ACOUSTIC CHIASM: EFFERENT PROJECTIONS OF THE LATERAL SUPERIOR OLIVE¹

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Abstract

The efferent connections of the cat's lateral superior olive (LSO) were examined first with kainic acid-induced anterograde degeneration and tritiated leucine autoradiography and then by systematic repetition of HRP and fluorescent dye retrograde tract-tracing techniques. The results show that virtually all LSO cells have axons ascending either contralaterally or ipsilaterally to high pontine and midbrain levels of the brainstem. Most terminate in the ventrolateral division of either the ipsilateral or contralateral central nucleus of the inferior colliculus, some terminate in the ipsilateral or contralateral dorsal nucleus of the lateral lemniscus, and a small number terminate in the ipsilateral intermediate nucleus of the lateral lemniscus. Only a small proportion (<5%) of LSO cells project to both sides via axon collaterals.

The ipsilateral, contralateral, and bilateral projections arise from three overlapping subpopulations of cells within LSO: Those projecting ipsilaterally are concentrated in its lateral limb; those projecting contralaterally are concentrated in its medial limb; the few projecting bilaterally are thinly scattered throughout. Therefore, a lateral-medial gradient is present across LSO based on the laterality of its cell's efferent targets. This gradient parallels LSO's tonotopic gradient: The higher the characteristic frequency of an LSO cell, the more likely it is to project contralaterally.

This arrangement of LSO's ascending projections, with most of its lateral cells projecting ipsilaterally and most of its medial cells projecting contralaterally, is similar to the arrangement of the optic chiasm in animals with overlapping eye-fields. Its presence seems to provide an anatomical basis for some recent electrophysiological and behavioral reports of chiasm-like properties of the superior olivary complex.

It has long been known that the auditory system of mammals is bilateral in the sense that each ear is represented on both sides of the ascending pathway from upper medulla to auditory cortex (Held, 1893; van Gehuchten, 1906; Ramón y Cajal, 1909; Rosenzweig, 1961). Despite this *anatomical* bilaterality, however, it has been shown recently that the system is *functionally* unilateral in the sense that the vast majority of auditory cells in the midbrain or forebrain are driven only or driven best by sounds in the contralateral sound field and, perhaps more telling, that unilateral transection of the ascending

pathway results in sound localization deficits confined to the hemifield contralateral to the lesion (Strominger, 1969; Knudsen and Konishi, 1978; Thompson and Masterton, 1978; Jenkins and Masterton, 1979, 1982; Middlebrooks and Pettigrew, 1980; Jenkins and Merzenich, 1981). Since both the anatomical bilaterality and the functional contralaterality of the system begin at the trapezoid body-superior olivary complex of the medulla, it now appears that this complex may serve audition in somewhat the same manner as the optic chiasm serves vision or the spinal and bulbar decussations serve somesthesia—each structure resulting in the left and right hemifields of sensory space to be represented on the opposite side of the midbrain and forebrain (Jenkins and Masterton, 1982).

Because in vision or somesthesia the sensory field is topographically represented on the receptor surface, the contralateral representation of each sensory hemifield in these modalities can be achieved merely by the shunting or sorting of the appropriate ascending fibers. In the auditory system, however, the topography of sensory

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space is not represented on the surface of the receptor; therefore, a simple sorting of the fibers arising at the ears cannot accomplish the same task.

It follows that the acoustic chiasm is fundamentally different from the optic or somesthetic chiasms. Where the optic or somesthetic chiasms are structures, the acoustic chiasm is a process. As a process it requires, first, the convergence and analysis of activity arising at the two ears and only then, the appropriate distribution of the binaural analysis to the left or right sides of the system. It is the binaural convergence required by the first stage of this two-stage process that makes the system anatomically bilateral, and it now seems likely that it is the potential for unilateral distribution in the second stage of the process that makes the system functionally contralateral.

One of the chief contributors to this two-stage process, and the focus of this report, is the lateral, or principal, superior olive. LSO³ gains second-order excitatory input from the ipsilateral ear and third-order inhibitory input from the contralateral ear (Goldberg and Brown, 1968, 1969; Boudreau and Tsuchitani, 1970). In addition, it is precisely tonotopic for both inputs with low frequencies represented laterally and high frequencies represented medially (Goldberg and Brown, 1968, 1969; Tsuchitani and Boudreau, 1969; Boudreau and Tsuchitani, 1970; Guinan et al., 1972). It follows from these characteristics that LSO probably serves the first, or analytical, stage of the two-stage chiasmatic process by acting as a binaural spectrum-difference analyzer—in effect, subtracting the sound-spectrum received at the contralateral ear from the sound-spectrum received at the ipsilateral ear. This binaural spectrum-difference is known to be an important cue for sound localization, especially for the higher frequencies in an animal's hearing range (e.g., Masterton et al., 1975).

However, LSO's contribution to the second stage of the two-stage process—the distribution of its spectrum-difference analysis to the higher levels of the auditory

system—is not so clear. Stotler (1953), using retrograde degeneration methods, showed that LSO projects to both inferior colliculi, and this conclusion has been verified with modern techniques for tracing fiber projections (Elverland, 1978; Roth et al., 1978; Adams, 1979; Brunso-Bechtold et al., 1981; Glendenning et al., 1981). From the outcome of these experiments one is led to the idea that the analysis of the binaural spectrum-difference accomplished by each LSO is probably transmitted bilaterally to both colliculi. However, this conclusion seems to stand in direct conflict with the functional contralaterality and chiasm-like properties of the system already demonstrated by ablation-behavior and electrophysiological experiments. For this reason, we undertook a re-examination of the efferent projections of LSO.

Because LSO is located deep within the medulla, surrounded by other auditory nuclei of the superior olivary complex and embedded in bypassing auditory fibers, it is difficult to study its efferents by anterograde tract-tracing methods. Therefore, we were forced to rely mostly on retrograde tract-tracing methods—placing either HRP or fluorescent dyes in each of its plausible efferent targets. In order to avoid the most obvious pitfall of this strategy—overlooking an unsuspected projection—we began by including as a potential target for LSO's efferents every nucleus from forebrain to spinal cord that showed any fiber or terminal degeneration whatever after a biochemical lesion of one entire superior olivary complex. We then narrowed this array of possible targets by eliminating those that did not show fiber or terminal labeling after injections of tritiated leucine centered in LSO. Each of the nuclei surviving this two-stage screening process, and a number of other nuclei suggested by previous investigators, were then studied with HRP or fluorescent dye retrograde tract-tracing techniques.

Materials and Methods

In a series of experiments on the neural connections of the brainstem auditory system, a total of 279 adult cats (aged 1 to 3 years) have been used to date. Of this total, 103 are relevant to the present report. Of these, 66 are new to this report and 37 others have been either published or referred to previously. Of the 66 new cases, 8 received either tritiated leucine or kainic acid injections in LSO; 27 received HRP injections either in cochlear nucleus, superior olivary complex, inferior colliculus, or medial geniculate nucleus; 4 received HRP injections in superior colliculus; 9 others received HRP applied directly to the cut fibers of either the cerebellar peduncles, the commissure of the inferior colliculus, the lateral lemniscus, or after hemisection of the spinal cord at the C1-C2 junction; and 18 others received paired injections of two different fluorescent dyes, one in each inferior colliculus. The 37 cases published or referred to elsewhere received microinjections of HRP either in the ventral, intermediate, or dorsal nuclei of the lateral lemniscus or in one of the subdivisions of the central nucleus of the inferior colliculus; one received a large kainic acid ablation of one superior olivary complex, and another received an injection of tritiated leucine centered in LSO (e.g., Masterton et al., 1979, 1981, 1982; Glendenning

³ The abbreviations used are: AVCN, anteroventral cochlear nucleus; BIN, nucleus of brachium of inferior colliculus; CP, cerebral peduncle; Cun, cuneiform nucleus; DCN, dorsal cochlear nucleus; DLL, dorsal nucleus of lateral lemniscus; dm, dorsomedial division of central nucleus of inferior colliculus; DMPO, dorsomedial periolivary nucleus; EX, external nucleus of inferior colliculus; HRP, horseradish peroxidase; IC, inferior colliculus; ILL, intermediate nucleus of lateral lemniscus; LGd, lateral geniculate nucleus, dorsal division; LGv, lateral geniculate nucleus, ventral division; LP, lateral posterior nucleus; LSO, lateral superior olive; LTB, lateral nucleus of trapezoid body; MD, medial dorsal nucleus; MG, medial geniculate nucleus; MV, medial vestibular nucleus; MSO, medial superior olive; MTB, medial nucleus of trapezoid body; N 3, oculomotor nucleus or nerve; N 5, spinal trigeminal nucleus or nerve; N 6, abducens nucleus or nerve; N 7, facial nucleus or nerve; PBG, parabigeminal nucleus; PC, pericentral nucleus of inferior colliculus; PPO, posterior periolivary nucleus; PVCN, posteroventral cochlear nucleus; RB, restiform body; RN, red nucleus; Sag, sagulum; SC, superior colliculus; SGI, stratum griseum intermediale of superior colliculus; SGP, stratum griseum profundum of superior colliculus; SGS, stratum griseum superficiale of superior colliculus; SN, substantia nigra; VB, ventrobasal complex; vl, ventrolateral division of central nucleus of inferior colliculus; VTB, ventral nucleus of the trapezoid body; VLL, ventral nucleus of the lateral lemniscus; ZI, zona incerta.

and Masterton, 1980; Brunso-Bechtold et al., 1981; Glendenning et al., 1981).

Details of the surgical, histological, analytic, and quantification techniques are described separately in the sections following.

Anterograde tract-tracing methods

Anterograde degeneration resulting from kainic acid injections and autoradiographic labeling from tritiated leucine injections centered in or including LSO were used to provide a first approximation of LSO's efferent projections.

Autoradiographic technique. Autoradiographic procedures followed those of Cowan et al. (1972). Briefly, tritiated leucine was prepared by N₂ evacuation, reconstituted in sterile physiological saline to concentrations of 20 to 75 $\mu\text{Ci}/\mu\text{l}$, and injected in the superior olivary complex under stereotaxic guidance. Survival times ranged from 2 to 15 days in an attempt to label the fiber pathways via both fast and slow axoplasmic transport systems.

