Projection of the Marginal Shell of the Anteroventral Cochlear Nucleus to Olivocochlear Neurons in the Cat

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ABSTRACT

The marginal shell of the anteroventral cochlear nucleus is anatomically and physiologically different from its central core. Previous studies suggest that neurons in the marginal shell are well suited to encode the intensity of acoustic stimuli. To investigate the projections of the marginal shell, a focal injection (<100 nl) of a mixture of biotinylated dextran amine (BDA) and $^3$H-leucine was made into the marginal shell of the cat combined with injection of cholera toxin subunit-B (CTB) into the cochleas. Following a 7-day survival, the cats were perfused. Axons and swellings labeled with BDA and olivocochlear neurons labeled with CTB were immunocytochemically stained black and brown, respectively. $^3$H-leucine labels were visualized by autoradiography. Labeled neural structures were examined via light microscopy. We found that swellings labeled with BDA, sometimes doubly labeled with BDA and $^3$H-leucine, were in close apposition with dendrites and/or somata of olivocochlear neurons identified with CTB labeling. Double labeling with BDA and $^3$H-leucine signifies that the label was anterogradely transported. The results support the conclusion that the anteroventral cochlear nucleus projects to medial olivocochlear neurons bilaterally and to lateral olivocochlear neurons ipsilaterally. Furthermore, the results are consistent with the interpretation that the marginal shell provides a source of the above-mentioned projections. Together with information in the literature, the present anatomical results support a hypothesis that the marginal shell provides information about stimulus intensity as a part of a reflex (or feedback gain control) system comprising the cochlea, cochlear neurons, cochlear nucleus, medial olivocochlear neurons, and cochlear outer hair cells. J. Comp. Neurol. 420: 127–138, 2000. © 2000 Wiley-Liss, Inc.

Indexing terms: cochlear mechanics; feedback; reflex; cholera toxin subunit B; biotinylated dextran amine; tritiated leucine

The marginal shell of the ventral cochlear nucleus (VCN) consists of the granule cell layer, the small cell cap, and the “cell poor rind,” which encapsulate the VCN central core of large cells. The granule cell layer covers the surface of the VCN, and the small cell cap (Osen, 1969) is subjacent to the granule cell layer. The cell-poor rind is usually located along the medial and ventral borders of the VCN (Liberman, 1993). The small cell cap in the human (Moore and Osen, 1979; Paxinos and Huang, 1995) is considerably expanded and constitutes a large portion of the cochlear nucleus (CN).

The marginal shell of the VCN is morphologically different from its central core (Cant, 1993). Liberman (1991, 1993) found that nearly all cat type I auditory nerve fibers innervating the small cell cap have low spontaneous rates. In contrast, the VCN core receives a mixture of auditory nerve fibers having high and low spontaneous rates. Collaterals of type II auditory nerve fibers, which originate from the outer hair cells, often terminate in the VCN marginal shell in rodents (Brown et al., 1988a; Brown and Ledwith, 1990). Besides ascending inputs, the VCN marginal shell also receives descending inputs from the superior olivary complex (Shore and Moore, 1998), including collaterals of medial olivocochlear neurons (Brown et al.,...
1988b; Benson and Brown, 1990), and from the auditory midbrain (Shore and Moore, 1998) and cortex (Feliciano et al., 1995; Weedman and Ryugo, 1996). There is little evidence for termination of medial olivocochlear collaterals in the VCN central core. The VCN marginal shell also receives vestibular (Burian and Gatoettner, 1988; Kevetter and Perachio, 1989; Zhao et al., 1995) and somatosensory (Itoh et al., 1987; Weinberg and Rustioni, 1987; Wright and Ryugo, 1996) inputs.

