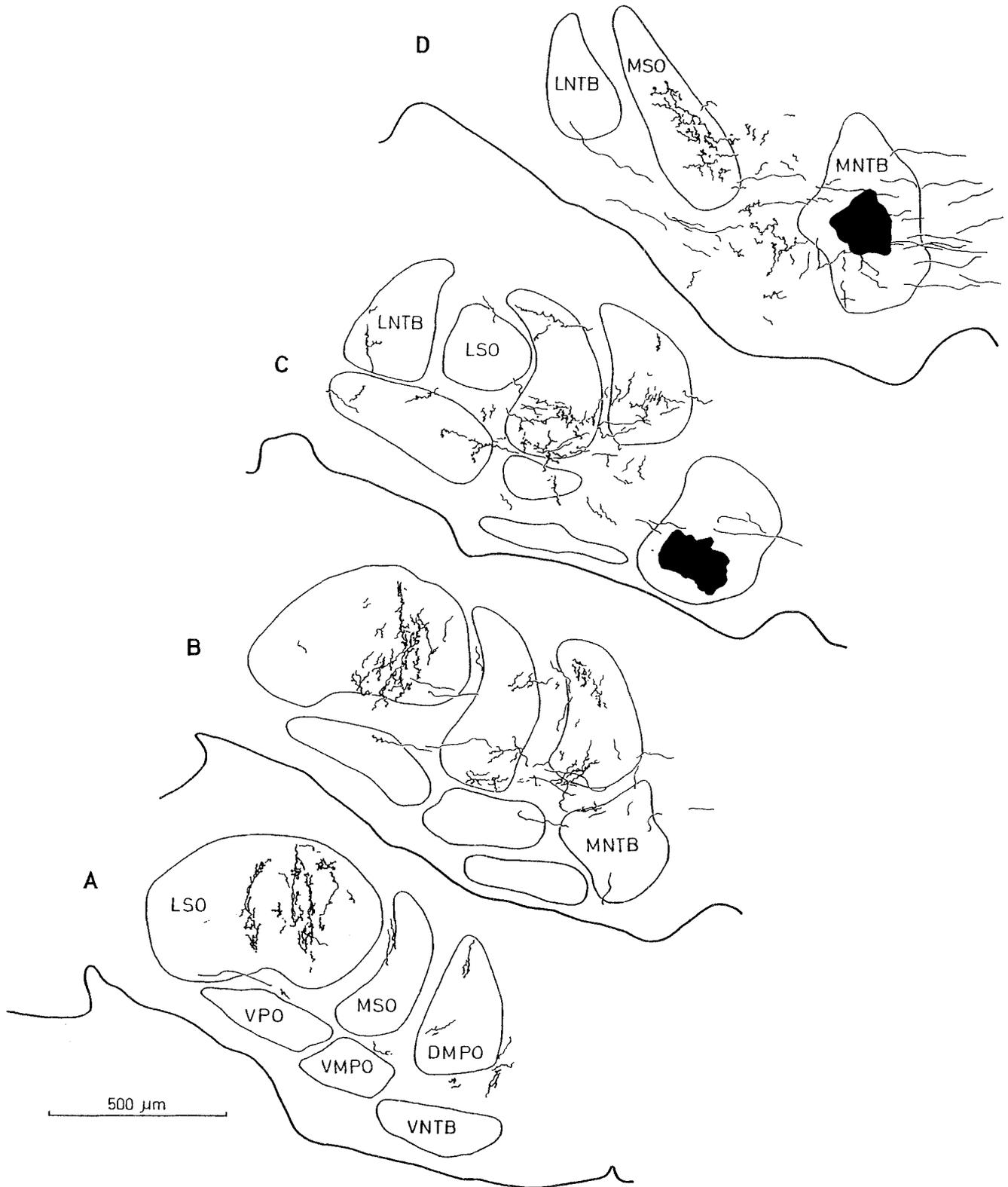


Fig. 7. **A–D:** Labeling pattern in the ipsilateral SOC after injections of HRP in two different regions of the MNTB (blackened areas in C and D) as it appears in DAB-reacted material. A is the most caudal, D the most rostral section. Note the prominent projections to LSO, MSO, and DMPO.

nantly in the medial half of the nucleus. The terminals within the MSO were largely in the ventral portion of the nucleus, and provided innervation along much of the rostrocaudal extent of the ventromedial quadrant of the nucleus. This portion of the MSO presumably represents high frequencies which correspond to the high frequency areas of the MNTB in which the injections were made.

There were also projections to the DMPO. The projections to DMPO were less prominent than those to the MSO and LSO. The fibers projecting to the DMPO entered the nucleus ventrally and the terminals were not restricted to a defined area.

The main target of the MNTB was the ipsilateral LSO. The HRP-filled fibers projecting to the ipsilateral LSO were arranged in small bundles and entered the nucleus ventrally and dorsally. The fibers extended throughout the width of the nucleus, and thus appeared to provide innervation to most, if not all, of the cells in two distinct frequency contours. Those presumably represented 42 and 63 kHz, which corresponded to the best frequencies at the injection sites.

Fibers and terminals were also seen in the ipsilateral VNLL and INLL, although the projection was less pronounced than that to MSO and DMPO. Finally, weak projections were also seen close to the injection sites in the ipsilateral VNTB and VMPO.

HRP injections in the AVCN

To investigate the projections of the AVCN to the nuclei of the superior olivary complex, we made small injections of HRP in the AVCN of six bats. There were several chief targets of the AVCN, which include the MSO, LSO, MNTB, VNLL, INLL, and inferior colliculus. In addition, there were weaker projections to other targets that include DMPO, LNTB, VPO, VMPO, and VNTB (Fig. 8). The results were consistent among the animals.

We turn first to the projections to the MSO, which were bilateral. The ipsilateral projections penetrated the MSO laterally and terminated largely in the ipsilateral half of the nucleus. The contralateral projections, on the other hand, entered the MSO ventromedially, and terminated mainly in its medial half. Injections into the low frequency region of the AVCN led to terminals more dorsally in the MSO, and injections into the high frequency part of the AVCN to more ventral labeling. There were also clear projections from the AVCN to the contralateral DMPO, where terminals extended throughout the entire nucleus. However, the projections from AVCN to DMPO were less prominent than they were to either MSO or LSO that are described next. Projections to the ipsilateral DMPO were unclear. The reason is that although in most experiments some fibers were stained in the ipsilateral DMPO, we could clearly see a few terminals in the sections from only one animal. If there is an ipsilateral projection from AVCN to DMPO, it apparently is very weak.

There were prominent projections to the ipsilateral LSO. The fibers entered the nucleus ventrally, dorsally and laterally. The terminals were restricted to bands that appeared to correspond to frequency contours that spanned the width of the nucleus. The loci of the bands varied with the frequencies of the unit clusters in the AVCN where the injections were made in the AVCN; injections in lower frequency regions of AVCN produced labeled fibers and terminals in the lateral limb of the LSO, whereas injections

in higher frequency regions of the AVCN produced labeling in the medial limb of LSO.