After the appropriate survival time, these animals were deeply anesthetized and perfused intracardially, first with saline, then with 10% formalin. Five series of frozen sections of 25 μm thickness were cut, mounted, defatted, and dipped in Kodak NTB2 nuclear-track emulsion, diluted 1:1 in a 1% detergent solution. They were then dried, packed in light-tight boxes, and stored with desiccant at 6°C for 1, 3, 5, 7, or 11 weeks. After exposure, the slides were developed in fresh, concentrated Kodak D19 developer for 2 min at 12°C, then fixed and stained with cresyl violet acetate.

Degeneration technique. Biochemical lesions were induced in the superior olivary complex by stereotaxically guided microinjections of 0.02 μl of 1% kainic acid in saline, except for one case (#177) in which a 30% concentration was used. Evidence that such injections result in little or no damage to the bypassing fibers of the trapezoid body is presented here and elsewhere (Coyle et al., 1978; Masterton et al., 1979; Glendenning et al., 1981). Postoperative survival periods ranged from 5 to 7 days. The cats were then deeply anesthetized and perfused with saline followed by 10% formalin. Their brains were removed, and frozen sections cut at 40 μm . At least two series of sections were impregnated with silver using modified Fink-Heimer techniques (e.g., Ebbesson, 1970). One series was then bleached and counterstained with cresyl violet (Kawamura and Niimi, 1972).

Retrograde tract-tracing methods

Injections of horseradish peroxidase, HRP-labeled wheat germ agglutinin, or fluorescent dyes, and direct HRP fills of freshly cut axons were used as retrograde tract-tracing techniques to verify specific LSO projections suggested by the previous anterograde tract-tracing methods.

HRP technique. Following HRP or HRP-wheat germ agglutinin injections or applications, the cats were allowed to survive for 48 to 72 hr. They were then deeply anesthetized and sacrificed by intracardial perfusion of physiological saline followed by a 3.0% glutaraldehyde fixative made up in a 0.1 M phosphate buffer with 3%

sucrose. The brains were removed, placed in the fixative for 4 hr, and then refrigerated for 24 hr in a 20% sucrose-phosphate buffer. They were then sectioned at 50- μm thickness on a freezing microtome. For the cases done early in the study, the sections were processed immediately by the diaminobenzidine method (LaVail and LaVail, 1972). Later cases were processed either with benzidine dihydrochloride or cold tetramethylbenzidine (Mesulam, 1976, 1978; de Olmos, 1977; Mesulam and Brushart, 1979; Mesulam and Rosene, 1979). Incubation times were adjusted from 10 to 60 min to accommodate the slowed reactions caused by the cold solutions. Adjacent sections were saved in refrigerated phosphate buffer and processed as needed.

Fluorescent dye, double-labeling technique. Most of the fluorescent dye cases received multiple injections totaling 1.0 to 2.0 μl of 2.5% fast blue in one inferior colliculus and an equal amount of 1.0 to 2.0% Nuclear Yellow in the other inferior colliculus (de Olmos and Heimer, 1980). However, in a few cases bisbenzimidazole, propidium iodide, or diamidino-2-phenylindol-dihydrochloride were used (Steward, 1981). These tracers were suspended in water and injected with a Hamilton syringe. Although survival times were systematically varied from 6 to 168 hr, the optimal survival time for visualization of double-labeled neurons in LSO was found to be about 72 hr for fast blue and 10 hr for Nuclear Yellow. Thus, the most frequently used sequence required the Nuclear Yellow to be injected 62 hr after the fast blue injection, and sacrifice followed the Nuclear Yellow injection after 10 hr.

The animals were sacrificed with an overdose of anesthetic followed by an intracardial perfusion of saline and then 10% formalin made up in a 0.1 M phosphate buffer (pH 7.4) with 3% sucrose. The brains were removed, immediately transferred to a freezing microtome, and cut transversely in sections 40 μm thick. The sections were collected in distilled water and immediately mounted and air dried. The first set of every third section was air dried, the second set was air dried and coverslipped, and the third set was refixed in 2% formalin and stained with cresyl violet. Following fluorescent photomicrography and the plotting and counting of the labeled cells, sections from the first set were also often refixed and stained with cresyl violet.

Surgical methods

Regardless of the particular surgical procedure to be performed, the animals were first anesthetized with halothane and then maintained with intravenous injections of sodium thiamylal (Suritol, Parke-Davis).

Injection technique. Either pressure or electrophoretic injections of the tracer substances were made. Pressure injections of 0.01 to 2.0 μl were made by means of a Hamilton syringe through a glass pipette. Electrophoretic injections were made by passing either a pulsed or direct current through a glass pipette. For HRP electrophoresis, an anodal current was pulsed at 2 Hz for 45 min. For labeled amino acid electrophoresis, a cathodal current was pulsed at 2 Hz for 60 min.

HRP and fluorescent dye injections in thalamic, midbrain, or pontine targets (i.e., MG, SC, IC, DLL, ILL, VLL). For injections into the inferior colliculus, caudal

retraction of the cerebellum through an entry just caudal to the tentorium permitted direct visualization and proper placement of the micropipette. For the medial geniculate and superior colliculus, entry was made rostral to the tentorium and the cerebellum retracted rostrally. To have direct visualization for proper pipette placement for injections of the nuclei of the lateral lemniscus, it was necessary to make a dorsolateral entry caudal to the tentorium and to retract the cerebellum both medially and caudally.

Kainic acid and tritiated leucine injections in superior olivary complex. In cases requiring injection of the superior olivary complex, a micropipette attached to a Hamilton syringe was angled stereotactically from far caudal. This approach avoided direct section of the efferents of the superior olivary complex as they coursed dorsally and rostrally into the lateral lemniscus.

HRP fills of cerebellar peduncles, spinal cord, commissure of inferior colliculus, or lateral lemniscus. In the cerebellar cases, dried flakes of raw HRP were applied directly to the cut stumps of the cerebellar peduncles after radical aspiration of the cerebellum itself. In the collicular commissure, lateral lemniscus, and spinal cord cases, raw HRP was applied to the ends of the cut fibers immediately after their section with a knife blade.

Analytic and quantification methods

Plotting technique. Sections of the brainstem of each animal were systematically studied with light and, when appropriate, darkfield or fluorescent optics. The locations of labeled neurons or of degenerated or labeled fibers and terminals were plotted on magnified drawings with the aid of an X-Y pantograph. Nuclear boundaries were drawn from adjacent Nissl-stained sections with the aid of a microprojector.

Photographic technique for fluorescent dye cases. One set of sections from the fluorescent dye cases was examined, and a second set was systematically photographed under epifluorescent illumination with a Leitz-Orthomat-W fluorescent microscope with an excitation wavelength of 390 nm. Following photography, the locations of labeled neurons were plotted with the aid of an X-Y pantograph attached to a fluorescent microscope stage. The sections were then stained with cresyl violet, and the borders of LSO and other relevant structures were transferred to the plots with the aid of a microprojector.

Cell-counting and statistical techniques. To quantify the presence and distribution of asymmetric labeling in LSO after injections in the inferior colliculus, the neurons labeled with either fluorescent dye or HRP were counted and tabulated. To demonstrate the gradients of labeled cells across LSO, four arbitrary lines were drawn dividing LSO into sectors—two vertical lines, one bisecting the dorsal and the other bisecting the ventral hilus; and two horizontal lines, one projecting laterally through the bottom of the dorsal hilus and the other projecting medially through the top of the ventral hilus. The four lines divided the S-shaped LSO into five sectors from dorsolateral to ventromedial and allowed unambiguous assignment of each LSO cell to one and only one sector. The asymmetry of retrograde cell labeling in

corresponding sectors of ipsilateral and contralateral LSO after inferior colliculus injections is one of the chief results in the present report.

Because none of the fluorescent dye injections completely filled the inferior colliculus nor labeled every cell in LSO, the counts of the labeled cells were also analyzed statistically. In order to eliminate chance or stochastic variation as an alternative explanation for the asymmetric retrograde labeling seen in LSO, χ^2 analyses were performed on each case alone, on each LSO alone, and on each tracer alone, as well as on the sum of the cell counts in the entire set of cases.

Terminology

Terminology for the divisions of the cochlear nucleus is adapted from that of Brawer et al. (1974), for the superior olivary complex from Warr (1975), for nuclei of the lateral lemniscus from Glendenning et al. (1981), and for the inferior colliculus from Rockel and Jones (1973).

Results

The pattern of fiber and terminal degeneration resulting from a large injection of kainic acid centered in the right superior olivary complex is shown in Figure 1. The injection resulted in complete cell loss throughout LSO, MSO, and the periolivary nuclei on the right side. The only neurons escaping the lesion were a few in the most rostral part of MTB. In addition to this virtually total destruction of the right superior olivary complex, the kainic acid lesion extended into the most rostral part of the principal nucleus of the facial nerve while the injection pipette itself made a thin lesion in the cerebellum and caudal tegmentum as it approached the superior olivary complex from a dorsal and caudal entry point.

In this and two other cases with more restricted kainic acid lesions, and in six other cases with injections of tritiated amino acids that included LSO, extensive anterograde terminal or fiber degeneration or anterograde autoradiographic labeling was seen bilaterally in the cochlear nuclei, in the medial and lateral band of the ipsilateral lateral lemniscus, in the medial band of the contralateral lateral lemniscus, bilaterally in the dorsal and intermediate nuclei of the lateral lemniscus, and bilaterally in the inferior colliculi (Fig. 1). In addition, lighter terminal or fiber degeneration or autoradiographic labeling was seen in the commissure of the inferior colliculus, the ipsilateral ventral nucleus of the lateral lemniscus, the ipsilateral facial nerve, brachium pontis, brachium conjunctivum, and scattered through bulbar, pontine, and midbrain tegmentum.

In none of the nine cases using anterograde tract-tracing techniques was evidence found for LSO fibers or terminals in either the external or pericentral nuclei of the inferior colliculus, in the dorsomedial division of the central nucleus of the inferior colliculus, nor in any structure more rostral. Furthermore, the contralateral superior olivary complex and the nuclei of the 3rd, 4th, 5th, 6th, 9th, 10th, and 12th nerves showed neither degenerated nor labeled fibers or terminals. The 7th nerve was seen to contain marked fibers only in the one case shown in Figure 1 and in one autoradiographic case in which the tritiated leucine also intruded on the 7th

Figure 1. Distribution of degeneration following a large injection of kainic acid in the right superior olivary complex. Arrows indicate injection site. Note degenerating terminals in ipsilateral ILL, bilaterally in DLL, and bilaterally in the ventrolateral division of the central nucleus of the inferior colliculus. Also note the lack of terminal degeneration in the dorsomedial division of the central nucleus and in the pericentral and external nuclei of the inferior colliculus. In this and all following figures, sections are numbered from caudal (lower left) to rostral (upper right).

nerve nucleus itself. The spinal cord showed no degeneration or labeling in any case.