Physiological characteristics of the marginal shell of the anteroventral cochlear nucleus (AVCN) are likewise different from those of the AVCN core. A large majority of marginal-shell neurons exhibit low spontaneous rates and wide dynamic ranges (as wide as 89 decibels), whereas a large majority of central-core units exhibit narrow dynamic ranges (Ghoshal and Kim, 1996a; 1997). Some neurons of the marginal shell are not driven by sounds, whereas essentially all neurons of the central core are (Ghoshal and Kim, 1996b). Because auditory nerve fibers with low spontaneous rates have wider dynamic ranges than their high spontaneous-rate counterparts (Sachs and Abbas, 1974; Evans and Palmer, 1980), the afferent input nearly exclusively from low spontaneous-rate nerve fibers to the marginal shell (Liberman, 1991) is thus expected to enhance encoding of the acoustic stimulus intensity in this region (Kim et al., 1995). Moreover, the input of medial olivocochlear neurons' collaterals to the marginal shell is expected to counteract the suppressive effects of the medial olivocochlear neurons on the output of the cochlear amplifier (Davis, 1983; Neely and Kim, 1983), thereby helping marginal-shell neurons to represent accurately the absolute stimulus intensity (Brown et al., 1988b; Kim et al., 1995; 1998). In brief, the unique afferent and efferent inputs to the VCN marginal shell should make this region ideally suited to encode intensity of the acoustic stimulus.

Although the descending portion of the cochlear feedback gain-control circuit, from medial olivocochlear neurons to the cochlea, has been well documented (see, e.g., Warr, 1992), the ascending portion of the circuit, from the CN to medial olivocochlear neurons, is poorly understood. The goal of this study is to evaluate the hypothesis that the AVCN marginal shell projects to medial olivocochlear neurons, thereby conveying stimulus intensity information.

**MATERIALS AND METHODS**

**General procedures**

This study used four laboratory cats, which were pigmented, female, of body weight 2.0—3.5 kg, and with clean ears and no history of diseases or hereditary abnormalities. The cats were purchased from a commercial breeder of research cats. The procedures used in this study followed the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Animal Care Committee, University of Connecticut Health Center. Each cat was initially anesthetized with an intraperitoneal injection of sodium pentobarbital, 35 mg/kg. An intravenous (IV) catheter and an intratracheal tube were installed. Deep anesthesia was maintained by administering sodium pentobarbital through the IV catheter or by applying halothane, 0.1—0.6%, mixed with nitrous oxide and oxygen, through the intratracheal tube. Animal body temperature was maintained near 38°C.

A craniotomy was performed on the left posterior fossa, and a portion of the cerebellum overlying the left CN was aspirated. Using surface landmarks of the AVCN, a micropipette was guided toward the AVCN using a microdrive. Insofar as the target injection site was the marginal shell of the AVCN, a shallow structure compared to the AVCN central core, it was important to ascertain accurately when the electrode tip first touched the surface of the AVCN. For this purpose, we applied a swept-tone stimulus and monitored the voltage signal picked up by the electrode (the same micropipette containing tracers with an inner diameter of about 25 μm) as it gradually advanced toward the AVCN surface. The appearance of detectable evoked neural activities of multunits signified the initial contact of the electrode with the AVCN surface. We advanced the electrode a short distance (100—200 μm) beyond that point. Recording multunit responses also allowed us to determine the characteristic frequency of the injection site in each of the cats, except for one as a result of equipment malfunction. A small amount (<0.1 μl) of a mixture of biotinylated dextran amine (BDA) (Molecular Probes Inc., Eugene, OR), 10%, 10,000 MW, and 3H-leucine (L-leucine-4,5-3H; ICN Biomedicals, Inc., Irvine, CA), 50 μCi/μl, was injected into the marginal shell of the AVCN using a pressure injector (Picospritzer; General Valve Corp., East Hanover, NJ), 20—100 psi, 40—200 msec duration, several pulses. Injection of a mixture of BDA and 3H-leucine was used previously by Warr and Beck (1996). The bulla was opened, and chola toxin subunit-B (CTB; List Biological Laboratories, Inc., Campbell, CA), 8—10 μl, 1%, was injected into the cochlear scala tympani through a micropipette (inner diameter about 30 μm), which impaled the round window membrane. Injection of CTB was made into both cochleas of each cat using the pressure injector described above, 25 psi, 50 msec pulse duration, over a period of about 20 minutes for each cochlea. Upon withdrawal of the micropipette, the round window membrane sealed by itself without showing any detectable fluid leakage. The experimental approach of this study is summarized in Figure 1.

