Acoustic Stria: Anatomy of Physiologically Characterized Cells and Their Axonal Projection Patterns

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ABSTRACT

The mammalian cochlear nucleus (CN) has been a model structure to study the relationship between physiological and morphological cell classes. Several issues remain, in particular with regard to the projection patterns and physiology of neurons that exit the CN dorsally via the dorsal (DAS), intermediate (IAS), and commissural stria. We studied these neurons physiologically and anatomically using the intra-axonal labeling method. Multipolar cells with onset chopper (OC) responses innervated the ipsilateral ventral and dorsal CN before exiting the CN via the commissural stria. Upon reaching the midline they turned caudally to innervate the opposite CN. No collaterals were seen innervating any olivary complex nuclei. Octopus cells typically showed onset responses with little or no sustained activity. The main axon used the IAS and followed one of two routes occasionally giving off olivary complex collaterals on their way to the contralateral ventral nucleus of the lateral lemniscus (VNLL). Here they can have elaborate terminal arbors that surround VNLL cells. Fusiform and giant cells have overlapping but not identical physiology. Fusiform but not giant cells typically show pauser or buildup responses. Axons of both cells exit via the DAS and take the same course to reach the contralateral IC without giving off any collaterals en route. J. Comp. Neurol. 482:349–371, 2005. © 2005 Wiley-Liss, Inc.

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Projection neurons of the cochlear nucleus (CN) use two primary pathways to reach higher auditory structures (see Cant and Benson, 2003, for review). In the cat, the ventromedially situated trapezoid body (TB) carries axons arising primarily from bushy cells of the anteroventral cochlear nucleus (AVCN), which project to the superior olivary complex (SOC), and from multipolar cells throughout the ventral cochlear nucleus (VCN) that are classified as type 1 (Cant, 1981) and that project to the inferior colliculus. In other species cochlear root neurons also use this pathway (Lopez et al., 1999). A second pathway, the dorsally situated/directed acoustic stria, has three main components. The dorsal acoustic stria (DAS) is situated between the dorsal cochlear nucleus (DCN) and the caudal aspect of the posteroventral cochlear nucleus (PVCN) and carries the axons of the pyramidal (fusiform) and giant cells of the DCN from the CN to the contralateral inferior colliculus. Medial and slightly caudal to the DAS is the intermediate acoustic stria (IAS) primarily composed of the axons of VCN octopus cells that project to the contralateral ventral nucleus of the lateral lemniscus (VNLL). Finally, axons of type 2 multipolar cells that project across the brainstem to the opposite cochlear nucleus form a commissural stria whose axons are initially mingled with those of the IAS. As the IAS and commissural axons head dorsally out of the cochlear nucleus, the two populations separate, with the commissural axons running rostral to the IAS (Osen, 1969a,b).
There have been several single-cell labeling studies in different species where individual members of the various subtypes of CN projection neurons were injected with either horseradish peroxidase (HRP) or Neurobiotin (Vector Labs, Burlingame, CA) (Rhode et al., 1983a,b; Rouiller and Ryugo, 1984; Smith and Rhode, 1985, 1987, 1989; Priauf and Ostwald, 1988; Ostapoff et al., 1994; Hancock and Voigt, 2002a,b; Palmer et al., 2003; Arnott et al., 2004). In these experiments the injection site has typically been at or close to the cell soma where the labeling substance may fill the cell body, dendritic tree, and local axon collaterals but rarely labels the axons past the boundaries of the CN. Studies in the trapezoid body (Spirou et al., 1990; Smith et al., 1991, 1993a,b) have successfully used intra-axonal recording methods to label the terminal portions of AVCN axons. Other than our preliminary report (Joris et al., 1992), no such study has been done on the acoustic stria. Thus, our knowledge about the projection patterns of axons using these pathways is based mainly on degeneration studies or retrograde and anterograde labeling studies where gross injections label populations of axons that are followed back to their cell bodies or forward to their termination sites (e.g., Warr, 1969, 1972, 1982; Adams and Warr, 1976; Adams, 1979; Schofield, 1985; Schofield and Cant, 1990a,b; for review, see Cant and Benson, 2003). In this study we used the intra-axonal recording and labeling method to get a more detailed account of the physiology, course, and termination sites of the cell populations that use the dorsal, intermediate, and commissural stria.