Summarizing the results of these anterograde tract-tracing cases and confirming the results from many previously published reports using similar techniques: Efferents originating from the superior olivary complex in the vicinity of LSO and, therefore, possibly from LSO project bilaterally to the ventrolateral division of the central nucleus of the inferior colliculus, to the cochlear nuclei, and to the dorsal and intermediate nuclei of the lateral lemniscus, and possibly to the ipsilateral ventral nucleus of the lateral lemniscus and the cerebellum (Barnes et al., 1943; Rasmussen, 1946; Goldberg and Moore, 1967; Browner and Webster, 1975; Brunso-Bectold and Thompson, 1976, 1978; Elverland, 1978). In the retrograde tract-tracing cases described below, each of these possible projections is either verified or ruled out as originating in LSO.

Of equal interest, the *negative* results of these cases suggest that LSO probably does *not* project directly to

the pericentral or external nuclei, nor to the dorsomedial division of the central nucleus of the inferior colliculus, to any structure rostral to the inferior colliculus, to the nuclei of the contralateral superior olivary complex, nor to the motor nucleus of any cranial nerve excepting possibly the 7th. Except for the superior colliculus, the medial geniculate, and the subdivisions of the inferior colliculus, these structures are not discussed further.

Absence of LSO projections to cochlear nuclei

In addition to the heavy ascending projections of LSO, the cases based on anterograde transport and degeneration techniques just described suggested the possibility of a descending projection from LSO to the cochlear nuclei. To investigate the possibility that LSO itself may contribute fibers terminating in the cochlear nuclei, four cases were prepared with large injections of HRP in one cochlear nucleus. Figure 2 illustrates the results of one such injection in which HRP invaded the DCN, PVCN, and AVCN divisions of the right cochlear nucleus.

This injection resulted in labeled cells ranging from the level of the inferior colliculus rostrally to the caudal pole of MTB. Although the majority of labeled cells were found ipsilateral to the injection, a large number were also found contralaterally. Many were found *bilaterally* in VTB and MTB, in DMPO, and in the dorsal hilus of

Figure 2. Distribution of labeled cells after a large HRP injection in the cochlear nucleus. Note labeled cells surrounding LSO but the absence of labeled cells within LSO itself. In this and the following cases with HRP injections, sections are 50 μ m thick.

LSO. In addition, labeled cells were found *ipsilaterally* in the dorsolateral and anterolateral periolivary nuclei and just outside the borders of LSO—so-called marginal cells. Since each of these nuclei is known to give rise to olivocochlear fibers (e.g., Rasmussen, 1946; Warr, 1975, 1978), the possibility remains that these cells were labeled either by direct damage and filling of their axons as they passed through the cochlear nucleus on the way to the cochlea, or by uptake by terminals of axons collateral to the olivocochlear axons.

But in addition to the nuclei known to be origins of the olivocochlear bundles, clearly labeled cells were also found bilaterally in LTB and PPO. A few labeled cells were also found ipsilaterally in the ventromedial and dorsal periolivary nuclei and contralaterally in the VLL and IC. Inasmuch as these nuclei are *not* known as sources of the olivocochlear bundles, they may send direct projections to the cochlear nucleus itself (Farley and Warr, 1981).

Most important for the present purpose, however, is the fact that in none of the four cases with large injections of HRP in the cochlear nuclei were any labeled cells found in LSO itself either ipsilaterally or contralaterally. This complete absence of labeled cells within LSO, amidst many labeled cells in surrounding nuclei, suggests that the projections to the cochlear nuclei demonstrated by the anterograde tract-tracing techniques described above probably resulted entirely from the spread of the tracer or the neurotoxin beyond the confines of LSO. Thus, we have no evidence of a direct projection from LSO to the cochlear nucleus of either side and, within the confidence limits placed on the HRP technique, some evidence that in the cat such a projection probably does not exist.

Projections from LSO to nuclei of the lateral lemniscus

Although the several origins of the afferents ascending to the ventral, intermediate, and dorsal nuclei of the lateral lemniscus have been described previously, LSO's specific contribution to these projections is detailed here in the interest of completeness (Glendenning et al., 1981).

Scarcity of LSO projections to VLL. Figure 1 shows that a lesion of the entire superior olivary complex results in only very light degeneration in the ventral nucleus of the lateral lemniscus. In the eight other cases using anterograde tract-tracing techniques, no evidence for anything beyond a very sparse projection to VLL was seen. Nevertheless, two cases with HRP microinjections in VLL that had been prepared for a previous report were re-examined for the possibility of labeled cells within LSO (Glendenning et al., 1981). These two cases showed that of a total of 1100 HRP-labeled cells found in bulbar auditory structures, only 1 was in LSO. Therefore, the anterograde and retrograde materials agree that if direct projections from LSO to VLL are present at all, they are probably negligible in number.

Ipsilateral projection from LSO to ILL. Following injections of tritiated leucine or kainic acid that included LSO but were not confined to it (e.g., Fig. 1), labeled or degenerating fibers were often seen in the ILL. For this reason two previously prepared cases with HRP microin-

jections in ILL were re-examined for labeled cells in LSO (Glendenning et al., 1981). Of a total of 3315 labeled cells in hindbrain auditory structures in these cases, 69 (2%) were located in ipsilateral LSO and none were found in contralateral LSO. This low level of retrograde labeling could result from direct damage and filling of LSO axons on their way to still higher structures. But a small projection from LSO to ipsilateral ILL cannot be ruled out.

Bilateral projection from LSO to DLL. As seen in Figure 1 and in every other case using anterograde tract-tracing techniques, evidence was seen of a dense terminal field in both the ipsilateral and contralateral DLL.

To explore the terminations of LSO in DLL specifically, five previously prepared cases with HRP microinjections in DLL were re-examined (Glendenning, et al., 1981). Of a total of 353 labeled cells in hindbrain auditory structures in these cases, 92 were found either in ipsilateral or contralateral LSO. This number of labeled cells in LSO amounts to 26% of the total number of auditory cells labeled by HRP injections in DLL. Thus, the results of the anterograde and retrograde tract-tracing techniques agree that a substantial projection from LSO to DLL is present.

In three of these five cases with microinjections of HRP in DLL, more labeled cells were found in the ipsilateral than in the contralateral LSO, in another case the numbers in each LSO were about equal, and in the remaining case more labeled cells appeared in contralateral LSO. From these cases and the anterograde materials described above, it is safe to conclude, as have others, that the projection from LSO to DLL is bilateral (Brunso-Bechtold and Thompson, 1978; Elverland, 1978; Glendenning and Masterton, 1980; Zook and Casseday, 1982). However, we do not have sufficient evidence to conclude that these bilateral projections are symmetrical either in density or in exact locus or mode of termination within DLL. We will return to the question of bilateral symmetry in LSO projections below.

Absence of direct projections from LSO to motor structures

Absence of projections from LSO to cerebellum. Because of unavoidable involvement of the cerebellum in each of the nine cases using anterograde tract-tracing techniques, the possibility of direct projections from LSO to cerebellum could not be ruled out. For this reason, three cases were prepared in which the cerebellum was surgically ablated deeply enough on one side to expose the cut surfaces of the restiform body, the brachium pontis, and the brachium conjunctivum. Raw HRP was then placed on the freshly cut stumps of each of the three peduncles.

In each of these cases, a very large number of vividly labeled cells was found in nonauditory nuclei throughout the brainstem. However, for the present purpose, it is important to note only that in no case were labeled cells found in LSO nor in any other nucleus known to be auditory. Therefore, we have no evidence for a direct projection of LSO to the cerebellum and some evidence that, in the cat at least, such a projection probably does not exist.

Absence of projections from LSO to the spinal cord. Because of our interest in the role of the superior olivary complex in orienting reflexes to sound, the possibility of direct efferent projections from LSO to the spinal cord was also examined (Thompson and Masterton, 1978). The spinal cord was hemisectioned at the C1-C2 junction and the cut surfaces coated with HRP. This procedure resulted in many labeled cells in the medullary, pontine, and mesencephalic reticular formation, the vestibular nuclei, the deep cerebellar nuclei, the red nucleus, the dorsal paraventricular nucleus of the hypothalamus, and in layer V of motor and somatosensory areas of cerebral cortex. Furthermore, labeled cells were found in the tegmentum just dorsal to the superior olivary complex. However, no labeled cells were found in LSO or any other nuclei of the superior olivary complex either ipsilateral or contralateral to the hemisection. Thus, LSO probably does not project to the spinal cord.

Absence of projections from LSO to superior colliculus and beyond. Although the dorsomedial periolivary nucleus of the superior olivary complex has been included in the vast inventory of nuclei projecting to the deeper layers of the superior colliculus (Edwards et al., 1979; Henkel et al., 1979; Spangler and Henkel, 1982), none of the nine cases with kainic acid lesions or tritiated leucine injections yielded clear evidence of a substantial projection to the superior colliculus or beyond. Nevertheless, the theoretical importance of a direct projection from LSO to the superior colliculus or medial geniculate, if they did exist, led us to prepare four cases with injections of HRP (or HRP-labeled wheat germ agglutinin) in the superior colliculus and three with injections in the medial geniculate. One superior colliculus case is shown in Figure 3. In this case extracellular HRP was clearly present in each layer of the right superior colliculus and intruded into the intercollicular nucleus and the rostral extension of the external nucleus of the inferior colliculus (RoBards, 1979). At the same time, very little if any extracellular HRP was seen in the central nucleus of the inferior colliculus.