**Immunohistological processing**

After a survival period of 7 days, each cat was anesthetized as described above and perfused transcardially, initially with saline and subsequently with a solution containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.1 M sodium phosphate-buffed saline (PBS), pH 7.4, at room temperature. The brain tissue was kept in 30% sucrose for 1 day. Frozen sections were cut at 50 μm in the transverse plane. The procedure for processing BDA label was similar to that of Veenman et al. (1992). The brain sections were incubated in 0.3 % H2O2 for 15—20 minutes and rinsed in 0.1 M PBS plus 0.1% Triton X-100. The sections were incubated in ABC solution (Vector Laboratories, Inc., Burlingame, CA) overnight at 4°C, and rinsed in 0.1 M PBS. The sections were treated with diaminobenzidine intensified with cobalt chloride and nickel ammonium sulfate (Adams, 1981). Thus, BDA-labeled structures were visualized with grayish black reaction products.

The sections were then incubated with 5% normal horse serum (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) for 2 hours at room temperature and in a primary antibody (goat anti-CTB, List Biological Labora-
tories, Inc.), diluted to 1:20,000, plus 2.5% normal horse serum, 4°C, under agitation for 3–4 days. The sections were then rinsed and incubated with a secondary antibody (biotinylated anti-goat IgG, made in horse; Vector Laboratories, Inc.) diluted to 1:2,000 in 0.1 M PBS, plus 1% normal horse serum, 24–48 hours, 4°C, under agitation. The sections were incubated in ABC overnight at 4°C and treated with diaminobenzidine without metal intensification. Thus, CTB-labeled structures were visualized with reddish brown reaction products.

Alternate sections (one half of the total set) were processed by autoradiography (Oliver, 1984) with an exposure time of 8 weeks at 4°C. A subset of the sections (usually three-fourths of the total set) was counterstained with cresyl violet, and the remainder was cleared.

**Microscopic examination**

A light microscope (Leitz Diaplan, Leica, Inc., Allendale, NJ) was used to identify axons and axonal swellings labeled with BDA (grayish black) and/or 3H-leucine (silver grains) and somata and dendrites of olivocochlear (OC) neurons labeled with CTB (reddish brown). Close appositions between BDA-labeled axonal swellings and CTB-labeled dendrites/somata were examined under a 100× oil-immersion objective (Leitz PL Fluotar, NA = 1.32). The criterion for acceptance as close apposition between an axonal swelling and a dendrite/soma was that the two structures should be in the same focal plane with no visible gap between the two under a 100× objective. Images were digitized through a color video camera connected to a computer. Camera lucida drawings were made for CTB-labeled OC neurons with closely apposed BDA-labeled axons and swellings. An injection site was defined as the area identified by the presence of dense and uniform deposits of BDA reaction product throughout the neuropil at the time of examination. A computer-interfaced microscope system (Neurolucida, Microbrightfield, Colchester, VT) was used to trace injection sites. The same system was also used to measure sizes of axonal swellings and thicknesses of axons (for the axonal segments between swellings) contacting OC neurons under a 100× objective.

**Regions containing OC neurons**

By using Warr and Guinan’s (1979) criteria, OC neurons were divided into medial and lateral olivocochlear (MOC and LOC) neurons. We designated the locations of OC neurons into various regions. In defining these regions of OC neurons, we used the nomenclature of the superior olivary complex nuclei described by Guinan et al. (1972). We found that OC neurons were located not only inside various cell-dense periolivary nuclei but also in cell-poor areas, which are not included in any periolivary nucleus. This difference between the nature of a periolivary nucleus and that of an OC region led us to introduce a new set of terminology of OC regions, shown in Figure 2 and described below.