The AVCN also provided strong projections to the contralateral MNTB. These were characterized by large, calyciform synapses on the MNTB cell bodies. Our data did not exhibit a clear tonotopic organization of the nucleus.

There were also substantial projections to the contralateral INLL, VNLL and inferior colliculus. In addition, there were terminals in ipsilateral VPO, VMPO and LNTB and in the contralateral VNTB, VMPO, and in a small band of cells medial to the nuclei of the lateral lemniscus.

Many retrogradely labeled neurons were found contralateral to the injection site in VNTB, VMPO, dorsal to the LSO in the DPO, and a few cells in VPO. In some experiments a few cells were also labeled in the ipsilateral VNTB. No labeled cells were found in the DMPO on each side.

HRP injections in the PVCN

HRP injections were made in the PVCN of two bats. One injection was at a ventrolateral (low frequency) and the other at a dorsomedial (high frequency) position. The results from these two experiments were consistent.

The major projections in both cases were to the MSO, LSO, MNTB, VNLL, INLL, and inferior colliculus. There were also fibers and terminals in the other divisions of the cochlear nucleus. Lesser projections were to the DMPO, LNTB, VPO, and VNTB.

Projections to the MSO were bilateral, where the contralateral projection was stronger than the ipsilateral projection. In each bat, the only ipsilateral projection observed was one fiber that entered the MSO, but in both bats clear terminals from the fibers were seen. The projection from PVCN to DMPO was exclusively to the contralateral DMPO.

The projections to LSO were entirely ipsilateral, and terminated in an apparent tonotopic fashion, i.e., fibers and terminals were in different portions of the LSO depending upon the injection site in the PVCN. There was a heavy projection to the contralateral MNTB where the fibers made synaptic connections on MNTB cells with large calyciform terminals. There were no projections to the ipsilateral MNTB.

Ipsilaterally there were labeled fibers and terminals in the DCN, AVCN and PVCN. The terminals in the cochlear nucleus may have been partly due to retrograde filling of ascending auditory nerve fibers that bifurcated and thus provided innervation to other portions of the cochlear nucleus.

There were also a few fibers and terminals in the ipsilateral VMPO, VPO, LNTB, and bilaterally in the VNTB. The projection to the VNTB was stronger contralaterally than ipsilaterally.

Retrogradely labeled neurons were found only contralateral to the injection site in VNTB, VMPO and a few cells in VPO. No labeled neurons were found in the DMPO.

DISCUSSION

The main finding of this study is that the superior olivary complex in the Mexican free-tailed bat is similar in many key respects to the superior olivary complex of other mammals. Below, we discuss the major features that distinguish each of the principal nuclei in other mammals which have been traditional subjects for studies of the mammalian auditory system. We then show that the features of superior olivary nuclei in the free-tailed bat compare favorably

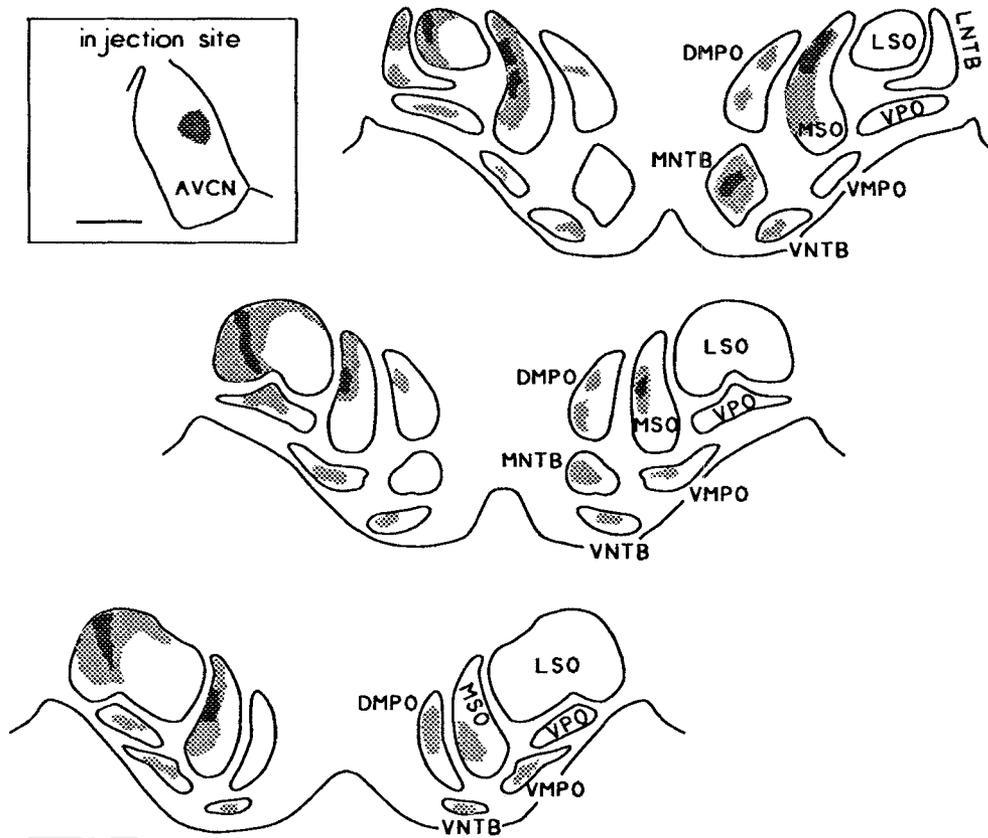


Fig. 8. Anterograde labeling pattern in the SOC after a HRP injection in the medial part of the AVCN (BF: 26 kHz, **inset** shows the injection site). Hatched areas indicate regions that contained labeled terminals. Darkly hatched fields display the areas of the highest density of labeled terminals. Calibration bars = 500 μ m.

with those generally acknowledged for the principal olivary nuclei in other mammals, which leads us to conclude that the MSO, DMPO, LSO, MNTB, and the other periolivary nuclei are homologous to the superior olivary nuclei of other animals.

MNTB and LSO

The anatomical organization of the free-tailed bat's MNTB and LSO are comparable in all major respects to those of other mammals. Turning first to the MNTB, the prominent projections from the contralateral AVCN terminate axosomatically on the MNTB cells as calyces of Held, as in other mammals (Morest, 1968a,b; Nakajima, 1971). MNTB neurons project mainly to the ipsilateral LSO, although there are weaker efferent projections to the MSO and DMPO. (Figure 9 summarizes the connection patterns of the SOC.)