MATERIALS AND METHODS

The surgical approach and general stimulus and analysis procedures are as described in Joris (1998) and Joris and Smith (1998). The basic details for intra-axonal recording and labeling are as described previously for experiments on the trapezoid body and medial nucleus of the trapezoid body (Smith et al., 1991, 1993a,b, 1998). The experimental protocol was approved by the University of Wisconsin Animal Care and Use Committee and conforms to NIH guidelines.

Young adult cats were anesthetized with intramuscular acepromazine (0.2 mg/kg) and ketamine (20 mg/kg) followed by intravenous infusion of sodium pentobarbital. A tracheal cannula was inserted. Rectal temperature was maintained at 37–38°C. Both pinnae were removed, the external auditory meati cut transversely, and metal earpieces inserted for delivery of acoustic stimuli. To maintain normal middle ear pressure polyethylene tubing was glued into a small hole made in each bulla. The posterior fossa was exposed and the cerebellum was carefully aspirated at the midline until the fourth ventricle was visible. Cerebellar aspiration was continued laterally until the dorsalmost aspect of left and right CN was noted. The striae could usually be visualized as they exited the CN and curved over the restiform body as they left the cochlear nucleus and very shallow in the dorsoventral dimension. In addition, the stria briefly runs superficially over the top of the restiform body but then quickly dives below the brain surface. Nevertheless, it was usually possible to move a sufficient distance away from the first successful penetration to attempt one or two subsequent penetrations. If at least one injection was accomplished on one side, an injection was attempted on the opposite side of the brain.

Acoustic stimuli and data collection

Calibrated acoustic stimuli, under Digital Microvax computer control, were delivered through hollow earpieces connected to earphones (Radio Shack Super Tweeters). Spikes were converted to standard pulses with a peak detection circuit, which were sampled with a 1-μs precision. The general stimulus paradigm was as follows. A search stimulus of tone bursts ranging from 100–40,000 Hz was presented until a unit was encountered. An automated tracking algorithm was used to obtain the threshold tuning curve from which characteristic frequency (CF, the frequency of lowest rate threshold), Q10 (a measure of sharpness of tuning), and spontaneous rate were determined. The responses of some cells, giant cells in particular (see Results), were too sluggish for the tracking algorithm to be practical. In such cases CF or BF (frequency of maximal response rate) was estimated with iso-SPL tone bursts stepped or swept in frequency. Responses to 25 or 50 ms duration tones at CF (STCF, interferstimulus interval 100 or 200 ms, 200 repetitions, rise-fall times 3.9 ms) were obtained at various sound pressure levels (measured as decibels re. 20 μPa, dBSPL), usually in 10-dB steps, and visualized as rate-level functions and peristimulus time histograms (PSTHs). A rate-level function to digitally generated pseudorandom broadband noise bursts was also obtained (typical parameters: 100 ms bursts every 500 ms, 10-dB steps, 40 repetitions). On the basis of these data, particularly the shape of the PSTH at multiple suprathreshold levels, the cell was physiologically classified and it was decided whether we would attempt to label it.

If time allowed, responses to additional stimuli, sometimes presented contralaterally, were presented to quantify temporal properties, including low-frequency tones, click trains at various rates, and amplitude-modulated tones (see Joris and Smith, 1998). Responses to these stimuli are not further discussed here.

Tissue processing

Perfusion, Neurobiotin reaction, and plastic embedding. After the last penetration the animal was maintained in an arreflexic state for 18–24 hours. Then, following a lethal dose of sodium pentobarbital, it was perfused with saline followed by two concentrations of
and the cells with ON response features are the same multipolar cells projecting to the opposite cochlear nucleus of the CN. The only direct evidence that the glycinergic sal and ventral cochlear nuclei before heading dorsally out provide collateral innervation to both the ipsilateral dorsal and ventral cochlear nuclei (Cant et al., 1992). That cell is included in the population described here.