Four regions of MOC neurons were defined as follows: 1) R-MOC, a rostral SOC region, rostral to the medial superior olive (MSO); it includes a rostral part of the medial nucleus of the trapezoid body and a part of the ventral nucleus of the laterallemniscus; some of the MOC neurons in this region are located as lateral as LOC neurons (Adams, 1983; Warr, 1992); 2) V-MOC, a region ventral to the MSO; it includes the ventral nucleus of the trapezoid body and a surrounding area; the rostral end of the MSO represents a landmark that separates V-MOC from R-MOC; 3) VM-MOC, a region ventromedial to the MSO; it includes most of the medial nucleus of the trapezoid body and the cell-poor area between the MSO and the medial nucleus of the trapezoid body; 4) DM-MOC, a region dorsomedial to the MSO; it includes the dorsomedial periolivary nucleus and a surrounding area.

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**Fig. 1.** Schematic diagram of the experimental approach used in this study. AVCN, anteroventral cochlear nucleus; BDA, biotinylated dextran amine; cereb., cerebellum; CTB, cholera toxin subunit B; 3H-leucine, tritiated leucine; LOC, lateral olivocochlear neuron; LSO, lateral superior olive; MOC, medial olivocochlear neuron; MSO, medial superior olive; RW, round window; 4th Vent, fourth ventricle; 5ST, spinal trigeminal tract; 6, abducens nerve root; 7, facial nerve root.
Analogously, five regions of LOC neurons were defined as follows: 1) M-LOC, a marginal region of the lateral superior olive (LSO); it includes the immediate margins surrounding the LSO and the dorsal hilus of the LSO; Warr et al. (2000) introduced the designation of a subpopulation of the cat LOC neurons to be those in the M-LOC region; 2) R-LOC, a region rostral to the LSO; it includes the anterolateral periolivary nucleus and a rostral part of the lateral nucleus of the trapezoid body and surrounding areas; R-LOC lies between the rostral ends of the LSO and MSO; 3) VL-LOC, a region ventrolateral to the LSO; it includes most of the lateral nucleus of the trapezoid body and a surrounding area; 4) D-LOC, a region dorsal to the LSO; it includes the dorsolateral periolivary nucleus and a surrounding area; the rostral end of the LSO represents a landmark that separates R-LOC from VL-LOC and D-LOC; 5) C-LOC, a region caudal to the LSO; it includes the posterior periolivary nucleus and a surrounding area; the caudal end of the LSO represents a landmark that separates C-LOC from D-LOC and VL-LOC.

**RESULTS**

The BDA injection sites are shown in Figure 3. The centers of the injection sites were located in the dorsolateral part of the marginal shell of the AVCN in all of the four animals. Nomenclature and parcellation of the CN are adapted from Brawer et al. (1974) and Liberman (1991, 1993).