The latter two were described recently for several mammals and are known to be collaterals of the MNTB-LSO projection (Kiss and Majorossy, 1983; Spangler et al., 1985; Kuwabara and Zook, 1992). Additionally, the projections to the VNLL and INLL in free-tailed bats have also been found in other mammals (Glendenning et al., 1981; Spangler et al., 1985).

The LSO in the free-tailed bat is also typically mammalian. Thus, its neuronal architecture is consistent with the classical description of the orderly arrangement of its

fusiform cells with dendrites oriented perpendicular to the curvature of the nucleus, and the afferent and efferent connections of the free-tailed bat's LSO are virtually identical to those reported for other mammals (Ramón y Cajal, 1909; Scheibel and Scheibel, 1974; Spangler et al., 1985; Cant and Casseday, 1986). We also point out that the presence of cholinergic neurons within the main body of the LSO is not inconsistent with findings of other studies (Altschuler et al., 1983). In general, cholinergic neurons within the SOC are known to belong to the efferent system (Warr, 1992). The loci of the efferent cell groups are highly variable among mammals, and efferent neurons are found in the LSO of several animals (Brown and Howlett, 1972; Warr, 1975; Aschoff and Ostwald, 1987; Aschoff et al., 1988; Vetter and Mugnaini, 1992).

MSO

One major impetus for this study was to determine whether the free-tailed bat has an "MSO" homologous to the MSO of other mammals. Below we show that the nucleus that we refer to as the MSO shares a number of features with the MSO of the cat, but we also point out a number of features that are not entirely consistent with the traditional view of the MSO. As we will show, the features that appear to be different in the free-tailed bat's MSO are actually differences in the degree to which those features

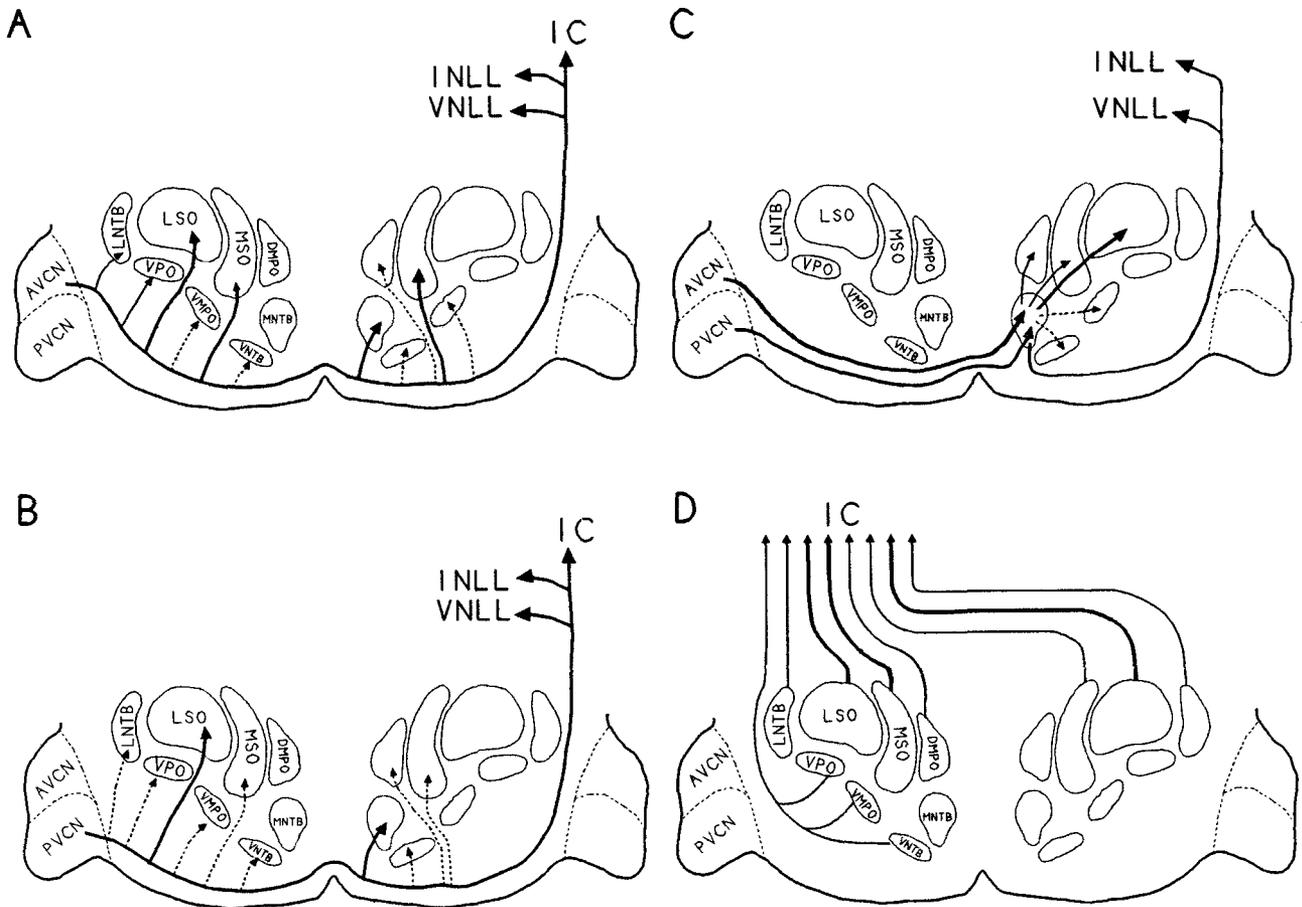


Fig. 9. Schematic drawings of the afferent and efferent projections of the SOC in the free-tailed bat. **A:** Projections from the AVCN. **B:** Projections from the PVCN. **C:** Connections of the MNTB. **D:** Ascending projections to the inferior colliculus.

are present rather than the appearance of new features that are not present in the MSO of other mammals.

The MSO in the free-tailed bat is a clearly defined cell group situated medial to the LSO and dorsolateral to the MNTB, exactly where the MSO is found in other mammals. Its principal cells are predominantly fusiform and their primary dendrites are oriented mediolaterally, and thus orthogonal to the major axis of the nucleus, which is also consistent with the type and arrangement of MSO principal cells in other mammals (Ramón y Cajal, 1911; Stotler, 1953; Irving and Harrison, 1967; Scheibel and Scheibel, 1974; Schwartz, 1977; Schofield and Cant, 1991). Additionally, the free-tailed bat's MSO receives about equal afferent inputs from both the ipsi- and contralateral AVCN, as well as projections from the ipsilateral MNTB (Stotler, 1953; Warr, 1966; Goldberg and Brown, 1968; Kiss and Majorosy, 1983; Spangler et al., 1985; Cant and Casseday, 1986; Covey et al., 1991; Kuwabara and Zook, 1992; Kandler and Friauf, 1993; Grothe and Sanes, 1993). Finally, there are no descending projections to the cochlear nucleus from the MSO in the free-tailed bat, and the absence of any positive staining for ACHE also suggests that the MSO does not send fibers to the cochlea. The absence of descending projections is a feature that distinguishes the MSO from DMPO or from the superior paraoli-

vary nucleus (SPN), which have descending projections (see below).