In this case labeled cells were found *bilaterally* in the parabigeminal nucleus, zona incerta, substantia nigra, dorsomedial periolivary nucleus, pretectal area, cuneiform nucleus, sagulum, locus ceruleus, lateral parabrachial nucleus, perihypoglossal nucleus, and external nucleus of the inferior colliculus; *ipsilaterally* in the ventral lateral geniculate nucleus, raphe nucleus, the pericentral nucleus of the inferior colliculus, and layer V of cerebral cortex; *contralaterally* in the intermediate and deep layers of the superior colliculus, lamina IV of the spinal cord, in the sensory and spinal trigeminal nuclei, in the nucleus of the brachium of the inferior colliculus, in the lateral cervical, gracile, and cuneate nuclei. Also, a few labeled cells were found in the vestibular nuclei and periaqueductal gray, and, possibly because the HRP intruded in the rostral inferior colliculus, a very few labeled cells were found in the dorsal and posteroventral cochlear nuclei and in the nuclei of the lateral lemniscus. Each of these sources of afferent input to the superior colliculus, the intercollicular nucleus or the external nucleus of the inferior colliculus, have been reported previously (e.g., Harting et al., 1973; Edwards et al., 1979; Henkel et al.,

Figure 3. Distribution of labeled cells after a large HRP injection in superior colliculus. Although a large number of labeled cells were found in structures ranging from striate cortex to spinal cord, no labeled cells were found in either LSO.

1979; RoBards, 1979; Distel and Fries, 1981; Schechter et al., 1981).

However, despite this labeling of a large number of cells in a wide variety of nominally visual, auditory, somatosensory, and motor nuclei, no labeled cells were found in LSO either ipsilaterally or contralaterally. Indeed, no labeled cells were found in the entire superior olivary complex except the few in or near the dorsomedial periolivary nucleus. This result confirms that of Edwards et al. (1979) and Henkel et al. (1979).

Turning to the three cases with large injections of HRP centered in the medial geniculate but spreading throughout the posterior thalamus and pretectum, a similarly negative result was obtained. Many well labeled cells were found in the inferior colliculus and in auditory cortex in each case. In addition, probably because the injection sites included posterior and ventrobasal nuclei of the thalamus, labeled cells were found in the dorsal column and trigeminal nuclei and also in somatosensory cortex. A few labeled cells were also found in the nuclei of the lateral lemniscus and in the case with the most posterior spread to the injection, a labeled cell was found in DMPO. But once again, no labeled cells were found in LSO in any case.

Thus, we could find no evidence either in the antero-grade or retrograde tract-tracing cases for a direct projection from LSO to the superior colliculus, the intercol-

licular nucleus, the rostral extension of the external nucleus of the inferior colliculus, or the posterior thalamus.

Projections of LSO to the inferior colliculi

Absence of projections from LSO to the pericentral nucleus and the dorsomedial division of the central nucleus of the inferior colliculus. The auditory projections ascending to the inferior colliculus are the subject of many reports from this and other laboratories (e.g., Barnes et al., 1943; Rasmussen, 1960, 1964; Harrison and Warr, 1962; Harrison and Irving, 1966; Warr, 1966, 1969, 1972; van Noort, 1969; Strominger and Strominger, 1971; Osen, 1972; Rockel and Jones, 1973; Browner and Webster, 1975; Brunso-Bechtold and Thompson, 1976; Elverland, 1978; Roth et al., 1978; Adams, 1979; Brunso-Bechtold et al., 1981; Zook and Casseday, 1982). Here, we focus only on LSO's specific contribution to these projections.

In each case in which anterograde tract-tracing techniques were used in the present study, large numbers of degenerating or labeled fibers in the lateral lemniscus were traced beyond DLL to their entry into the central nucleus of both the ipsilateral and contralateral inferior colliculi (see Fig. 1). Dense clusters of marked terminals were invariably found in the ventrolateral division of the central nucleus, and marked fibers were sometimes found in the commissure of the inferior colliculus. However, in none of the cases were degenerating or labeled fibers or terminals seen in either the external or pericentral nucleus or in the dorsomedial division of the central nucleus. These findings are similar to those reported by Rockel and Jones (1973), who found that lemniscal fibers have few terminations in the dorsomedial division of inferior colliculus and little or none in the pericentral or external nuclei. Nevertheless, the importance of these possible projections led us to explore the subdivisions of the inferior colliculus and its commissure more precisely using HRP technique.

A total of 14 cases were prepared with injections of HRP in the inferior colliculus, and 3 additional cases were examined in which HRP flakes were placed on the cut axons of the fibers of the commissure of the inferior colliculus. In these latter 3 cases, labeled cells were found in the central nucleus of the inferior colliculus, sagulum, all three nuclei of the lateral lemniscus, the cuneiform nucleus, the nucleus of the posterior commissure, pontine gray, periaqueductal gray, and the reticular formation. However, no labeled cells were located in LSO nor in any other hindbrain auditory nucleus.

Turning now to the 14 cases with injections of HRP in the inferior colliculus, three serve to illustrate the essential results (Figs. 4 to 6). Figure 4 shows a case with an HRP injection involving mostly the pericentral nucleus and the dorsomedial division of the central nucleus. The HRP that invaded the ventrolateral division of the central nucleus was confined to its most dorsal part. In this case many labeled cells were seen in the contralateral inferior colliculus in the vicinity of the commissure (sections 180 and 212 in Fig. 4) and in each of the three chief divisions of the contralateral cochlear nucleus (sections

Figure 4. Distribution of labeled cells after HRP injection in the pericentral nucleus and the dorsomedial division of the central nucleus of the inferior colliculus. The injection site encroached on the most dorsal part of the ventrolateral division of the central nucleus. Note the absence of labeled cells in either LSO.

68, 104, and 116). In addition, labeled cells were also seen bilaterally in the dorsal nuclei of the lateral lemniscus, ipsilaterally in the intermediate and ventral nuclei of the lateral lemniscus and in MSO, bilaterally in deep layers of the superior colliculus, ipsilaterally in areas medial to the medial geniculate, in the nucleus of the brachium of the inferior colliculus, and the nucleus of the peripeduncular nucleus.

For the present purpose, however, the important feature of this and three similar cases is the absence of labeled cells in either LSO. Although in some cases labeled cells were found thinly scattered among the nuclei surrounding LSO (for example, in the posterior periolivary nucleus, in the lateral nucleus of the trapezoid body, and in the medial superior olive), none were found in LSO itself.

In sharp contrast, cases such as those shown in Figures 5 and 6 had many labeled cells in LSO. In the case shown in Figure 5, the HRP injection was centered in the dorsal part of the ventrolateral division of the central nucleus. In Figure 6, the injection was centered in the ventromedial part of the ventrolateral division. Each of these two cases had many labeled cells in hindbrain auditory structures including the cochlear nuclei, the nuclei of the lateral lemniscus, and the nuclei of the superior olivary

Figure 5. Distribution of labeled cells after HRP injection centered in the dorsolateral part of the ventrolateral division of the central nucleus of the inferior colliculus. Note labeled cells concentrated in lateral limbs of both LSOs and with more on the side *ipsilateral* to the injection.

Figure 6. Distribution of labeled cells after HRP injection centered in the ventromedial part of the ventrolateral division of the central nucleus of the inferior colliculus. Note labeled cells concentrated in the medial limbs of each LSO and, in contrast to Figure 5, with more on the side *contralateral* to the injection.

complex. Each case also had many labeled cells in LSO.

The idea that LSO's projections to the inferior colliculus might be confined to the ventrolateral division of its central nucleus was verified by a re-examination of 12 cases with microinjections of HRP in the inferior colliculus that had been reported previously (Brunso-Bechtold et al., 1981). These results, combined with the results from the anterograde tract-tracing cases, suggest that LSO's projections to the inferior colliculus probably do not invade the pericentral or external nuclei of the inferior colliculus nor the dorsomedial division of its central nucleus. Instead, they are probably confined to the ventrolateral division of the central nucleus.

Asymmetry in LSO projections to the inferior colliculus

By comparing the cases in Figures 4, 5, and 6, we have seen that the projections from LSO to the inferior colliculus are probably confined to the ventrolateral division of its central nucleus. By comparing the cases in Figures 5 and 6, two other observations can be made. *First*, it can be seen that, corresponding to the difference in the location of the injection sites in the inferior colliculus in the two cases, the location of labeled cells within LSO is quite different. In Figure 5, the injection site was located dorsally (the low frequency region) in the inferior colli-

culus and most of the labeled cells in LSO were found in its lateral limb (the low frequency region of LSO). In the case shown in Figure 6, the injection was located ventrally (the high frequency region) in the inferior colliculus and most of the labeled cells in LSO were found in its medial limb (the high frequency region of LSO). This result, verified in every inferior colliculus case using a retrograde tract-tracing technique, confirms the conclusions of many anatomical and electrophysiological investigations: the cochleotopicity (or tonotopicity) of LSO is preserved in its projections to the inferior colliculus (e.g., Rose et al., 1966; van Noort, 1969; Clopton and Winfield, 1974; Adams, 1975; Aitken et al., 1975; Brunso-Bechtold and Thompson, 1976; Beyerl, 1978; Knudsen and Konishi, 1978; Roth et al., 1978; Semple and Aitken, 1979; Harris et al., 1980; Syka et al., 1981).

The *second* observation that can be made by comparing the cases shown in Figures 5 and 6 is that there is a marked asymmetry in labeling between the ipsilateral and contralateral LSO. That is, in the case with the dorsolateral injections and labeled cells mostly in the *lateral* limb of LSO (Fig. 5), there were more labeled cells in the LSO *ipsilateral* to the injection site than there were in the LSO *contralateral* to the injection site (166 ipsilateral versus 64 contralateral in the four sections shown). However, in the case with the ventromedial

injection and labeled cells in the *medial* limb of LSO (Fig. 6), more labeled cells were found in the LSO *contralateral* to the injection site than there were in the LSO ipsilateral to the injection site (78 contralateral versus 30 ipsilateral in the four sections shown).

This clear asymmetry in the origins of the crossed and uncrossed pathways from LSO to the inferior colliculus was also seen in every other collicular case in which the HRP injection was sufficiently small and eccentric to label cells in only one part of LSO. However, the HRP technique used here leaves open the possibility that this asymmetrical labeling may have been due to some uncontrolled variation in the combination of survival times, differing lengths of the crossed and uncrossed pathways, and the exact locations of the injection sites, or may even have been due to simple stochastic processes in the HRP technique. For this reason, we prepared a number of collicular cases using simultaneous retrograde double-labeling techniques.