We labeled eight axons in the acoustic stria that showed clear onset-chopper response features consistent with the larger population described previously (Rhode et al., 1983a; Smith and Rhode, 1989). These axons could be traced retrogradely back to the cell body in the cochlear nucleus. The consistent physiological features of these cells to tones at CF included spike rates that increased over a wide dynamic range of intensities and the characteristic onset chopper PSTH. Figure 1 illustrates PSTHs of all eight of these labeled cells (Fig. 1C–G, I–K) as well as an O2 response from another multipolar cell axon (Fig. 1H, see below) and an O3 response from an unlabeled axon in the acoustic stria whose response was driven by contralateral stimulation (Fig. 1L). Tuning curves (Fig. 1A) and rate-level functions (Fig. 1B) of these same cells are also shown. All of the O2 responses are characterized by regular firing at tone onset, generating a multi-peaked histogram followed by no activity or a lower level of sustained, less regular activity. Figure 2 shows six examples (cells 1–5, 7) of the cell body and dendritic tree of O2 cells and their locations within the cochlear nucleus and of one cell (Fig. 2, cell 6) with an O3 PSTH. One of the O2 cells was located within the deep DCN (Fig. 2, cell 1) while the rest were found around the rostrocaudal locations in the VCN. The location, cell size, and dendritic tree configuration of the O2 cells in the VCN is in good agreement with the distribution of cells labeled in previous intracellular studies in the cat (Smith and Rhode, 1989) and with the location of most of the cells previously shown in gross injection studies to project to the opposite CN (Cant and Gaston, 1982; Shore et al., 1992; Schofield and Cant, 1996b; Alibardi, 1998). The gross injection studies also indicated that a few cells projecting to the opposite CN are in the deep DCN, which fits with our one labeled O2 cell at this location.

We examined three of these cells at the EM level to compare them with a previous report (Smith and Rhode, 1989). Both the cell body and proximal dendritic tree showed a dense synaptic coverage (Figs. 3A, B, 4A, D), a common feature of this cell type (Smith and Rhode, 1989). The axons of all the cells located in the VCN had collaterals in both the VCN and DCN. Most of the collateral field in the DCN innervated both fusiform and deep layers in and around the frequency region of the DCN that, based on reported frequency maps (Spirou et al., 1993), corresponds to the CF of the O2 cell (Fig 3D). In the DCN the synaptic terminals could be seen on large dendrites as well as cell bodies (Fig. 3E) and contained nonround vesicles. The O2 cell located in the deep DCN also had collateral branches that innervated the deep DCN and fusiform cell layer in and around the location of the parent cell body (Fig. 4C, E). We could not find any axon collaterals of this cell that headed for the VCN.

After giving off collateral branches within the ipsilateral cochlear nucleus, the O2 axon projected dorsally over the restiform body, with other strial axons. In all but two cases the axon could also be traced in an anterograde direction from the injection site just dorsal to the restiform body. Axons that could be followed ran medially and rostrally within the DAS bundle of fibers crossing cranial nerve VII just below the genu (Fig. 5B, C) and then rostrally, medially, and ventrally toward the midline. After crossing the midline dorsal to the superior olivary com-

phosphate-buffered, calcium-containing glutaraldehyde/paraformaldehyde fixative. The brain was then removed and stored overnight in the lower concentration fixative. Seventy-μm sections were cut on a vibratome, the Neurobiotin visualized using the ABC reagent method (Vector Labs) and the Adams (1981) DAB-nickel/cobalt intensification method, and then prepared for light and/or electron microscopy (EM). For light microscopy, sections were mounted on glass slides, counterstained with cresyl violet, and coverslipped. For EM, the vibratomed, Neurobiotin-reacted sections were fixed in 2% osmium tetroxide, dehydrated, and flat-embedded in Epon-Araldite plastic resin between two sheets of plastic film. After a camera lucida or a computer-aided 3D drawing of the injected axon and its parent cell body were made, section(s) containing pertinent portions of the injected cell were flat-mounted and resectioned into 5-μm sections. The appropriate 5-μm section was selected, remounted, trimmed, and thinned, then counterstained with uranyl acetate and lead citrate and observed with a JEOL 100CX electron microscope.