However, in the MSO of some other mammals, such as the cat and gerbil, the fusiform cells are arranged as a highly ordered dorsoventral cell column, whereas in the free-tailed bat the fusiform cells have a less orderly arrangement and are intermingled with many multipolar cells. We suggest that the difference in the orderliness of the cellular arrangement is more a difference in the degree than of kind. A similar argument applies to the large number of multipolar cells. Although we did not quantitatively assess the population of multipolar cells in free-tailed bats, it is our impression that it is somewhat larger than has been observed in the cat or gerbil, but this is also a quantitative rather than a qualitative difference.

A potentially more significant difference is that MSO neurons in most mammals project only ipsilaterally to the inferior colliculus (Beyerl, 1978; Elverland, 1978; Roth et al., 1978; Schweizer, 1981; Zook and Casseday, 1982b; Nordeen et al., 1983; Schofield and Cant, 1991), whereas in the free-tailed bat more than 30% of individual MSO cells send collaterals to both the ipsi- and contralateral inferior colliculi. However, the absence of any contralateral projection may not be as absolute as most previous reports suggest. For example, with large HRP injections in the

inferior colliculus of the cat and guinea pig, a few labeled cells were often seen in the contralateral MSO (D. Oliver personal communication and R. Saint Marie, personal communication). This is reinforced by Adams (1979), and Brunso-Bechtold et al. (1981) who presented some evidence for a bilateral MSO projection in the cat. This suggests that, like the orderliness of the cellular arrangement and the population of multipolar cells, the projection from the contralateral MSO to inferior colliculus represents a quantitative rather than a qualitative difference among species. This suggestion is further supported by studies of the mole (Kudo et al., 1990) and methaterian opossum (Willard and Martin, 1984). In those species, like the free-tailed bat, there are also prominent bilateral MSO projections to the inferior colliculus. Taken together, these considerations lead us to conclude that this nucleus is indeed an MSO and is homologous to the MSO of other mammals.

DMPO

In the cat, the DMPO is a large nucleus located medial to the MSO and dorsolateral to the MNTB (Morest, 1968a). However, in some rodents a nucleus in the same location has been referred to as the SPN (Harrison and Feldman, 1970; Olo and Schwartz, 1979; Nordeen et al., 1983; Schofield, 1991). There is considerable confusion in the literature with regard to whether the DMPO and SPN are separate nuclei or whether they are homologous, as has been suggested by several authors (e.g., Nordeen et al., 1983; Schwartz, 1992). Indeed, in some reports of the guinea pig's superior olive, this nucleus is called the SPN (Schofield, 1991; Schofield and Cant, 1991), whereas in other reports the same nucleus in the same animal is referred to as the DMPO (Robertson and Winter, 1988; Saint Marie and Baker, 1990).

Judging from the descriptions of SPN and DMPO in the literature, both are composed of a variety of different cell types, among which are very large multipolar cells. The arrangement of the cells is less orderly than in LSO, MSO, or MNTB. Both DMPO and SPN not only have various cell types, but they are also composed of more than one subpopulation of cells, where each subpopulation has a defined afferent and efferent projection pattern, and presumably a corresponding function (Morest, 1968a; Harrison and Feldman, 1970; Olo and Schwartz, 1979; Adams, 1983; Nordeen et al., 1983; Aschoff and Ostwald, 1987; Bishop and Henson, 1987; Robertson and Winter, 1988; Saint Marie and Baker, 1990; Schofield, 1991; Schofield and Cant, 1991, 1992; Thompson and Thompson, 1991). What is unclear is whether the SPN and DMPO have the same complement of neuronal subpopulations, and are in reality the same nucleus, or whether they have different subpopulations, and are distinctly different nuclei. In a previous report, the presence of ACHE-positive cells was considered as a possible feature that distinguished DMPO from SPN (Olo and Schwartz, 1979). This is illustrated by the cat DMPO, which has a large number of cholinergic cells (Rasmussen, 1946), whereas the SPN in the rat stains poorly for ACHE (Brown and Howlett, 1972), and in mice the SPN is ACHE-free (own unpublished results). This comparison indicates that there is at least one identifiable cell group which is always represented in the DMPO, but is missing in the mouse and probably in rat SPN. Based on these features, we refer to a nucleus situated medial to the MSO, that has the other properties described above, and a distinct population of cholinergic neurons, as a DMPO.

Thus, we call the nucleus in the free-tailed bat, situated medial to the MSO, the DMPO since it has the features described above which characterize that nucleus. Among these are a variety of cell types that include large multipolar cells, bilateral inputs from the cochlear nuclei and bilateral projections to inferior colliculus. Furthermore, the predominance of projections to the inferior colliculi are ipsilateral, and are in close agreement with the predominance of ipsilateral projections to the inferior colliculus from the DMPO of the guinea pig (Saint Marie and Baker, 1990; Schofield and Cant, 1992), chinchilla (Saint Marie and Baker, 1990) and cat (Adams, 1983). Since this would also apply to the SPN, the defining feature for calling this nucleus in the free-tailed bat a DMPO is the presence of ACHE-positive cells, which suggests that it contributes to the olivocochlear system, as does the DMPO in other mammals. The contribution to the olivocochlear system is further supported by the findings of Aschoff and Ostwald (1987), who injected fast blue in the cochlea of free-tailed bats and found labeled cells in precisely the area in which the DMPO is located.

Functional considerations

The functional significance of the DMPO is poorly understood, since no systematic electrophysiological investigations of DMPO neurons have been undertaken. Thus, we only can state that the projection patterns indicate that at least some cells in this nucleus are involved in binaural processing, and that some neurons convey information to higher centers, whereas others contribute to the efferent system.

The LSO in the free-tailed bat is almost certainly involved in the processing of binaural information for the localization of high frequency sounds. The principal cue for localizing high frequency sounds is the interaural intensity disparity (IID). In all mammals, IIDs are initially compared in the LSO by neurons that receive a direct excitatory input from the ipsilateral cochlear nucleus and an inhibitory input from the contralateral cochlear nucleus via the projection from the MNTB (for review see: Tsuchitani and Johnson, 1991; Irvine, 1992). As mentioned previously, the LSO of free-tailed bats has similar connections, which strongly suggests that its LSO, like that of other mammals, is dominated by EI neurons. This suggestion is further supported by a study of the LSO in a closely related species, *Molossus ater*, in which LSO cells were almost exclusively excitatory-inhibitory (Harnischfeger et al., 1985).