Gradient in origin of crossed and uncrossed projections to inferior colliculus. Because of the overall bilaterality of LSO's projections to the inferior colliculus and the possibility that the apparent asymmetry of these projections seen in the HRP cases might be the artifactual result of a combination of technical peculiarities, a total of 18 cases were prepared using retrograde fluorescent dye techniques. We reasoned that if one dye was injected into one colliculus and another dye into the other colliculus, a lateral to medial gradient in one LSO and a medial to lateral gradient in the other LSO should appear with each dye simultaneously. This simultaneous labeling of LSO cells with one or the other dye would serve to rule out the possibility that an asymmetry in the labeling by one dye was due merely to differing axon lengths, transport speeds, survival times, or individual cell peculiarities.

Each fluorescent dye case was essentially similar in preparation. Fast blue (or bisbenzimidazole) was injected in one inferior colliculus, Nuclear Yellow was injected into the other, and the cells labeled with each dye were plotted. The plots of each LSO were then divided into five sectors ranging from dorsolateral to ventromedial, and the cells in each sector were counted and classified as projecting either ipsilaterally, contralaterally, or, if double labeled, bilaterally.

The distribution of labeled LSO cells in one of these cases can be seen in Figure 7. In this case, the asymmetrical labeling can best be seen in the distribution of the *black dots*—each dot representing one cell labeled with the dye injected into the *left* colliculus. Taking only the two extreme sectors of LSO (i.e., the dorsolateral and the ventromedial sectors) as an example, Figure 7 shows more black dots in the dorsolateral sector of the left LSO (79) than in the dorsolateral sector of the right LSO (15). At the same time there are more black dots in the ventromedial sector in the right LSO (24) than there are in the ventromedial sector in the left LSO (17). The mirror image result obtains if the *open circles* are counted. These results for each dye alone confirm those based on single HRP injections illustrated earlier (Figs. 5 and 6).

Possibly because of slightly asymmetric sectioning of

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Figure 7. Distribution of labeled cells following injection of fast blue in the left and Nuclear Yellow in the right inferior colliculus. *Black dots*, cells labeled with fast blue. *Open circles*, cells labeled with Nuclear Yellow. Sections are 40 μ m thick. *Bottom left* section of LSO shows lines used to divide it into five sectors. Note that the *black dots* in the left LSO represent cells originating *uncrossed* fibers, but in the right LSO they represent cells originating *crossed* fibers. Conversely, the *open circles* in the left LSO originate *crossed* fibers, but in the right LSO they originate *uncrossed* fibers.

these brains, a similar difference in labeling was *not* present in every sector in every section of every case (for example, see the two ventromedial sectors in *section 208*). However, summed across all the sections through LSO in any one case, the asymmetry in labeling is clear and statistically highly reliable. In every case, there were more cells labeled with ipsilateral dye in the lateral limbs and more cells labeled with contralateral dye in the medial limbs. The consistency of this result can be seen in Table I, where the cell counts in the five cases with the largest fluorescent dye injections are shown. The table shows the same result obtained for each case as well as for the sum of the cases: The origin of the uncrossed projection is concentrated in LSO's lateral limb and the crossed projection in its medial limb.

The changing proportions of cells labeled with the ipsilateral dye versus those labeled with the contralateral dye progressed across each LSO in a dorsolateral to ventromedial gradient. This result is summarized in Figure 8, which shows that the shift in origins of the crossed projections in the medial limb to the origins of the

TABLE I

Number of neurons labeled with ipsilateral (uncrossed) dye or contralateral (crossed) dye in each sector of LSO in the five cases with the largest collicular injections

Cat No.		LSO Sector					Subtotal	Total	χ^2
		DL	VL	M	DM	VM			
64	Uncrossed	180	96	195	73	45	589	1009	79.64 ($p < 0.00001$)
	Crossed	67	52	111	106	84	420		
111	Uncrossed	64	34	55	69	12	234	514	85.10 ($p < 0.00001$)
	Crossed	17	15	68	106	74	280		
112	Uncrossed	139	163	368	166	63	899	1644	129.62 ($p < 0.00001$)
	Crossed	31	85	276	211	143	745		
116	Uncrossed	115	97	195	102	42	551	875	42.63 ($p < 0.001$)
	Crossed	36	33	120	74	61	324		
117	Uncrossed	218	69	165	99	62	613	985	126.20 ($p < 0.00001$)
	Crossed	47	15	101	92	117	372		
Total	Uncrossed	716	459	978	509	224	2886	5028	448.56 ($p < 0.00001$)
	Crossed	198	200	676	589	479	2142

Figure 8. Lateral to medial gradient in the percentage of LSO cells originating crossed and uncrossed projections. Mean and entire range of the five cases with the largest fluorescent dye injections.

uncrossed projections in the lateral limb grades smoothly across the intermediate sectors of LSO.

Presence of cells in LSO projecting to both inferior colliculi via axon collaterals. Although the primary motive for the use of fluorescent dyes in the present experiment was to confirm the existence of a gradation in the origins of LSO's crossed and uncrossed projections to the inferior colliculus, these cases also allowed the examination of LSO for double-labeled cells, and, indeed, clearly double-labeled cells were found.

The number of double-labeled cells in the 18 cases ranged from 0 to a maximum of 5% with a mean of about 3%. In none of the cases was there an obvious segregation of double-labeled cells within LSO. They appeared thinly scattered throughout the rostral-caudal extent of the nucleus and were more prominent neither in the medial nor lateral limbs. Although the double-labeling technique

used here probably underestimates the true number of cells projecting bilaterally via collaterals, the present results suggest that such cells, although present, are probably not prevalent in LSO.

Route of LSO projections crossing to contralateral inferior colliculus. To visualize the path traced by the LSO fibers projecting to the contralateral side, we reviewed our previously prepared cases with microinjections of tritiated leucine in LSO. One with an injection centered in the medial limb of the right LSO is shown in Figure 9. Because the tracer spread outside the medial limb into the adjacent MSO and local periolivary nuclei, the ipsilateral projections in this case are contaminated. However, since the retrograde materials previously described revealed few if any contralateral projections to the inferior colliculus from the superior olivary complex other than those from LSO, it follows that the contralateral projections to inferior colliculus seen in Figure 9 arise mostly, if not entirely, from LSO. The remaining few labeled fibers leaving the injection site toward the contralateral side could be followed into the olivocochlear bundle crossing toward the contralateral cochlear nucleus under the floor of the IVth ventricle.

Figure 9 shows that the fibers crossing to the contralateral inferior colliculus exit LSO dorsally and medially and then turn almost horizontally until crossing the midline. They then turn ventrally as if headed toward the rostral pole of the contralateral LSO. Just before reaching the contralateral LSO, however, they turn rostrally and dorsally once more, collecting medial to the ventral nucleus of the lateral lemniscus. The efferent projections from LSO remain in the medial band of the lateral lemniscus until reaching their targets in the dorsal nucleus of the lateral lemniscus and the inferior colliculus. This route is virtually identical to the one shown by Elverland in similar materials (1978, Fig. 6).

This somewhat meandering route of the crossed LSO pathway is far dorsal to the trapezoid body and even dorsal to the intermediate acoustic stria. At the same

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Figure 9. Distribution of labeled fibers and terminals following an injection of tritiated leucine centered in but not confined to the medial limb of the right LSO (arrows). Spread of tracer into MSO and surrounding periolivary nuclei contaminates ipsilateral LSO projections but not its contralateral projections to the inferior colliculus. The crossed projection leaves LSO dorsomedially, turns horizontally, descends toward the contralateral LSO, and then turns dorsally to join the medial band of the lateral lemniscus. This path closely parallels the dorsal acoustic stria over most of its course.

time, it is ventral to the fibers of the crossed olivocochlear bundle emanating from the periolivary nuclei, and far caudal and ventral to the commissure of Probst interconnecting the two dorsal nuclei of the lateral lemniscus (e.g., Probst, 1902; Rasmussen, 1946; Warr, 1978; Kudo, 1981). Perhaps surprisingly, the crossing LSO fibers follow the same general course across the midline and through the contralateral tegmentum as the fibers of the dorsal acoustic stria and may, indeed, be comingled (e.g., von Monakow, 1890; Held, 1893; Fernandez and Karapas, 1967).

Summary of results

Combining the results based both on anterograde and retrograde tract-tracing techniques, five tentative conclusions are suggested: *First*, the vast majority of efferent projections from LSO ascend dorsally and rostrally via the ipsilateral and contralateral lateral lemniscus to terminate bilaterally mostly in the dorsal nucleus of the

lateral lemniscus and in the ventrolateral division of the inferior colliculus. *Second*, the projections to the inferior colliculus stem from one of three overlapping subpopulations of cells in each LSO—one large population concentrated laterally projecting only to the ipsilateral side, another large population concentrated medially projecting only to the contralateral side, and a third small and scattered population projecting to both sides via collaterals. *Third*, few if any fibers probably project directly from LSO to either the pericentral or external nuclei or to the dorsomedial division of the central nucleus of the inferior colliculus, nor to any structures more rostral. *Fourth*, there is probably no significant projection from LSO to the cochlear nucleus of either side nor to the contralateral superior olivary complex. *Fifth*, there is probably no significant direct projection from LSO to the spinal cord, to the cerebellum, to any cranial nerve nucleus except possibly the 7th, nor to any layer of the superior colliculus.

Discussion

In the present investigation virtually all of the efferent projections of LSO were found to ascend to more rostral levels of the brainstem. To begin with, no evidence of descending projections from LSO to lower medulla or spinal cord was found even though it was specifically sought. The only evidence of a descending projection whatever was found in the anterograde materials: Injections of tritiated amino acids or kainic acid centered in LSO often result in labeled or degenerating terminals in the cochlear nucleus bilaterally. Nevertheless, very large injections of HRP in the cochlear nucleus that resulted in many labeled cells surrounding LSO, failed to label cells within LSO itself.

Several previous reports have discussed possible projections from LSO to the cochlear nucleus as well as to the cochlea itself, and such projections may well exist in some degree in some animals (e.g., Held, 1893; Rasmussen, 1960, 1964; Osen and Roth, 1969; Borg, 1973; Browner and Webster, 1975; Adams and Warr, 1976; J. Persing and E. Rubel, personal communication). However, despite many attempts in cat, descending or reciprocal projections from LSO to the cochlear nuclei have not been demonstrated with modern tract-tracing techniques. Thus, at present, the weight of evidence derived from the most unequivocal methods indicates that, in cat, LSO itself does not project to the cochlear nucleus. Furthermore, the same evidence is entirely consistent with the idea that fibers terminating in the cochlear nucleus as well as in the cochlea itself originate in the periolivary nuclei (e.g., Osen and Roth, 1969; van Noort, 1969; Warr, 1975, 1978; Elverland, 1978; Farley and Warr, 1981).