For viewing the axon in three dimensions we used the Neurolucida system (MicroBrightField, Colchester, VT), which allows the tracking of various structures through serial tissue sections. The outlines of the coronal brainstem sections containing the labeled axon were entered as well as labeled pieces of axon and various pertinent structures. These data were then merged to form a 3D image that could be rotated to any plane. Negatives of the electron micrographs were scanned on a Duoscan T1200 scanner (AGFA) using FotoLook software (AGFA) and any adjustments of tone or contrast were made using this software. Micrographs were then imported into Powerpoint (Microsoft) where figures were created.

RESULTS

This article focuses primarily on the anatomical rather than the physiological features of labeled cells whose axons use the acoustic stria. Only the basic physiological response properties of these cells will be described here. A more detailed account of the physiology of labeled and unlabeled axons will be presented in a subsequent article.

Multipolar cells

Onset choppers. The combined evidence from several different studies (Cant and Gaston, 1982; Wentholt, 1987; Kolston et al., 1992; Shore et al., 1992; Schofield and Cant, 1996b; Alibardi, 1998; Needham and Paolini, 2003; Palmer et al., 2003; Arnott et al., 2004) suggests that the large VCN multipolar cells whose response to short tones have been labeled onset-chopper (O2; Rhode et al., 1983a; Smith and Rhode, 1989) closely resemble a population of large glycinergic multipolar cells whose response to short tones at CF included spike rates that increased over a wide dynamic range of intensities and the characteristic onset chopper PSTH. Figure 1 illustrates PSTHs of all eight of these labeled cells (Fig. 1C–G, I–K) as well as an O2 response from another multipolar cell axon (Fig. 1H, see below) and an O3 response from an unlabeled axon in the acoustic stria whose response was driven by contralateral stimulation (Fig. 1L). Tuning curves (Fig. 1A) and rate-level functions (Fig. 1B) of these same cells are also shown. All of the O2 responses are characterized by regular firing at tone onset, generating a multi-peaked histogram followed by no activity or a lower level of sustained, less regular activity. Figure 2 shows six examples (cells 1–5, 7) of the cell body and dendritic tree of O2 cells and their locations within the cochlear nucleus and of one cell (Fig. 2, cell 6) with an O3 PSTH. One of the O2 cells was located within the deep DCN (Fig. 2, cell 1) while the rest were found around the rostrocaudal locations in the VCN. The location, cell size, and dendritic tree configuration of the O2 cells in the VCN is in good agreement with the distribution of cells labeled in previous intracellular studies in the cat (Smith and Rhode, 1989) and with the location of most of the cells previously shown in gross injection studies to project to the opposite CN (Cant and Gaston, 1982; Shore et al., 1992; Schofield and Cant, 1996b; Alibardi, 1998). The gross injection studies also indicated that a few cells projecting to the opposite CN are in the deep DCN, which fits with our one labeled O2 cell at this location.

We examined three of these cells at the EM level to compare them with a previous report (Smith and Rhode, 1989). Both the cell body and proximal dendritic tree showed a dense synaptic coverage (Figs. 3A, B, 4A, D), a common feature of this cell type (Smith and Rhode, 1989). The axons of all the cells located in the VCN had collaterals in both the VCN and DCN. Most of the collateral field in the DCN innervated both fusiform and deep layers in and around the frequency region of the DCN that, based on reported frequency maps (Spirou et al., 1993), corresponds to the CF of the O2 cell (Fig 3D). In the DCN the synaptic terminals could be seen on large dendrites as well as cell bodies (Fig. 3E) and contained nonround vesicles. The O2 cell located in the deep DCN also had collateral branches that innervated the deep DCN and fusiform cell layer in and around the location of the parent cell body (Fig. 4C, E). We could not find any axon collaterals of this cell that headed for the VCN.