The function of the MSO in the free-tailed bat, however, is an open question. There are actually two questions that we address below. The first question is whether MSO neurons in the free-tailed bat can process binaural information in the same manner as MSO neurons do in other mammals. The second question is, if the processing is comparable, does the MSO have the same functional role in free-tailed bats as it presumably does in animals that hear low frequencies.

We turn first to the question of information processing in the MSO. The classic view of the MSO is that it is a nucleus whose neurons code for interaural time differences (ITDs) of low frequency signals (Masterton and Diamond, 1967; Goldberg and Brown, 1969; Guinan et al., 1972b; Caird and Klinke, 1983; Langford, 1984; Yin and Chan, 1990). These signals generate discharges in the auditory nerve and cochlear nucleus that are phase-locked to the cycle-by-cycle action of the stimulus (Galambos and Davis, 1943; Rose et

al. 1967; Johnson, 1980). MSO neurons compare the relative arrival times of the excitatory and inhibitory inputs from the two ears, and express the comparisons through variations in its firing rate (Goldberg and Brown, 1969; Caird and Klinke, 1983; Langford, 1984; Yin and Chan, 1990; Grothe and Sanes, 1994). Thus, MSO neurons discharge maximally for a particular ITD, and less so or minimally for others.

The problem with the so-called coincidence hypothesis (Jeffress, 1948) for the free-tailed bat is that it has poor low frequency hearing (Schmidt et al., 1990), and that its small headwidth generates very small ITDs that are, at most, only about 50 μ sec (Pollak, 1988). The MSO in the free-tailed bat, however, could still process high frequency signals binaurally in a manner similar to that described above for low frequencies in other animals. Coincidence detection of the arrival times of discharges evoked by the two ears could be achieved by converting the large interaural intensity disparities generated at the ears into latency differences, that is, through a time-intensity trade (Galambos et al., 1959; Moushegian et al., 1964; Hall, 1965; Brugge et al., 1969; Goldberg and Brown, 1969; Kitzes et al., 1980; Caird and Klinke, 1983; Yin et al., 1985; Pollak, 1988). The latency differences generated in this way would produce arrival time disparities at the MSO neurons that are much larger than those that could be generated by the interaural time disparities. Thus, an MSO neuron would still receive excitatory and inhibitory inputs from both ears, and its discharge rate would be determined by their relative arrival times. The major difference is that in the case of mammals that hear low frequencies, the timing of the discharges would be governed by the arrival times of the signals at the two ears, whereas in free-tailed bats, it would be governed by the intensity differences at the two ears that are converted into latency differences in the nervous system.

Although there have been no neurophysiological studies of MSO neurons in the Mexican free-tailed bat, there is some information about the response properties of MSO cells in *Molossus ater* (Harnischfeger et al., 1985). Of significance is that MSO neurons in *Molossus ater* are binaural. About half of the cells are EE (receiving excitatory inputs from both ears) and the other half are EI cells (excitatory projections from the contralateral ear and inhibitory projections from the ipsilateral ear). Several of these cells, moreover, are sensitive to interaural time disparities in the range of tens of microseconds, and they exhibited time-intensity trading. The temporal sensitivity of some MSO cells in *Molossus ater* are, therefore, similar to those reported for MSO cells in other mammals. These features suggest that the manner with which binaural signals are processed in the MSO of molossid bats is similar to the binaural processing in the MSO of other mammals.

The next question we address is why it is that animals which hear both high and low frequencies partition the processing of cues for localization to two nuclei, the MSO for low frequencies and the LSO for high frequencies, whereas molossid bats, which hear primarily high frequencies, apparently employ two nuclei for binaural processing of the same range of frequencies. We point out in this regard that the generally held notion that the MSO processes only low frequencies and that the LSO processes only high frequencies is oversimplified. For example, while the MSO has an overrepresentation of neurons tuned to low frequencies, about one-third of MSO neurons in the cat and dog are tuned to frequencies above 4–5 kHz (Goldberg and

Brown, 1969; Guinan et al., 1972b). We consider these to be “high frequencies” since phase-locking to sinusoidal stimulation fails completely in this frequency range (e.g., Kiang et al., 1965). It seems reasonable, therefore, to suggest that a substantial population of MSO cells in the cat and other mammals is concerned with the binaural processing of high frequency stimuli. Similarly, the LSO is dominated by neurons tuned to high frequencies, but there is also a substantial population tuned to low frequencies, which evoke phase-locked discharges (Finlayson and Caspary, 1991). In the cat, low frequency LSO neurons are apparently monaural (Boudreau and Tsuchitani, 1968; Guinan et al., 1972b), but in the chinchilla, low frequency LSO neurons are EI, process binaural phase-disparities, and exhibit time-intensity trading (Finlayson and Caspary, 1991). Thus, it would appear that in some mammals at least, localization of low frequency sounds is partially accomplished by binaural processing in the LSO.

Given these features, we suggest that if the MSO of the free-tailed bat is involved in processing binaural information for localization of high frequencies, it may simply be a feature that it shares with the MSO of other mammals but in free-tailed bats it is expressed in a more pronounced form. However, it leaves unanswered the question of why molossid bats employ these two nuclei for binaural processing of localization information, while other bats have only a vestigial (Irving and Harrison, 1967) or a monaural MSO (Grothe et al., 1992).

An alternative hypothesis is that the MSO could be involved in the processing of monaural as well as binaural signals, but the degree to which monaural or binaural processing dominates may vary among animals. Through modifying the degree of excitatory and inhibitory innervation, the coding features of MSO neurons could be adjusted (Grothe et al., 1992; Grothe and Sanes, 1994). An extreme modification of this sort is seen in the MSO of the mustached bat. The characteristics of the MSO in this bat are similar to those of other mammals, but the ipsilateral inputs are reduced or absent, and the inhibitory inputs from the MNTB are enlarged (Grothe, 1990; Covey et al., 1991; Grothe et al., 1992; Vater, 1993). The MSO neurons are, thus, monaural, but their responses depend on the timing of the excitatory and inhibitory inputs they receive from the contralateral ear. The nature of this dependency is highly similar to that seen in the brain slices of the gerbil MSO neurons when driven with contralateral stimulation (Grothe and Sanes, 1994). In the mustached bat, the temporal interaction between the excitatory and inhibitory inputs from the contralateral ear plays a key role in shaping the temporal response patterns evoked by amplitude-modulated signals presented monaurally (Grothe, 1994). In other words, the interplay between the excitation and inhibition creates a “filter” that determines the range of amplitude modulation rates to which the neuron will respond. The MSO of the free-tailed bat also receives a prominent inhibitory input from the MNTB, but in addition, has inputs from the ipsilateral ear. One consequence of this arrangement might be that its MSO neurons code both for some acoustic feature and for a certain region of space. However, the bilateral inputs should decrease the specificity of the filter, shifting the filter properties as a function of the interaural temporal disparity. If this is correct, then coding for two or more attributes, even if degraded by binaural inputs, could provide some advantage to molossid bats. The mustached bat, however, relies to a

great extent on modulation patterns for the recognition of objects in its acoustic space (Henson et al., 1987). The elimination of inputs from one side could enhance the stability of the filter and allow these neurons to encode certain features of a acoustic stimulus without contamination from inputs from the other ear, and thus code for that feature or features independent of the position of the sound source in space.