Absence of direct projections from LSO to motor structures. Because of our interest in orienting reflexes to sound, specific examination was also made of the possibility of direct projections from LSO to the spinal cord, cerebellum, and superior colliculus (Thompson and Masterton, 1978). No cells were found in LSO either following the direct application of HRP to the cut fibers of the cerebellar peduncles or the spinal cord, or after large

injections into the superior colliculus, although each of these treatments results in a very large number of labeled cells elsewhere. These findings are consistent with those reported by others (e.g., Syka and Strashill, 1970; Kuypers and Maisky, 1975; Aitkin and Boyd, 1975; Edwards et al., 1979; Henkel et al., 1979). When these results are combined with the results of anterograde tract-tracing materials, both of ours and of others, each denying LSO projections to cranial nerve nuclei or tegmentum, it seems reasonable to conclude that LSO probably does not project directly to brainstem structures known to be motor (Barnes et al., 1943; Borg, 1973; Browner and Webster, 1975; Elverland, 1978).

LSO projections to pontine auditory nuclei. Given that few, if any, long fibers descend from LSO or project directly to brainstem motor structures, attention can be focused on the several possible auditory targets of the ascending projections—specifically, the three nuclei of the lateral lemniscus (VLL, ILL, and DLL), the several subdivisions of the inferior colliculus, and beyond.

In general, the projections of the entire superior olivary complex to the three nuclei of the lateral lemniscus are minor in size compared to its main projections to the inferior colliculus. In agreement with others, the present results indicate that the projections from LSO itself are no exception to this more general rule (e.g., Browner and Webster, 1975; Elverland, 1978; Zook and Casseday, 1979; Glendenning et al., 1981). By either anterograde or retrograde tract-tracing techniques, LSO's projections to VLL are slight and to ILL modest at best.

The efferents from LSO to both ipsilateral and contralateral DLL are by far the heaviest of any of its projections to the nuclei of the lateral lemniscus. Bilateral projections from LSO to DLL have been demonstrated previously both by anterograde and retrograde tract-tracing techniques (e.g., Rasmussen, 1946; Stotler, 1953; Browner and Webster, 1975; Brunso-Bechtold and Thompson, 1978; Zook and Casseday, 1979; Glendenning et al., 1981). The present results are consistent with those reports. However, even these substantial projections from LSO to DLL are far less extensive than the projections from LSO to the inferior colliculi and may, in fact, be mostly if not entirely collateral to those continuing to the colliculi.

In sum, LSO's projections to the pons seem to be restricted mostly, if not entirely, to one and possibly two of the three nuclei of the lateral lemniscus. With few fibers terminating in VLL, perhaps somewhat more in ipsilateral ILL, and quite a few bilaterally in DLL, LSO's contribution to the nuclei of the lateral lemniscus seems to increase as the midbrain is approached.

Restriction of LSO's midbrain terminations to the ventrolateral division of the central nucleus of the inferior colliculus. Before turning to the chief projections of LSO to the ventrolateral division of the central nucleus of the inferior colliculus, it is worth repeating that neither the anterograde nor retrograde tract-tracing techniques yielded any evidence whatever of an LSO projection to either the pericentral or external nuclei of the inferior colliculus or to the dorsomedial division of the central nucleus of the inferior colliculus. Specifically, HRP injections either confined to one of these three nuclei or to

their several combinations, or applied directly to the cut fibers of the commissure of the inferior colliculus, invariably failed to label any LSO cells whatever while labeling many other cells elsewhere. Nor did the tritiated leucine or kainic acid cases show labeled or degenerating terminals in these nuclei. Instead, both kinds of evidence indicate that LSO's efferents are confined to the ventrolateral division of the central nucleus. These results are consistent with previous studies employing either anterograde or retrograde tract-tracing techniques (e.g., Barnes et al., 1943; Goldberg and Moore, 1967; Borg, 1973; Adams, 1975, 1979; Browner and Webster, 1975; Brunso-Bechtold and Thompson, 1976, 1978; Harrison, 1978; Roth et al., 1978; Brunso-Bechtold et al., 1981). They are also consistent with electrophysiological surveys of the response characteristics of cells within the inferior colliculus: Only the ventrolateral division of the central nucleus contains cells with LSO-like response characteristics (Rose et al., 1966; Roth et al., 1978; Semple and Aitkin, 1979).

Organization of LSO's projections to the inferior colliculus

Since Stotler's (1953) study of the superior olivary complex, it has been known that each LSO contributes efferents heavily and about equally to each inferior colliculus. This conclusion has been verified many times since, including recent studies using the most modern techniques for tracing fiber projections (Borg, 1973; Browner and Webster, 1975; Elverland, 1978; Roth et al., 1978; Adams, 1979; Brunso-Bechtold et al., 1981; Zook and Casseday, 1982). For example, in previously published studies it has been shown that filling one inferior colliculus with a large amount of HRP labels about the same number of cells in both the ipsilateral and contralateral LSO (Roth et al., 1978; Adams, 1979; Brunso-Bechtold et al., 1981).

In the absence of evidence to the contrary over the 30 years since Stotler's study (1953), it has been assumed that since each LSO as a whole projects about equally to the ipsilateral and contralateral inferior colliculi, each part of LSO projects about equally to both colliculi. The present results indicate that this assumption is probably incorrect. Specifically, the results show that for any collicular injection small enough and eccentric enough to label cells in either the lateral or the medial limb of LSO, the resultant retrograde labeling is asymmetrical. If the injection is centered in the ventromedial inferior colliculus, the labeling is heavier in the LSO contralateral to the injection; if the injection is centered more dorso-laterally, the labeling is heavier in the LSO ipsilateral to the injection.

The asymmetry in retrograde labeling found here is also present in the figures contained in most previously published HRP studies of the afferents to the inferior colliculus in cat. For example, in Figure 3 of Roth et al. (1978), in Figures 3 and 4 of Adams (1979), and in Figures 1 and 3 of Brunso-Bechtold et al. (1981), HRP injections in ventromedial inferior colliculus yielded more labeled cells in contralateral LSO than in ipsilateral LSO; and dorsolateral injections yielded more labeled cells in ipsilateral LSO than in contralateral LSO—the same result

Figure 10. Schematic drawing of LSO's projections to the inferior colliculus showing chiasm-like arrangement.

as obtained here both by HRP and fluorescent dye techniques. Thus, the data from at least three laboratories using at least two different techniques are in good agreement: Most of LSO's crossed and uncrossed projections arise from different parts of the nucleus.

Although we have referred to the projection from LSO to the two colliculi as "asymmetrical" because of the obvious asymmetry seen in retrograde materials, it is noteworthy that from LSO's point of view the locations and concentration gradients of cells originating the crossed and uncrossed projections are quite symmetrical. Most cells in the lateral limbs of both LSO's project ipsilaterally, most cells in the medial limbs of both LSO's project contralaterally. Thus, it is *only* from the collicular point of view (and, therefore, only in the retrograde materials) that this projection appears to be asymmetrical.

Finally, it may be of interest to students of auditory system function that this arrangement of LSO's crossed and uncrossed projections is reminiscent of the optic chiasm (Fig. 10). Because of LSO's known involvement in sound localization, this similarity may be no accident.

Functional consequences of LSO's efferent projections

The gradient in the origins of crossed and uncrossed fibers across LSO has relatively immediate physiological corollaries. As already mentioned, both the LSO and the central nucleus of the inferior colliculus are tonotopically organized with the dorsolateral central nucleus and the lateral limb of LSO containing cells most sensitive to low frequencies, whereas the ventromedial central nucleus and the medial limb of LSO contain cells most sensitive to high frequencies (e.g., Boudreau and Tsuchitani, 1970; Guinan et al., 1972; Roth et al., 1978; Semple and Aitken, 1979). As shown in Figure 9 this lateral to medial gradient for frequency in LSO parallels its gradient of origins of crossed and uncrossed projections. It follows that most of LSO's high frequency cells (e.g., above 16 kHz) project contralaterally, and most of its low frequency cells (e.g., below 4 kHz) project ipsilaterally. Thus, despite the structural similarity of LSO's projections to the partial decussation seen in the optic

chiasm, the physiological basis for the crossed and uncrossed fibers is fundamentally different: The optic chiasm is strictly arranged according to the spatial characteristics of the contributing visual cells, the chiasm-like arrangement of LSO's projections is based mostly on the frequency characteristics of its auditory cells. It is only when these characteristics are referred to the physiological basis of position on the two receptor surfaces (i.e., space in the retina versus frequency in the cochlea) that an abstract similarity between the functional bases of the two arrangements appears once more.

Nevertheless, the chiasm-like anatomical arrangement of projections from LSO helps to explain some of the electrophysiological peculiarities of collicular cells. Specifically, electrophysiological surveys of cells in the inferior colliculus have shown that many LSO-like cells are present in its central nucleus, most are sensitive to high frequencies, and, most important, they are driven only by the contralateral ear. This last characteristic is usually expressed in Goldberg and Brown's (1969) notation of "EI," meaning a cell *Excited* by stimulation of the ipsilateral ear and *Inhibited* by stimulation of the contralateral ear. This notation can be extended to distinguish between EI and IE responses, where "IE" means a cell of opposite allegiance: *Inhibited* by stimulation to the ipsilateral ear and *Excited* by stimulation to the contralateral ear. Using this notation for the present purpose, the vast majority of LSO-type cells found in the colliculus are of the IE type (Rose et al., 1966; Roth et al., 1978; Semple and Aitken, 1979). In contrast, almost all of the cells in LSO are of the EI type (Tsuchitani and Boudreau, 1969; Boudreau and Tsuchitani, 1970; Tsuchitani, 1977; Brownell et al., 1979).

This wide surplus of IE cells over EI cells in the colliculus does *not* follow from the idea that each LSO projects about equally and homogeneously to each colliculus. However, it does seem to be explained by the present results. Since the LSO-type cells in the colliculus are usually ventral, high frequency cells, the present results suggest that they gain most of their LSO input from the contralateral side. This being the case, it need only be noted that when an "EI" axon from LSO crosses the midline, it becomes an "IE" axon. Thus, the high frequency cells of each colliculus receive mostly IE input from the contralateral LSO and, apparently, reflect this IE input in their own IE responses. In contrast, the low frequency cells in the colliculus receive overlapping input: EI input from the ipsilateral LSO, OE input (ipsi—none; contra—excitatory) from the contralateral cochlear nuclei, and EE input (ipsi—excitatory; contra—excitatory) from the ipsilateral MSO. It is not unlikely that the convergence and integration of these excitatory inputs tends to obscure the contribution from ipsilateral LSO alone.