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plex, the axons of the DAS continued rostrally, just lateral to the contralateral lateral lemniscus, whereas five of the $O_C$ axons instead turned and headed caudally and laterally toward the opposite cochlear nucleus, a trajectory best appreciated in the horizontal plane (Fig. 5E). In two cases we were able to follow the axon as it headed under the spinal trigeminal tract and nucleus and into the contralateral cochlear nucleus (Fig. 5D), where it began branching before fading. In three cases the axon faded just before entering the cochlear nucleus. None of these “typical” $O_C$ axons gave off any collaterals to nuclei in the superior olivary complex.

The axons of two multipolar cells in the VCN with $O_C$ physiology did not conform to this typical contralateral pathway. One axon (Fig. 2, cell 7) had “typical” collaterals in the ipsilateral cochlear nucleus and followed the same course as the $O_C$ axons described above until it reached the midline. Just after crossing the midline dorsal to the superior olivary complex it headed straight ventrally, within the confines of a single 70-µm section, to assume a position in the ventral aspect of the trapezoid body, and then crossed back over the midline to run caudally under the MSO and LSO in the trapezoid body, where it faded, heading toward the parent cochlear nucleus. A second cell (Fig. 2, cell 5) with $O_C$ physiology sent an axon across the ipsilateral brainstem after giving off collaterals in the ipsilateral CN and following the same course as the “typical” $O_C$ axons. After crossing the midline the axon branched as it approached the dorsomedial end of the contralateral MSO. The main axon (based on diameter) headed ventrally and around the medial side of the MSO and then coursed dorsally and rostrally until it became too light to follow lateral to the VNLL. The collateral branch headed over the contralateral MSO and faded medial to the rostral end of the MSO.

$O_L$ multipolar cell. We labeled one cell whose physiology differed somewhat from the $O_C$ multipolar cell population described above. The PSTH of this cell (Fig. 1H) shows a single onset peak followed by a pause and then a low level of sustained activity; this pattern is often referred to as onset with low sustained activity ($O_L$). In addition, the rate intensity function was compressive. The large cell body was located in the VCN and the dendritic tree was multipolar, like the $O_C$s (Fig. 2, cell 6). At the EM level the cell body resembled a type 2 multipolar cell in that a large percentage of the surface of the cell body and proximal dendrites was covered with synaptic terminals (64%, Fig. 6A,B). The axon was unusual in that it showed characteristics of both $O_C$ and octopus cell axons. It headed dorsally into the acoustic stria and gave off collaterals to the DCN (like an $O_C$ axon). At the EM level the terminals contained nonround vesicles and synapsed on dendrites and cell bodies in the DCN much like the $O_C$s terminals described above (Fig. 6C,D). The axon continued dorsally into the cochlear nucleus via the DAS and, after crossing the midline, it started heading caudally (like an $O_C$ axon). However, just after getting to the contralateral side it gave off a collateral that went to the contralateral DMPO (like some octopus cells). The main axon continued caudally and laterally (like an $O_C$) over the contralateral LSO, then, just lateral to the contralateral LSO, gave off a small but distinct collateral that headed into the contralateral cochlear nucleus (like an $O_C$, but usually the main axon heads there). The main axon turned rostrally to