Figure 4 shows color images of examples of CTB-labeled (reddish brown) dendrites and somata of OC neurons in close apposition with axonal swellings labeled with BDA (grayish black). The axons and axonal swellings of the AVCN marginal shell neurons were well labeled by BDA. Somata and long stretches of dendrites of OC neurons were well labeled with CTB in the present study, a result consistent with results of Vetter and Mugnaini (1992). BDA-labeled axons tended to be thin (mean diameter 0.5 \( \mu m \)). Many of the axonal swellings were small (see, e.g., Fig. 4F, J, M), whereas some were larger (see, e.g., Fig. 4C, K). Some of these axonal swellings and axons were doubly labeled with BDA and \(^3\)H-leucine (silver grains;
Because autoradiographic silver grains are present on the surface of a tissue slide, whereas neural structures (such as swellings) are generally within a tissue section, visualizing a structure and the corresponding silver grains required taking images at multiple focal planes. Double labeling of axons/swellings with BDA and $^3$H-leucine, as shown in Figure 4, provides evidence that the tracers were anterogradely transported, because $^3$H-leucine is specifically transported anterogradely (Cowan et al., 1972), whereas BDA is transported both antero- and retrogradely. These images demonstrate that the colors (brown and black) of the two reaction products (CTB and BDA) are clearly distinguishable. The OC cells shown in Figure 4A,E are LOC cells in the ipsilateral SOC. The rest of the OC cells are MOC cells on the contralateral side (Fig. 4B–D,G–I) and on the ipsilateral side (Fig. 4F,J–M). The characteristic frequency (CF) of each injection site was 13.8 kHz for Q87, unknown for Q88, 4.0 kHz for Q89, and 6.0 kHz for Q93. A, anterior part of PVCN; AA, anterior part of the anterior division of AVCN; AN, auditory nerve; AP, posterior part of the anterior division of AVCN; CG, cerebellar granular cell layer; D, dorsal; DAS, dorsal acoustic stria; DCN, dorsal cochlear nucleus; G, granule cell layer; L, lateral; PD, posterodorsal part of the AVCN; PV, posteroventral part of the AVCN; R, cell-poor rind; S, small cell cap.
Fig. 4. Examples of images of axons (arrows) and axonal swellings (arrowheads) that were labeled with BDA (black) and in close apposition with dendrites or somata (open arrows) of OC neurons retrogradely labeled with CTB (reddish brown). Silver grains, which were taken at different focal planes, represent 3H-leucine labels. All images were taken with a 100× oil-objective lens, and dorsal is upward unless otherwise indicated. A1, A2: R-LOC, ipsi. Thin lines in A1 and A2 (and other panels) indicate that the image is a montage constructed with parts taken at multiple focal planes. B1, B2: VM-MOC, contra., rotated 45° clockwise. C1, C2: VM-MOC, contra. D1–D3: VM-MOC, contra. E1–E3: R-LOC, ipsi. F1, F2: V-MOC, ipsi. G1, G2: VM-MOC, contra. H: R-MOC, contra., rotated 90° clockwise. I: V-MOC, contra. J: V-MOC, ipsi., rotated 90° counterclockwise. K: V-MOC, ipsi., rotated 30° clockwise. L: VM-MOC, ipsi., rotated 30° clockwise. M: MOC neuron’s dendrite, R-MOC, rotated 35° clockwise.
camera lucida drawings represent structures in multiple focal planes superimposed and thus demonstrate more parts of each cell. BDA-labeled swellings were found in contact with proximal dendrites (see, e.g., Fig. 5E,H) distal dendrites (see, e.g., Fig. 5A,K) or somata (see, e.g., Fig. 5B,G) of MOC cells. A series of BDA-labeled axonal swellings connected by an axon, resembling stringed beads, was often observed traveling together and maintaining close contacts with an MOC neuron’s dendrite (see, e.g., Fig. 5D,K).

Camera lucida drawings of CTB-labeled LOC neurons and BDA-labeled axons/swellings on the ipsilateral side are shown in Figure 6. Two of the LOC neurons, shown in Figure 6A,C, are represented in Figure 4A,E, respectively. The LOC neurons tended to be smaller than the MOC neurons, and a group of LOC neurons were sometimes clustered together (see, e.g., Fig. 6D). The contacts of BDA-labeled axonal swellings on the LOC neurons tended to be on the somata (Fig. 6, all five neurons), although contacts of axonal swellings on both dendrites and somata were also observed (Fig. 6C). In the case of the neuron shown in Figure 6C (also Fig. 4E), an axon collateral (arrow, Fig. 6C) was seen coming off a parent axon and contacting the soma.

The axonal swellings contacting OC neurons have various sizes as noted above. Their distributions are shown in Figure 7A,B. The results in this figure represent swellings associated with a random sample of 11 MOC cells (the same as those shown in Figs. 4, 5) and 11 LOC cells SOC, respectively. These MOC neurons are also shown in color pictures in Figure 4. The locations of the neurons are given in the legend of Figure 4.