LSO's role in an acoustic chiasm

Finally, the chiasm-like arrangement of projections from LSO may also explain some of the unexpected results from recent behavioral experiments into sound localization—indeed, the results that inspired the present study. It has long been known that it is the high frequencies contained in a natural sound (due to their

efficient shadowing by head and pinna) that provide the binaural spectrum-difference cues for localizing a sound source (e.g., Rosenzweig, 1961; Masterton, 1975; Masterton et al., 1975). It is also known that it is LSO with its excitatory ipsilateral input and inhibitory contralateral input (i.e., its EI cells) that accomplishes the required analysis of the binaural spectrum-difference (Boudreau and Tsuchitani, 1970). These two facts, taken together with the chiasm-like arrangement of LSO projections shown here, suggest that LSO may segregate its output on the basis of the output's eventual utility for sound localization: concentrating the neural representation of the most useful cues in the projection to the contralateral side.

The same set of facts also suggests that section of one lateral lemniscus and the LSO efferents within it would deprive the higher levels of the auditory system of the spectrum-difference analysis only for sounds in the contralateral hemifield of auditory space, while the spectrum-difference analysis for the ipsilateral hemifield would remain available through the other, intact lemniscus. This inference is directly supported by the conclusions of the behavioral experiments: Unilateral section of the lateral lemniscus results in deficits in sound localization only in the hemifield contralateral to the lesion (Thompson and Masterton, 1978; Jenkins and Masterton, 1979, 1982).

By the partial sorting of its ascending output on the basis of its utility for sound localization, the chiasm-like arrangement of LSO projections seems to contribute to much the same sensory function as the optic or somesthetic chiasm. Each chiasm allows the two hemifields of its respective sensory space to be represented on the contralateral side of the brain. Insofar as unilateral lesions *below* either chiasm result in ipsilateral or bilateral field deficits while unilateral lesions *above* either chiasm result in deficits confined to the contralateral hemifield, the contribution of the three chiasm appears to be nearly identical (Jenkins and Masterton, 1982; Moore et al., 1974; Casseday and Neff, 1975; Casseday and Smoak, 1981).

At the same time, unlike the effects of unilateral section of the optic tract or medial lemniscus, unilateral section of the lateral lemniscus does *not* result in deafness either in the contralateral ear or in the contralateral sound field (Masterton et al., 1967; Masterton and Diamond, 1973; Neff et al., 1975). Thus, the functional contribution of LSO's chiasm-like projections probably extends beyond sound localization, possibly to a segregation and sorting of cues for other auditory discriminations as well.

References

- Adams, J. C. (1975) Ascending and descending projections to the inferior colliculus. *Soc. Neurosci. Abstr.* 1: 37.
- Adams, J. C. (1979) Ascending projections to the inferior colliculus. *J. Comp. Neurol.* 183: 519–538.
- Adams, J. C., and W. B. Warr (1976) Origins of axons in the cat's acoustic striae determined by injection of horseradish peroxidase into severed tracts. *J. Comp. Neurol.* 170: 107–122.
- Aitkin, L. M., and J. Boyd (1975) Responses of single units in cerebellar vermis of the cat to monaural and binaural stimuli. *J. Neurophysiol.* 38: 418–429.
- Aitkin, L. M., W. R. Webster, J. L. Veale, and D. C. Crosby (1975) Inferior colliculus. I. Comparison of response properties of neurons in central, pericentral and external nuclei of adult cat. *J. Neurophysiol.* 38: 1196–1207.
- Barnes, W. T., H. W. Magoun, and S. W. Ranson (1943) The ascending auditory pathway in the brainstem of the monkey. *J. Comp. Neurol.* 79: 129–152.
- Beyerl, B. D. (1978) Afferent projections to the central nucleus of the inferior colliculus in the rat. *Brain Res.* 145: 209–223.
- Borg, E. (1973) A neuroanatomical study of the brainstem auditory system of the rabbit. I. Ascending connections. *Acta Morphol. Neerl.-Scand.* 2: 31–48.
- Boudreau, J. C., and C. Tsuchitani (1970) Cat superior olive S segment cell discharge to tonal stimulation. In *Contributions to Sensory Physiology*, W. D. Neff, ed., Vol. 4, pp. 143–213, Academic Press, Inc., New York.
- Brawer, J. R., D. K. Morest, and E. S. C. Kane (1974) The neuronal architecture of the cochlear nucleus of the cat. *J. Comp. Neurol.* 155: 257–300.
- Brownell, W. E., P. B. Manis, and L. A. Ritz (1979) Ipsilateral inhibitory responses in the cat lateral superior olive. *Brain Res.* 177: 189–193.
- Browner, R. H., and D. B. Webster (1975) Projections of the trapezoid body and the superior olivary complex of the kangaroo rat (*Dipodomys merriami*). *Brain Behav. Evol.* 11: 322–354.
- Brunso-Bechtold, J. K., and G. C. Thompson (1976) Auditory hindbrain projections to the inferior colliculus as demonstrated by horseradish peroxidase in the cat. *Anat. Rec.* 184: 365.
- Brunso-Bechtold, J. K., and G. C. Thompson (1978) Ascending auditory projections to the nuclei of the lateral lemniscus as demonstrated by horseradish peroxidase. *Anat. Rec.* 190: 350.
- 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.
- Casseday, J. H., and W. D. Neff (1975) Auditory localization: Role of auditory pathways in brainstem of the cat. *J. Neurophysiol.* 38: 842–858.
- Casseday, J. H., and H. A. Smoak (1981) Effects of unilateral ablation of anteroventral cochlear nucleus on localization of sound in space. In *Neuronal Mechanisms of Hearing*, J. Syka and L. Aitkin, eds., pp. 277–282, Plenum Press, New York.
- Clopton, B. M., and J. A. Winfield (1974) Unit responses in the inferior colliculus of the rat to temporal patterns of tone sweeps and noise bursts. *Exp. Neurol.* 42: 532–540.
- Cowan, W. M., D. I. Gottlieb, A. E. Hendrickson, J. L. Price, and T. A. Woolsey (1972) The autoradiographic demonstration of axonal connections in the central nervous system. *Brain Res.* 37: 21–51.
- Coyle, J. T., M. E. Molliver, and M. J. Kuhar (1978) In situ injection of kainic acid: A new method for selectively lesioning neuronal cell bodies while sparing axons of passage. *J. Comp. Neurol.* 180: 301–324.
- de Olmos, J. S. (1977) An improved HRP method for the study of central nervous connections. *Exp. Brain Res.* 29: 541–551.
- de Olmos, J., and L. Heimer (1980) Double and triple labeling of neurons with fluorescent substances; the study of collateral pathways in the ascending raphe system. *Neurosci. Lett.* 19: 7–12.
- Distel, H., and W. Fries (1981) Sources of subcortical projections to the superior colliculus in the monkey. *Invest. Ophthalm. Vis. Sci. (Suppl.)* 20: 74.
- Ebbesson, S. O. E. (1970) The selective silver impregnation of degenerating axons and their synaptic endings in nonmam-