Fig. 1. Tuning curves (A), rate-level functions (B), and PST histograms to short tones at CF of nine labeled multipolar cells (C–K) and one contralaterally driven unlabeled axon with $O_c$ physiology (L). The $O_c$ responses (C–G,I–L) show regular firing at tone onset that generates multiple peaks followed by no activity or a lower level of sustained, less regular spiking. The $O_L$ PSTH (H) shows a single onset peak followed by a pause and a resumption of spontaneous activity. CFs (in kHz) are indicated on the PST histograms, which were obtained at 74 or 84 dB SPL, except for the high-threshold cell (E), where the level was 104 dB. Linestyles and symbols at CFs in A correspond with the other panels. For clarity of illustration, not all datapoints in B are plotted with a symbol. PST histograms have 500 bins. The morphology of the cells from which the first seven PST histograms were derived is shown in the same order in Figure 2.
Fig. 2. Camera lucida representations of seven large stellate cells in the CN, six with O$_2$ PSTHs (cells 1–5, 7) and one with a O$_1$ PSTH (cell 6), and their location in sections of the cochlear nucleus. Asterisks in sections indicate the cell body location: four in the AVCN/nerve root area, two in the PVCN, and one in the deep DCN. Arrows indicate axons. Cells and dendritic trees are oriented in the figure as they are in the sections. AN, auditory nerve; AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; OCA, octopus cell area; PVCN, posteroventral cochlear nucleus. Scale bar in 1 = 1 mm (applies to all drawings of tissue sections); scale bar in 5 = 100 μm (applies to all cell drawings).
Fig. 3. Electron microscopic features of an onset chopper unit. 
A: Micrograph of the labeled cell body (CB, cell 4 in Fig. 2). B: Electron micrograph of a primary dendrite (d) illustrating the dense synaptic coverage of the dendritic tree. C: Camera lucida drawings of the cell body and dendritic tree. D: Axon collaterals innervating the DCN. Arrows indicate path of an orthodromic spike. The main terminal field is confined to a fairly narrow region of the deep and fusiform cell layer of the DCN. The two insets illustrate the location of the terminal field (gray areas) in two representative sections of the DCN. Arrows indicate small pieces of the main axon in each section. E: Micrograph of labeled synaptic terminals (asterisks) from this DCN terminal field synapsing on a dendrite (d) and a small sparsely innervated cell body (CB) in the deep DCN. Scale bars = 5 μm in A; 2 μm in B; 1 mm in C; 200 μm in D; 2 μm in E.
reach the contralateral VNLL where it started branching/innervating this area (like an octopus cell axon).

Octopus cells

Octopus cells (Osen, 1969) have large tentacle-like dendrites which often arise from one side of the cell body and are oriented in one direction. They are located exclusively in the posteriormost aspect of the PVCN in an area designated the octopus cell area (OCA), and recordings here (Godfrey et al., 1975) indicate that these cells typically respond to short tones with an onset response. Response features from only three positively identified octopus cells have ever been reported in the cat. In one, the axon faded just after leaving the CN (Rhode et al., 1983a). In a second cell the axon was so lightly labeled that an axon could not be identified (Rouiller and Ryugo, 1984). The other was described in our preliminary report (Joris et al., 1992) and is included here. Thus, the information on octopus cell axonal projections comes primarily from retrograde and anterograde gross injection studies (e.g., Warr, 1969; Adams, 1997).

We labeled the three octopus cell axons that could be traced back to their cell body and two axons that could not be traced back to the cell body but whose physiology and axonal projection pattern would lead us to believe that they belong to octopus cells. Figure 7A illustrates tuning curves and PST histograms of the three octopus cells and the two axons. All cells showed onset responses followed by a low level or no sustained response. For four of these the onset response was very well timed and the sustained activity very low; for these the PSTH can be classified as O₁ (onset with a very low level of sustained activity). For the fifth cell (top right PSTH) the CF exceeded the equipment-calibrated frequency range and we were only able to use tone stimulation that was at most 15 dB above threshold; possibly, the PSTH would have been more like the other cells if higher stimulus levels could have been used. Several other important features of these cells, including their remarkable abilities to respond to short repetitive stimuli at very high rates, will be included in a subsequent article. Of the three recovered cell bodies, one was very darkly labeled and virtually all of the dendritic tree could be distinguished (Fig. 7B, cell 1). A second cell body was moderately well labeled and much of the primary and secondary dendritic tree could be distinguished (Fig. 7B, cell 2). The third cell was lightly labeled and only the primary and initial portions of the secondary dendrites could be seen (Fig. 7C, cell 3). All were in the OCA and had the typical oriented dendritic trees. In order to compare the synaptic inputs to these cells with descriptions in a previous report (Kane, 1973), EM of the most darkly labeled cell body and its primary dendrites was done. The cell body and primary dendrites showed significant synaptic terminal coverage (60%), with many resembling auditory nerve terminals (Fig. 8A–C).