Fig. 5. A–K: Camera lucida drawings of MOC neurons in close apposition with BDA-labeled axonal swellings. The left and right groups represent MOC neurons in the contralateral and ipsilateral SOC, respectively. These MOC neurons are also shown in color pictures in Figure 4. The locations of the neurons are given in the legend of Figure 4.

Fig. 6. Camera lucida drawings of LOC neurons, in the ipsilateral SOC, in close apposition with BDA-labeled axonal swellings. Two of these LOC neurons are shown as color pictures in Figure 4. The locations of the neurons are as follows. A: R-LOC. B: M-LOC. C: R-LOC. D: M-LOC. E: M-LOC.
The swellings in contact with MOC neurons (mean 5.2 \( \mu \text{m}^2 \)) were significantly \((P < 0.01, \text{analysis of variance})\) larger than those in contact with LOC neurons (mean 3.3 \( \mu \text{m}^2 \)). The difference in the size of the swellings suggests 1) that there may be a difference in functional characteristics of the two groups of putative synapses and/or 2) that there may be two different cell types in the AVCN marginal shell projecting to MOC or LOC neurons separately.

Distributions of BDA-labeled axonal thicknesses contacting MOC and LOC neurons are shown in Figure 7C,D. It is not known whether these axons are myelinated. The thickness of a BDA-labeled portion of an axon is expected to correspond to the axoplasm excluding any myelin sheath that may be present. The axons associated with MOC and LOC neurons had similar thicknesses with means of 0.54 and 0.52 \( \mu \text{m} \), respectively \((P > 0.05, \text{analysis of variance})\). The fact that these axons are thin suggests that they may be lightly myelinated or unmyelinated and that the axons transmit signals slowly, at least over their termination zones where the thicknesses were measured.

Figure 8 shows distributions of OC neurons, which are in close apposition with BDA-labeled axonal swellings, in various OC regions. C, caudal; D, dorsal; DM, dorsomedial; M, marginal; R, rostral; V, ventral; VL, ventrolateral; VM, ventromedial. This figure represents 116 OC neurons from three cats.
various OC regions. The largest component (52%) was contalateral MOC neurons. The next large component (29%) was ipsilateral MOC neurons. A relatively small proportion (19%) corresponded to ipsilateral LOC neurons. The numbers of these OC neurons in various OC regions are listed in Table 1. The findings of the present study are summarized schematically in Figure 9A.

**DISCUSSION**

**Effective and virtual injection sites**

An effective injection site is defined to be "the volume of tissue which has sustained the uptake and subsequent transport of a tracer", whereas a virtual injection site is the volume of tissue "which is identified by the presence of dense and uniform deposits of reaction product throughout the neuropil at the time of microscopic examination" (Mesulam, 1982, p 63–64). In results obtained with horseradish peroxidase as a tracer, a virtual injection site was observed to expand between 2 and 8 hours and contract beyond 18 hours following injection (Hedreen and McGrath, 1977; Vanegas et al., 1978; Mesulam, 1982).

Consequently, an effective injection site can be substantially different from a virtual injection site.

Although we are not aware of a corresponding study of BDA injection sites, it is possible that analogous phenomena may exist for BDA injection sites. The injection sites described above correspond to virtual injection sites. It is possible that the sizes of the effective injection sites may be underestimated by the virtual injection sites in the present study considering that a long (7-day) survival period was used. The reason for our choice of the 7-day survival period was based on a relatively slow axonal transport of BDA, 2.2 mm/day (Oliver and Beckius, 1993). Thus, it is possible that the effective injection sites of the present results might have encroached into the central core of the AVCN and that the axonal contacts with OC neurons described in this paper may partly, or even entirely, represent axons originating from the AVCN central core.