- malian species. In *Contemporary Research Methods in Neuroanatomy*, W. J. H. Nauta and S. O. E. Ebbesson, eds., pp. 132-161, Springer-Verlag, Berlin.
- Elverland, H. H. (1978) Ascending and intrinsic projections of the superior olivary complex in the cat. *Exp. Brain Res.* 32: 117-134.
- Farley G. R., and W. Warr (1981) Some recurrent projections of the superior olive to anteroventral and dorsal cochlear nuclei in cat. *Soc. Neurosci. Abstr.* 7: 56.
- Fernandez, C., and F. Karapas (1967) The course of the stria of Monakow and Held in the cat. *J. Comp. Neurol.* 131: 371-381.
- Glendenning, K. K., and R. B. Masterton (1980) Afferent and efferent connections of the lateral superior olivary nucleus in cat. *Anat. Rec.* 196: 63-64.
- 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: 673-704.
- 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.
- Goldberg, J. M., and R. Y. Moore (1967) Ascending projections of the lateral lemniscus in the cat and monkey. *J. Comp. Neurol.* 129: 143-155.
- Guinan, J. J., Jr., B. E. Norris, and S. S. Guinan (1972) Single auditory units in the superior olivary complex. II. Locations of unit categories and tonotopic organization. *Int. J. Neurosci.* 4: 147-166.
- Harris, L. R., C. Blakemore, and M. Donaghy (1980) Integration of visual and auditory space in the mammalian superior colliculus. *Nature* 288: 56-59.
- Harrison, J. M. (1978) Functional properties of the auditory system of the brain stem. In *Handbook of Behavioral Neurobiology*, R. B. Masterton, ed., Vol. 1, pp. 409-458, Plenum Press, New York.
- Harrison, J. M., and R. Irving (1966) Ascending connections of the anterior ventral cochlear nucleus in the rat. *J. Comp. Neurol.* 126: 51-64.
- Harrison, J. M., and B. Warr (1962) A study of the cochlear nucleus and ascending pathways of the medulla. *J. Comp. Neurol.* 119: 341-380.
- Harting, J. K., W. C. Hall, I. T. Diamond, and G. F. Martin (1973) Anterograde degeneration study of the superior colliculus in *Tupaia glis*: Evidence for a subdivision between superficial and deep layers. *J. Comp. Neurol.* 148: 361-386.
- Held, H. (1893) Die Zentrale Gehörleitung. *Arch. Anat. Physiol. Anat. Abt.* 17: 201-248.
- Henkel, C. K., S. B. Edwards, and K. S. Kersey (1979) Segregated origins for superior collicular projections based on their intended target structures. *Soc. Neurosci. Abstr.* 5: 372.
- Jenkins, W. M., and R. B. Masterton (1979) Contralateral representation of auditory hemifield in upper levels of brainstem auditory system. *Anat. Rec.* 193: 575-576.
- Jenkins, W. M., and R. B. Masterton (1982) Sound localization: Effects of unilateral lesions in central auditory system. *J. Neurophysiol.* 47: 987-1016.
- Jenkins, W. M., and M. M. Merzenich (1981) Lesions of restricted frequency representational sectors within primary auditory cortex produce frequency dependent sound localization deficits. *Soc. Neurosci. Abstr.* 7: 392.
- Kawamura, S., and K. Niimi (1972) Counterstaining of Nauta-Gygax impregnated sections with cresyl violet. *Stain Technol.* 47: 1-6.
- Knudsen, E. I., and M. Konishi (1978) Space and frequency are represented separately in auditory midbrain of the owl. *J. Neurophysiol.* 41: 870-884.
- Kudo, M. (1981) Projections of the nuclei of the lateral lemniscus in the cat: An autoradiographic study. *Brain Res.* 221: 57-69.
- Kuypers, H. G. J. M., and V. A. Maisky (1976) Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. *Neurosci. Lett.* 1: 9-14.
- LaVail, J. H., and M. M. LaVail (1972) Retrograde axonal transport in the central nervous system. *Science* 176: 1416-1417.
- Masterton, R. B. (1975) Adaptation for sound localization in the ear and brainstem of mammals. *Fed. Proc.* 33: 1904-1910.
- Masterton, R. B., and I. T. Diamond (1973) Hearing: Central neural mechanisms. In *Handbook of Perception*, E. C. Carterette and M. P. Friedman, eds., Vol. 3, pp. 409-448, Academic Press, Inc., New York.
- Masterton, R. B., J. A. Jane, and I. T. Diamond (1967) Role of brainstem auditory structures in sound localization. I. Trapezoid body, superior olive, and lateral lemniscus. *J. Neurophysiol.* 30: 341-359.
- Masterton, R. B., G. C. Thompson, J. K. Brunso-Bechtold and M. J. RoBards (1975) Neuroanatomical basis of binaural phase-difference analysis for sound localization: A comparative study. *J. Comp. Physiol. Psychol.* 89: 379-386.
- Masterton, R. B., K. K. Glendenning, and K. A. Hutson (1979) Preservation of trapezoid body fibers after biochemical ablation of superior olives with kainic acid. *Brain Res.* 173: 156-159.
- Masterton, R. B., K. K. Glendenning, and R. J. Nudo (1981) Anatomical-behavioral analyses of hindbrain sound localization mechanisms. In *Symposium on Neuronal Mechanisms of Hearing*, J. Syka and L. Aitkin, eds., pp. 263-275, Plenum Press, New York.
- Masterton, R. B., K. K. Glendenning, and R. J. Nudo (1982) Anatomical pathways subserving the contralateral representation of a sound source. In *Localization of Sound: Theory and Application*, W. Gatehouse, ed., pp. 113-125, Amphora Press, CT.
- Mesulam, M. -M. (1976) The blue reaction product in horseradish peroxidase neurohistochemistry: Incubation parameters and visibility. *J. Histochem. Cytochem.* 24: 1273-1280.
- 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.
- Mesulam, M. -M., and T. M. Brushart (1979) Transganglionic and anterograde transport of horseradish peroxidase across dorsal root ganglia: A tetramethylbenzidine method for tracing central sensory connections of muscles and peripheral nerves. *Neuroscience* 4: 1107-1117.
- Mesulam, M. -M., and D. L. Rosene (1979) Sensitivity in horseradish peroxidase neurohistochemistry: A comparative and quantitative study of nine methods. *J. Histochem. Cytochem.* 27: 763-773.
- Middlebrooks, J. C., and J. D. Pettigrew (1980) Functional classes of auditory cortical neurons distinguished by sensitivity to sound location. *Soc. Neurosci. Abstr.* 6: 333.
- Moore, C. N., J. H. Casseday, and W. D. Neff (1974) Sound localization: The role of the commissural pathways of the auditory system of the cat. *Brain Res.* 82: 13-26.
- Morest, D. K. (1973) Auditory neurons of the brain stem. *Adv. Oto-Rhino-Laryngol.* 20: 337-356.
- Neff, W. D., I. T. Diamond, and J. H. Casseday (1975) Behavioral studies of auditory discrimination: Central nervous

- system. In *Handbook of Sensory Physiology*, W. D. Keitel and W. D. Neff, eds., Vol. 2, pp. 307–400, Springer-Verlag, New York.
- Osen, K. K. (1972) Projection of the cochlear nuclei on the inferior colliculus in the cat. *J. Comp. Neurol.* 144: 355–372.
- Osen, K. K., and K. Roth (1969) Histochemical localization of cholinesterases in the cochlear nuclei of the cat, with notes on the origin of acetylcholinesterase-positive afferents and the superior olive. *Brain Res.* 16: 165–185.
- Probst, M. (1902) Experimentelle Untersuchungen über die Anatomie und Physiologie der Leitungsbahnen des Gehirnstammes. *Arch. Anat. Physiol. Suppl.* 47: 254.
- Ramón y Cajal, S. (1909) *Histologie du Systeme Nerveux de l'Homme et des Vertébrés*, Tome I, pp. 774–838, Instituto Ramón y Cajal, Madrid, 1952.
- Rasmussen, G. L. (1946) The olivary peduncle and other fiber projections of the superior olivary complex. *J. Comp. Neurol.* 84: 141–219.
- Rasmussen, G. L. (1960) Efferent fibers of the cochlear nerve and cochlear nucleus. In *Neuronal Mechanisms of the Auditory and Vestibular Systems*, G. L. Rasmussen and W. F. Windle, eds., pp. 105–115, Charles C Thomas, Springfield, IL.
- Rasmussen, G. L. (1964) Anatomic relationships of the ascending and descending auditory systems. In *Neurological Aspects of Auditory and Vestibular Disorders*, W. S. Fields and B. R. Alford, eds., pp. 5–23, Charles C Thomas, Springfield, IL.
- RoBards, M. J. (1979) Somatic neurons in the brainstem and neocortex projecting to the external nucleus of the inferior colliculus: An anatomical study in the opossum. *J. Comp. Neurol.* 184: 547–566.
- Rockel A. J., and E. G. Jones (1973) The neuronal organization of the inferior colliculus of the adult cat. I. The central nucleus. *J. Comp. Neurol.* 147: 11–60.
- Rose, J. E., N. B. Gross, C. D. Geisler, and J. E. Hind (1966) Some neural mechanisms in the inferior colliculus of the cat which may be relevant to localization of a sound source. *J. Neurophysiol.* 29: 288–314.
- Rosenzweig, M. R. (1961) Development of research on the physiological mechanisms of auditory localization. *Psychol. Bull.* 58: 376–389.
- Roth, G. L., L. M. Aitkin, R. A. Anderson, 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.
- Schechter, P. B., J. A. Hirsch, and T. C. T. Yin (1981) Auditory input to cells in the deep layers of the cat superior colliculus. *Soc. Neurosci. Abstr.* 11: 57.
- Seiple, M. N., and L. M. Aitkin (1979) Representation of sound frequency and laterality by units in central nucleus of cat inferior colliculus. *J. Neurophysiol.* 42: 1626–1639.
- Spangler, K. M., and C. K. Henkel (1982) Some efferent projections of the dorsomedial periolivary region. *Soc. Neurosci. Abstr.* 8: 347.
- Steward, O. (1981) Horseradish peroxidase and fluorescent substances and their combination with other techniques. In *Neuroanatomical Tract-tracing Methods*, L. Heimer and M. J. RoBards, eds., pp. 279–310, Plenum Press, New York.
- Stotter, W. S. (1953) An experimental study of the cells and connections of the superior olivary complex of the cat. *J. Comp. Neurol.* 98: 401–432.
- Strominger, N. L. (1969) Localization of sound in space after unilateral and bilateral ablation of auditory cortex. *Exp. Neurol.* 25: 521–533.
- Strominger, N. L., and A. I. Strominger (1971) Ascending brainstem projections of the anteroventral cochlear nucleus in the monkey. *J. Comp. Neurol.* 143: 217–232.
- Syka J., and M. Strashill (1970) Activation of superior colliculus neurons and motor responses after electrical stimulation of the inferior colliculus. *Exp. Neurol.* 28: 384–392.
- Syka, J., D. Druga, J. Popelář, and B. Kolinová (1981) Functional organization of the inferior colliculus. In *Neuronal Mechanisms of Hearing*, J. Syka and L. Aitkin, eds., pp. 137–154, Plenum Press, New York.
- Thompson, G. C., and R. B. Masterton (1978) Brain stem auditory pathways involved in reflexive head orientation to sound. *J. Neurophysiol.* 41: 1183–1202.
- Tsuchitani, C. (1977) Functional organization of lateral cell groups of cat superior olivary complex. *J. Neurophysiol.* 40: 296–318.
- Tsuchitani, C., and J. C. Boudreau (1969) Stimulus level of dichotically presented tones and cat superior olive S-segment cell discharge. *J. Acoust. Soc. Am.* 46: 979–988.
- van Gehuchten, A. (1906) *Le System Nerveux de l'Homme*, Ed. 4, Trois Rois Louvain.
- van Noort, J. (1969) *The Structure and Connections of the Inferior Colliculus*, Van Gorcum & Co., N. V., Netherlands.
- von Monakow, C. (1890) Striae acusticae und untere Schleife. *Arch. Psychiatr. Nervenkr.* 22: 1–26.
- Warr, B. (1966) Fiber degeneration following lesions in the anterior-ventral cochlear nucleus of cat. *Exp. Neurol.* 14: 453–474.
- Warr, B. (1969) Fiber degeneration following lesions in the posteroventral cochlear nucleus of the cat. *Exp. Neurol.* 23: 140–155.
- Warr, B. (1972) Fiber degeneration following lesions in the multipolar and globular cell areas in the ventral cochlear nucleus of the cat. *Brain Res.* 40: 247–270.
- 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, B. (1978) The olivocochlear bundle: Its origins and terminations in the cat. In *Evoked Electrical Activity in the Auditory System*, R. F. Naunton and C. Fernandez, eds., pp. 43–65, Academic Press, Inc., New York.
- Zook, J. M., and J. H. Casseday (1979) Connections of the nuclei of the lateral lemniscus in the mustache bat, *Pteronotus parnellii*. *Soc. Neurosci. Abstr.* 5: 34.
- Zook, J. M., and J. H. Casseday (1982) Origin of ascending projections to inferior colliculus in the mustache bat, *Pteronotus parnellii*. *J. Comp. Neurol.* 207: 14–28.