Before leaving the CN, the axon of the darkly labeled cell (Fig. 7B, cell 1, arrow) gave off two small collateral branches which innervated the interstitial nucleus of the stria of Held (Warr, 1969). The axons of the other two cells were lightly labeled while in the CN, so although we saw no local collaterals, we cannot rule out the possibility that they were too light to distinguish. All three axons headed dorsally in the acoustic stria. After coursing over the restiform body the axons separated from the DAS/commissural pathway by turning sharply ventral while slowly heading rostrally medial to the restiform body. From this point the axons followed one of two paths on their way to a common target, the VNLL. The course of one of these axons is shown in the coronal and horizontal plane in Figure 8D. Two of them (one is illustrated in Fig. 8D) ran medially while still heading rostrally dorsal to ipsilateral LSO and MSO. One of them continued ventrally and rostrally and ran beneath the LSO before heading dorsally again between the LSO and MSO. All three then continued medially and rostrally over and past the ipsilateral MSO. In two cases we could see ipsilateral collaterals. One axon had a collateral above the LSO which innervated the dorsolateral periolivary group. Just medial to this the same axon gave off a second collateral that headed ventrally, directly between the LSO and MSO before going under the MSO and branching into the region just medial to it. A second octopus cell axon gave off a collateral quite dorsal in the ipsilateral brainstem which could not be followed to its termination. The two axons whose cell bodies were not labeled but were most likely octopus cell axons were too lightly labeled on the ipsilateral side to see collaterals. The main axons of the three octopus cells and the two tentative octopus cell axons continued medially and rostrally crossing the midline dorsolateral to the superior olivary complex. On the contralateral side the axons headed rostrally and laterally going over MSO and LSO. Two of these axons gave off collaterals that headed into the DMPO and one gave off a collateral that headed toward the DLPO before fading. All five axons continued rostrally into the contralateral lateral lemniscus and could be followed into the VNLL before fading; three could be seen branching in the VNLL. The collaterals of one very well-labeled axon in the VNLL are illustrated in Figure 9. The large myelinated axon (Fig. 9A) began branching as it approached the VNLL (Fig. 9B). Some of the axon terminals were large. EM of one of these revealed that the terminal provided multiple synaptic contacts containing round synaptic vesicles (Fig. 9C) on a cell body in the VNLL (Fig. 9D) in a fashion similar to globular bushy calyceal terminals on cells in the MNTB.

DCN fusiform and giant cells

When recordings are made in the acoustic striae, the predominant PSTH pattern encountered is the pause or buildup pattern (P/B, an onset peak followed by a pause of variable length then a resumption of spike activity (P), or no onset and a buildup of spike activity after some delay (B)). The association, in the pentobarbital-anesthetized cat, of these PSTH patterns with fusiform cells was first proposed based on extracellular recordings (Kiang et al., 1965; Godfrey et al., 1975) and is now well established through intracellular labeling (Rhode et al., 1983b; Smith and Rhode, 1985; Ding et al., 1999; Hancock and Voigt, 2002a,b). Less well established is the physiology of giant cells. It has been concluded from extracellular recordings that DCN giant cells have the same physiology as fusiform cells (because 1) in deep DCN stable responses can be obtained which are indistinguishable from responses obtained in the fusiform cell layer (Godfrey et al., 1975; Young and Brownell, 1976; Voigt and Young, 1988; Spirou and Young, 1991), and 2) DCN projection cells that can be antidromically activated from the DAS have the same physiological properties as cells in the fusiform cell layer (Young, 1980). Oddly, some of our very first successfully labeled axons were from giant cells whose responses were...
Figure 4.
very different from those of fusiform cells. To increase our yield of labeled giant cells we therefore attempted injections primarily of axons with “unusual” responses. Labeling of axons with clear P/B responses was only attempted as a last resort (i.e., when several penetrations over several hours had not resulted in impalement of non-P/B fibers). Our sample of labeled neurons is thus biased physiologically and possibly also anatomically.