In a study conducted in our laboratory, it was observed that axons labeled with BDA injected into the marginal shell and those with BDA injected into the central core of the AVCN were markedly different. Axons associated with injection into the core were thicker (more than half were thicker than 1.5 μm) and projected to both the principal nuclei (i.e., lateral and medial superior olives) and peri-olivary areas. In contrast, those associated with injection into the shell were thinner (92% were thinner than 1.5 μm) and projected nearly exclusively to peri-olivary areas with little projection to the principal nuclei (Ye and Kim, 1998). The thicknesses and spatial distributions (within the superior olivary complex) of BDA-labeled axons of the present study, where the center of the virtual injection site was within the marginal shell of the AVCN in all four animals, closely correspond to those of the latter group of axons described above. These observations support the interpretation that the present results represent, to a large extent, projections from the AVCN marginal shell to OC neurons. Results obtained with injection of BDA and 3H-leucine into the AVCN central core combined with injection of CTB into the cochlea are presently unavailable. A future study of such a nature should be valuable in addressing the questions of whether both the marginal shell and central core of the AVCN project to OC neurons and what differences may exist between the projections from the shell and core of the AVCN to OC neurons.

**Main finding**

This study provides information about the ascending portion of the MOC and LOC reflex (or feedback) circuits. Together with the physiology of AVCN marginal-shell neurons (Ghoshal and Kim, 1996a, 1997) and afferent inputs to the marginal shell (Liberman, 1991, Liberman, 1993), the present anatomical results support a hypothesis that the AVCN marginal shell provides information about stimulus intensity to MOC neurons as a part of a feedback gain-control system consisting of the cochlea, cochlear neurons, cochlear nucleus, MOC neurons, and cochlear outer hair cells. A projection from the AVCN marginal shell to MOC neurons fulfills a requirement of the above hypothesis. The projection of the AVCN marginal shell to LOC neurons has come as an unexpected finding. This was not anticipated, because little experimental or theoretical insight is available regarding the function of the LOC reflex circuit.

**Ascending projections to OC neurons**

A few previous studies have described ascending projections of the CN to OC neurons. Robertson and Winter (1988) observed that the VCN projected to MOC neurons in the guinea pig. Because of a technical limitation of their method, they could not determine whether the VCN also projected to LOC neurons. Thompson and Thompson (1991) found, also in the guinea pig, that the postcentral CN projected to MOC and LOC neurons. Kim et al. (1995) reported preliminary results obtained with injection of BDA into the AVCN marginal shell of the cat combined with horseradish peroxidase injection into the floor of the fourth ventricle; BDA-labeled axonal swellings were observed in close apposition with OC neurons. The present paper is an extension of that of Kim et al. (1995). The present and earlier studies agree that the VCN projected to LOC neurons. However, the projection of the AVCN marginal shell to MOC neurons fulfills a requirement of the above hypothesis. The projection of the AVCN marginal shell to LOC neurons has come as an unexpected finding. This was not anticipated, because little experimental or theoretical insight is available regarding the function of the LOC reflex circuit.
the observed projection. The present study also extends information to another subdivision of the CN, i.e., from posteroventral CN to anteroventral CN, and another species, i.e., from guinea pig to cat.

The spatial distributions of OC neurons contacted by BDA-labeled axonal swellings were different among the animals of the present study, particularly for MOC neurons on the contralateral side (Table 1). For example, the MOC neurons on the contralateral side tended to be concentrated in V-MOC and VM-MOC for cats Q88 and Q89, respectively. The injection site of Q89 was more posterior to that of Q88. The characteristic frequency of Q89 was 4 kHz and that of Q88 was unknown. There may be a spatial relationship between the location of AVCN marginal-shell neurons and the OC neurons to which the marginal-shell neurons project. Further studies are needed to address this question.