The connections of the MSO in other mammals, such as the cat, apparently emphasize binaural processing of cues for sound localization. In this animal, MSO neurons are equally innervated by excitatory inputs from the two ears, but the inhibitory inputs are markedly weaker than in the mustached bat (Vater, 1993) or the free-tailed bat (this study). This arrangement might enhance the precision of coding for interaural time disparities while sharply reducing the filter properties that code for modulation rates or other acoustic features. The connections of the free-tailed bat's MSO suggest that it should have some of the functional properties outlined above for the mustached bat as well as some properties of the cat MSO. Thus, the MSO of the free-tailed bat receives a strong inhibitory input from the MNTB, as in the mustached bat, but in addition, it has bilateral excitatory inputs, similar to those in the cat. One consequence of this arrangement might be that MSO neurons in free-tailed bats could code both for some acoustic features of a sound and for the spatial location from which the sound emanates. However, the coding of either attribute in such a system would most likely be less specific than in systems that emphasize either monaural processing or binaural processing of cues for sound localization. For example, whatever filter properties are established for coding amplitude modulations or some other acoustic features, would also be affected by interaural disparities, and therefore might not convey information with the same accuracy as that provided by monaural processing in the mustached bat. The degree to which this hypothesis is correct remains to be determined. However, it provides a testable hypothesis that could account for some of the disparate features among the MSOs of various animals that only recently have become evident.

In conclusion, at least some of the nuclei of the superior olivary complex apparently possess an evolutionary plasticity that is expressed by their variable locations among different species and their relative sizes and connections. This is especially apparent for the cholinergic cell groups that contribute to the olivocochlear system, and for the MSO. Presumably the species-specific features evolved in response to various selective pressures and constraints. With regard to MSO, this proposition suggests that this nucleus is homologous, although not identical either connectionally or functionally, among different animals.

ACKNOWLEDGMENTS

The experiments reported here were conducted at the Zoologisches Institut Munich. We thank the v. Humboldt Foundation for granting a fellowship for George D. Pollak. We thank Gerhard Neuweiler for his support, and Claudia Schulte, Maike Pötke, and Friedl Althaus for technical assistance. The research was partly supported by Deutsche Forschungsgemeinschaft, SFB 204, and grant DC-20068 from the National Institute of Deafness and Other Communicative Disorders.

LITERATURE CITED

- Adams, J.C. (1977) Technical consideration on the use of horseradish peroxidase as a neuronal marker. *Neuroscience* 2:141-145.
- Adams, J.C. (1979) Ascending projections to the inferior colliculus. *J. Comp. Neurol.* 183:519-538.
- Adams, J.C. (1983) Cytology of periolivary cells and the organization of their projections in the cat. *J. Comp. Neurol.* 215:275-289.
- Altschuler, R.A., M.H. Parakkal, and J. Fex (1983) Localization of enkephalin-like immunoreactivity in acetylcholinesterase-positive cells in the guinea-pig lateral superior olivary complex that project to the cochlea. *Neuroscience* 9:621-630.
- Aschoff, A., and J. Ostwald (1987) Different origins of cochlear efferents in some bat species, rats, and guinea pigs. *J. Comp. Neurol.* 264:56-72.
- Aschoff, A., M. Müller, and H. Ott (1988) Origin of cochlear efferents in some gerbil species. A comparative anatomical study with fluorescent tracers. *Exp. Brain Res.* 71:252-261.
- Beyerl, B.D. (1978) Afferent projections to the central nucleus of the inferior colliculus in the rat. *Brain Res.* 145:209-223.
- Bishop, A.L., and O.W. Henson, Jr. (1987) The efferent cochlear projections of the superior olivary complex in the mustached bat. *Hearing Res.* 31:175-182.
- Boudreau, J.C., and C. Tsuchitani (1968) Binaural interaction in the cat superior olive S-segment. *J. Neurophysiol.* 31:442-454.
- Brown, J.C., and B. Howlett (1968) The facial outflow and the superior salivatory nucleus: An histochemical study in the rat. *J. Comp. Neurol.* 134:175-192.
- Brown, J.C., and B. Howlett (1972) The olivo-cochlear tract in the rat and its bearing on the homologies of some constituent cell groups of the mammalian superior olivary complex: A thiocholine study. *Acta Anat.* 83:505-526.
- Brugge, J.F., N.A. Dubrovsky, L.M. Aitkin, and D.J. Anderson (1969) Sensitivity of single neurons in auditory cortex of cat to binaural tonal stimulation: Effects of varying interaural time and intensity. *J. Neurophysiol.* 32:1005-1024.
- Brunso-Bechtold, J.K., G.C. Thompson, and R.B. Masterton (1981) HRP study of the organization of auditory afferents ascending to central nucleus of inferior colliculus in cat. *J. Comp. Neurol.* 197:705-722.
- Caird, D., and R. Klinke (1983) Processing of binaural stimuli by cat superior olivary complex neurons. *Exp. Brain Res.* 52:385-399.
- Cant, N.B. (1991) Projections to the lateral and medial superior olivary nuclei from the spherical and globular bushy cells of the anteroventral cochlear nucleus. In R.A. Altschuler, R.P. Bobbin, B.M. Clopton, and D.W. Hoffman (eds): *Neurobiology of Hearing: The Central Auditory System*. New York: Raven Press, pp. 99-119.
- Cant, N.B., and J.H. Casseday (1986) Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. *J. Comp. Neurol.* 247:457-476.
- Casseday, J.H., E. Covey, and M. Vater (1988) Connections of the superior olivary complex in the rufous horseshoe bat *Rhinolophus rouxi*. *J. Comp. Neurol.* 278:313-329.
- Covey, E., M. Vater, and J.H. Casseday (1991) Binaural properties of single units in the superior olivary complex of the mustached bat. *J. Neurophysiol.* 66:1080-1094.
- Elverland, H.H. (1978) Ascending and intrinsic projections of the superior olivary complex in the cat. *Exp. Brain Res.* 32:117-134.
- Finlayson, P.G., and D.M. Caspary (1991) Low-frequency neurons in the lateral superior olive exhibit phase-sensitive binaural inhibition. *J. Neurophysiol.* 65:598-605.
- Galambos, R., and H. Davis (1943) The response of single auditory-nerve fibres to acoustic stimulation. *J. Neurophysiol.* 6:39-57.
- Galambos, R., J. Schwartzkopf, and A. Rupert (1959) Microelectrode study of the superior olivary nuclei. *Am. J. Physiol.* 197:527-536.
- Gallyas, F. (1979) Silver staining of myelin by means of a physical development. *Neurosci. Res.* 1:203-209.
- Glendenning, K.K., J.K. Brunso-Bechtold, G.C. Thompson, and R.B. Masterton (1981) Ascending auditory afferents to the nuclei of the lateral lemniscus. *J. Comp. Neurol.* 197:673-703.
- Goldberg, J.M., and P.B. Brown (1968) Functional organization of the dog superior olivary complex: An anatomical and electrophysiological study. *J. Neurophysiol.* 31:639-656.
- Goldberg, J.M., and P.B. Brown (1969) Response of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: Some physiological mechanisms of sound localization. *J. Neurophysiol.* 32:613-636.

- Grothe, B. (1990) Versuch einer Definition des medialen Kerns des oberen Olivenkomplexes bei der Neuweltfledermaus *Pteronotus p. parnellii*. Ph.D. dissertation, University of Munich.
- Grothe, B. (1994) Interaction of excitation and inhibition in processing of pure tone and amplitude modulated stimuli in the medial superior olive of the mustached bat. *J. Neurophysiol.* 71(2).
- Grothe, B., and D.H. Sanes (1993) Bilateral inhibition by glycinergic efferents in the medial superior olive. *J. Neurophysiol.* 69:1192-1196.
- Grothe, B., and D.H. Sanes (1994) Inhibition influences the temporal response properties of gerbil medial superior olivary neurons: An *in-vitro* study. *J. Neurosci.* 70(3).
- Grothe, B., M. Vater, J.H. Casseday, and E. Covey (1992) Monaural interaction of excitation and inhibition in the medial superior olive of the mustached bat: An adaptation for biosonar. *Proc. Natl. Acad. Sci. USA* 89:5108-5112.
- Guinan, J.J., S.S. Guinan, and B.E. Norris (1972a) Single auditory units in the superior olivary complex. I: Responses to sounds and classifications based on physiological properties. *Int. J. Neurosci.* 4:101-120.
- Guinan, J.J., B.E. Norris, and S.S. Guinan (1972b) Single auditory units in the superior olivary complex. II: Locations of unit categories and tonotopic organization. *Int. J. Neurosci.* 4:147-166.
- Hall, J.L. (1965) Binaural interaction in the accessory superior olivary nucleus of the cat. *J. Acoust. Soc. Am.* 37:814-823.
- Hardy, H., L. Heimer, R. Switzer, and D. Watkins (1976) Simultaneous demonstration of horseradish peroxidase and acetylcholinesterase. *Neurosci. Lett.* 3:1-5.
- Harnischfeger, G., G. Neuweiler, and P. Schlegel (1985) Interaural time and intensity coding in superior olivary complex and inferior colliculus of the echolocating bat *Molossus ater*. *J. Neurophysiol.* 53:89-109.
- Harrison, J.M., and M.L. Feldman (1970) Anatomical aspects of the cochlear nucleus and superior olivary complex. In W.D. Neff (ed): *Contributions to Sensory Physiology 4*. New York: Academic Press, pp. 95-143.
- Harrison, J.M., and R. Irving (1966) Visual and nonvisual auditory systems in mammals. *Science* 154:738-743.
- Henson, O.W., Jr., A. Bishop, A. Keating, J. Kobler, M. Henson, B. Wilson, and R. Hansen (1987) Biosonar imaging of insect by *pteronotus p. parnellii*, the mustached bat. *Neotroph. Res.* 3:82-101.
- Irvine, D.R.F. (1992) Physiology of the auditory brainstem. In A.N. Popper and R.R. Fay (eds): *The Mammalian Auditory Pathway: Neurophysiology*. New York: Springer, pp. 153-231.
- Irving, R., and J.M. Harrison (1967) The superior olivary complex and audition: A comparative study. *J. Comp. Neurol.* 130:77-86.
- Jeffress, L.A. (1948) A place theory of sound localization. *J. Comp. Physiol. Psychol.* 41:35-39.
- Jen, Ph. H.-S. (1978) Electrophysiological properties of auditory neurons in the superior olivary complex of echolocating bats. *J. Comp. Physiol.* 128:47-56.
- Johnson, D.H. (1980) The relation between spike rate and synchrony in responses of auditory-nerve fibres to single tones. *J. Acoust. Soc. Am.* 68:1115-1122.
- Kandler, K., and E. Friauf (1993) Pre- and postnatal development of efferent connections of the cochlear nucleus in the rat. *J. Comp. Neurol.* 328:161-184.
- Kiang, N.Y.-S., T. Watanabe, C. Thomas, and L.F. Clark (1965) *Discharge patterns of single fibres in the cat's auditory nerve*. Cambridge, MA: MIT Press.
- Kiss, A., and K. Majorossy (1983) Neuron morphology and synaptic architecture in the medial superior olivary nucleus. Light- and electron microscope studies in the cat. *Exp. Brain Res.* 52:315-327.
- Kitzes, L.M., K.S. Wrege, and J.M. Cassady (1980) Patterns of response of cortical cells to binaural stimulation. *J. Comp. Neurol.* 192:455-472.
- Kudo, M., Y. Nakamura, H. Tokuno, and Y. Kitao (1990) Auditory brainstem in the mole (*mogera*): Nuclear configurations and the projections to the inferior colliculus. *J. Comp. Neurol.* 298:400-412.
- Kuwabara, N., and J.M. Zook (1992) Projections to the medial superior olive from the medial and lateral nuclei of the trapezoid body in rodents and bats. *J. Comp. Neurol.* 324:522-538.
- Langford, T.L. (1984) Responses elicited from medial superior olivary neurons by stimuli associated with binaural masking and unmasking. *Hearing Res.* 15:39-50.
- Masterton, B., and I.T. Diamond (1967) Medial superior olive and sound localization. *Science* 155:1696-1697.
- Mesulam, M.-M. (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *J. Histochem. Cytochem.* 26:106-117.
- Morest, D.K. (1968a) The collateral system of the medial nucleus of the trapezoid body of the cat, its neuronal architecture and relation to the olivo-cochlear bundle. *Brain Res.* 9:288-311.
- Morest, D.K. (1968b) The growth of synaptic endings in the mammalian brain: A study of the calyces of the trapezoid body. *Z. Anat. Entw. Gesch.* 127:201-220.
- Moushegian, G., A.L. Rupert, and M.A. Whitcomb (1964) Medial superior olivary unit response patterns to monaural and binaural clicks. *J. Acoust. Soc. Am.* 36:196-202.
- Nakajima, K. (1971) The structure of the medial nucleus of the trapezoid body of the bat with special reference to two types of synaptic endings. *J. Cell Biol.* 50:121-134.
- Nordeen, K.W., H.P. Killackey, and L.M. Kitzes (1983) Ascending projections to the inferior colliculus in the adult gerbil, *Meriones unguiculatus*. *J. Comp. Neurol.* 214:131-143.
- Ollo, C.H., and I.R. Schwartz (1979) The superior olivary complex in C57BL/6 mice. *Am. J. Anat.* 155:349-374.
- Pollak, G.D. (1988) Time is traded for intensity in the bat's auditory system. *Hearing Res.* 36:107-124.
- Raleigh, Lord (1907) On our perception of sound direction. *Philos. Mag.* 13:214-232.
- Ramón y Cajal, S. (1911) *Histologie du systeme nerveux de l'homme et des vertebres*. Paris: Maloine.
- Rasmussen, G.L. (1946) The olivary peduncle and other fibre projections of the superior olivary complex. *J. Comp. Neurol.* 84:141-219.
- Robertson, D., and I.M. Winter (1988) Cochlear nucleus inputs to olivocochlear neurones revealed by combined anterograde and retrograde labelling in the guinea pig. *Brain Res.* 462:47-55.
- Rose, J.E., J.F. Brugge, D.J. Anderson, and J.E. Hind (1967) Phase-locked response to low frequency tones in single auditory nerve fibres of the squirrel monkey. *J. Neurophysiol.* 30:769-793.
- Roth, G.L., L.M. Aitkin, R.A. Andersen, and M.M. Merzenich (1978) Some features of the spatial organization of the central nucleus of the inferior colliculus of the cat. *J. Comp. Neurol.* 182:661-680.
- Saint Marie, R.L., and R.A. Baker (1990) Neurotransmitter-specific uptake and retrograde transport of 3H glycine from the inferior colliculus by ipsilateral projections of the superior olivary complex and nuclei of the lateral lemniscus. *Brain Res.* 524:244-253.
- Scheibel, M.E., and A.B. Scheibel (1974) Neuropil organization in the superior olive of the cat. *Exp. Neurol.* 43:339-348.
- Schmidt, S., J. Thaller, and M. Pötke (1990) Behavioral audiogram and masked threshold in the free-tailed bat, *Tadarida brasiliensis*. In N. Elsner and G. Roth (eds): *Brain-Perception-Cognition*. Proc. 18th Göttingen Conf. Stuttgart: Thieme, p. 146.
- Schofield, B.R. (1991) Superior paraolivary nucleus in the pigmented guinea pig: Separate classes of neurons project to the inferior colliculus and the cochlear nucleus. *J. Comp. Neurol.* 312:68-76.
- Schofield, B.R., and N.B. Cant (1991) Organization of the superior olivary complex in the guinea pig. I. Cytoarchitecture, cytochrome oxidase histochemistry, and dendritic morphology. *J. Comp. Neurol.* 314:645-670.
- Schofield, B.R., and N.B. Cant (1992) Organization of the superior olivary complex in the guinea pig. II. Patterns of projection from the periolivary nuclei to the inferior colliculus. *J. Comp. Neuro.* 317:438-455.
- Schwartz, I.R. (1977) Dendritic arrangements in the cat medial superior olive. *Neuroscience* 2:81-101.
- Schwartz, I.R. (1992) The superior olivary complex and lateral lemniscal nuclei. In D.B. Webster, N.A. Popper, and R.R. Fay (eds): *The Mammalian Auditory Pathway*. Neuroanatomy. New York: Springer, pp. 117-167.
- Schweizer, H. (1981) The connections of the inferior colliculus and the organization of the brainstem auditory system in the greater horseshoe bat (*Rhinolophus ferrumequinum*). *J. Comp. Neurol.* 201:25-49.
- Spangler, K.M., W.B. Warr, and C.K. Henkel (1985) The projections of principal cells of the medial nucleus of the trapezoid body in the cat. *J. Comp. Neurol.* 238:249-261.
- Stotler, W.A. (1953) An experimental study of the cells and connections of the superior olivary complex of the cat. *J. Comp. Neurol.* 98:401-431.
- Thompson, A.M., and G.C. Thompson (1991) Posteroventral cochlear nucleus projections to olivocochlear neurons. *J. Comp. Neurol.* 303:267-285.
- Tsuchitani, C., and D.H. Johnson (1991) Binaural cues and signal processing in the superior olivary complex. In R.A. Altschuler, R.P. Bobbin, B.M.