**Cell types of the AVCN marginal shell**

The marginal shell of the AVCN has been described to contain multiple cell types: granule cells, Golgi cells, unipolar brush cells, chestnut cells, mit cells, elongate cells, and stellate (or multipolar) cells of small, medium, and large sizes (Cant, 1992, 1993; Weedman et al., 1996; Morest, 1997; Doucet and Ryugo, 1997; Ferragamo et al., 1998). It is not clear whether each of the above names uniquely corresponds to a distinct cell type or whether some of the multiple names correspond to a common cell type. Based on the results of the present study, it is not known which of these cell types in the marginal shell

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**Fig. 9.** A: Schematic diagram of the findings of the present study, illustrating ascending projections from the AVCN marginal shell to OC neurons. B: Descending projections from OC neurons to the left cochlea, adapted from Warren and Liberman (1989), with permission from Elsevier Science. AVCN, anteroventral cochlear nucleus; cereb., cerebellum; LOC, lateral olivocochlear neuron; LSO, lateral superior olive; MOC, medial olivocochlear neuron; MSO, medial superior olive; 4th Vent., fourth ventricle.
project to OC neurons. Future studies with injections of a retrograde tracer into a region containing OC neurons (e.g., VM- and V-MOC, VL- and D-LOC) should help to address this question.

**Comparison of ascending and descending projections involving OC neurons**

The components of the ascending projections of the AVCN marginal shell to OC neurons (Fig. 9A) are compared to the components of the descending projections of OC neurons to the cochlea in Figure 9B (adapted from Warren and Liberman, 1989). The descending projections to one cochlea include four components representing the two types of OC neurons on both sides of the brainstem. In contrast, the ascending projections from one AVCN marginal shell include only three components representing those to MOC neurons on both sides and to LOC neurons on the ipsilateral side; the results of Thompson and Thompson (1991) are consistent with this. Consequently, LOC neurons on the right side do not receive ascending projections from the left AVCN marginal shell but they do project to the left cochlea. The neural circuit implies that LOC neurons, in part, receive ascending signals from one cochlea but send descending signals to both cochleas. It is interesting to note that the contralateral to ipsilateral ratio for the ascending projections of the AVCN marginal shell to MOC neurons (1.8, representing 52%/29%; Fig. 9A) is comparable to the corresponding ratio of descending projections from MOC neurons to the cochlea (2.4, representing 26%/11%; Fig. 9B). The present results were obtained with injections of CTB into both cochleas in each animal in order to maximize the number of labeled OC neurons. Consequently, the results do not provide information about to which of the cochlea the labeled OC neurons project.

**Labeling with 3H-leucine**

The injection sites of 3H-leucine (not shown) were larger than those of BDA. Thus, axons labeled with 3H-leucine may have originated from an area larger than the BDA injection sites. Consistently with this, there were many axons that were labeled only with 3H-leucine and not with BDA. Because 3H-leucine was used in combination with BDA, however, axons and swellings doubly labeled by both tracers were interpreted to have originated from BDA injection sites.

As was described for Figure 4 above, some axons were doubly labeled with BDA and 3H-leucine. Other axons, however, were labeled with BDA only. One potential reason for the absence of 3H-leucine label is that the axons may have been located too far away from the surface of the tissue section, such that the radioactive emission from 3H was too weak to produce silver grains in the emulsion on the tissue surface. Another potential reason why some axons were labeled with BDA only is that the BDA label in some cases may be retrogradely transported. Consistently with this, we did observe that some somata in the SOC were labeled with BDA. However, the numbers of such BDA-labeled somata was quite small. We believe that the first reason indicated above (i.e., axons being away from the surface) accounts for most of the axons being labeled with BDA only in the present material.

**Future studies**

Conducting a study similar to the present one with injection of a retrograde tracer into one cochlea (some ipsilaterally and others contralaterally) would be desirable to obtain further information about the reflex circuits involving the two cochleas. Electron microscopic examination of synaptic ultrastructures of the contacts between AVCN marginal shell and OC neurons, demonstrated only by light microscopy in this study, would also be desirable in future studies. Such electron microscopic studies plus neurotransmitter-related immunocytochemical studies should help to elucidate whether these contacts consist of excitatory or inhibitory synapses and which neurotransmitters are involved in this projection.

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**LITERATURE CITED**


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