- Clopton, and D.W. Hoffman (eds): *Neurobiology of Hearing: The Central Auditory System*. New York: Raven Press, pp. 163–194.
- Vater, M. (1993) Synaptic organisation of the mustached bat's medial superior olive. (Abstr.) Proceedings of the 21th Göttingen Neurobiol. Conference.
- Vater, M., and A.S. Feng (1990) Functional organization of ascending and descending connections of the cochlear nucleus of horseshoe bats. *J. Comp. Neurol.* 292:373–395.
- Vetter, D.E., and E. Mugnaini (1992) Distribution and dendritic features of three groups of rat olivocochlear neurons: A study with two retrograde cholera toxin tracers. *Anat. Embryol.* 185:1–16.
- Warr, W.B. (1966) Fiber degeneration following lesions in the anteroventral cochlear nucleus of the cat. *Exp. Neurol.* 14:453–474.
- Warr, W.B. (1975) Olivocochlear and vestibular efferent neurons of the feline brain stem: Their location, morphology and number determined by retrograde axonal transport and acetylcholinesterase histochemistry. *J. Comp. Neurol.* 161:159–182.
- Warr, W.B. (1992) Organization of olivocochlear efferent systems in mammals. In D.B. Webster, N.A. Popper, and R.R. Fay (eds): *The Mammalian Auditory Pathway: Neuroanatomy*. New York: Springer, pp. 410–448.
- Willard, F.H., and G.F. Martin (1984) Collateral innervation of the inferior colliculus in the North American opossum: A study using fluorescent markers in a double labeling paradigm. *Brain Res.* 303:171–182.
- Yin, T.C.T., and J.C.K. Chan (1990) Interaural time sensitivity in medial superior olive of cat. *J. Neurophysiol.* 64:465–488.
- Yin, T.C.T., J.A. Hirsch, and J.C.K. Chan (1985) Response of neurons in the cat's superior colliculus to acoustic stimuli. II A model of interaural intensity sensitivity. *J. Neurophysiol.* 53:746–758.
- Zook, J.M., and J.H. Casseday (1982a) Cytoarchitecture of auditory system in lower brainstem of the mustached bat, *Pteronotus parnellii*. *J. Comp. Neurol.* 207:1–13.
- Zook, J.M., and J.H. Casseday (1982b) Origin of ascending projections to inferior colliculus in the mustached bat, *Pteronotus parnellii*. *J. Comp. Neurol.* 207:14–28.
- Zook, J.M., and P.A. Leake (1989) Connections and frequency representation in the auditory brainstem of the mustached bat, *Pteronotus parnellii*. *J. Comp. Neurol.* 290:243–261.