We labeled 15 axons that could be traced back to fusiform cells in the DCN and eight axons that were traced...
Fig. 6. Electron microscopic features of the O1 unit. A: Electron micrograph (left) and camera lucida (right) of a primary dendrite (d) illustrating the dense synaptic coverage. B: Electron micrograph (left) and camera lucida (right) of the cell body (CB) illustrating the dense synaptic innervation. C,D: Electron micrographs of two examples of the cell’s synaptic terminals (asterisks) synapsing on a dendrite (d) and a cell body (CB) in the deep DCN. Scale bars = 2 μm in A; 10 μm in B; 2 μm C (applies to C,D).
back to giant cells in the deep DCN. Figure 10 illustrates the physiology of eight labeled fusiform cells to short tone bursts. The tuning curves (Fig. 10A) show that these cells have low thresholds to tones (note that for two cells the CF was above the calibration limit of our equipment, so that the absolute threshold is unknown). A second important property is that the rate-level curves to short CF tones typically show a nonmonotonicity at intermediate SPLs (Fig. 10B). The PSTHs shown are at SPLs in this range. Five labeled fusiform cells showed the P/B pattern that is typically observed in the pentobarbital-anesthetized cat. PSTHs for four of these cells are shown in Figure 10C–F. In seven of the labeled cells the pause following the onset peak was shorter and less profound (Fig. 10G–I). Nevertheless, their PSTHs still show some resemblance to the P/B pattern. Figure 10J illustrates a fusiform cell response that was unusual in that it had a well-timed chopping pattern at all SPLs. Another unusual cell (not illustrated) had a high threshold (~70 dB), gave poor responses to tones, and was inhibited by broadband noise.

Figure 11 illustrates the physiology of seven labeled giant cells. This physiology showed considerable diversity that cannot be captured in a unified description. Moreover, very limited data are available from these cells because the contact time with these fibers tended to be short and also because of the physiological response properties themselves. In very broad terms, the online evaluation of these cells indicated high CFs, poor frequency tuning, high thresholds, a predominance of inhibition rather than excitation, and sluggish responses with low and regular
Fig. 8. Electron microscopy of an octopus cell and axonal course. 
A: Electron micrograph of the cell body (CB, cell 1 in Fig. 7). B: Electron micrograph of a proximal dendrite (d). C: Drawings of the cell body and dendrite from the sections in A and B illustrating the dense synaptic coverage of these regions, a common feature of this cell type. D: Frontal (upper) and horizontal (lower) sections illustrating the course of the whole axon (arrows) across the brainstem. Cochlear nucleus containing the cell body is labeled "ipsi." Abbreviations as in Figure 5. Scale bars = 10 μm in A,B; 2 mm in D.
Fig. 9. Octopus cell innervation of the contralateral VNLL.
A: Electron micrograph of the myelinated axon (asterisk). B: Camera lucida drawing of the axon collateral system within the VNLL. Curved arrow indicates location of terminal shown in D.
C: Electron micrograph showing an octopus cell synaptic terminal on a cell body (CB) and spine (s) in the VNLL. D: Low-power electron micrograph of a cell in the VNLL (CB) receiving multiple synaptic terminals (arrows) from the octopus cell axon. Scale bars = 100 μm in B; 1 μm in C (applies to A,C); 10 μm in D.
firing rates. Figure 11A illustrates the high thresholds and wide tuning. In four cells a tuning curve could not be obtained due to the sluggishness of the response, and iso-level tones were used to obtain an estimate of frequency tuning (Fig. 11B,E,I). With one exception (Fig. 11H), none of these cells showed the characteristic P/B pattern and nonmonotonic behavior typical of fusiform cells in this preparation. By the same token, the PSTHs certainly fall in the range of “atypical” response patterns that can be observed in fusiform cells (Fig. 10G–J). Several of the giant cells show sequential modes in their PSTHs, i.e., “chopping.” In two cases with high spontaneous activity (Fig. 11B,E,J) the response to broadband noise was inhibitory at threshold but excitatory at higher levels.
Thus, with the necessarily limited sample size of labeled cells available, it appears that there are global differences in physiological properties of giant vs. fusiform cells, but that there is also overlap, so that for a given cell the physiology is less predictive of anatomical type than for several other CN cell classes. Cells with low thresholds, clear P/B pattern and a nonmonotonic rate curve are likely to have fusiform morphology. Cells with high thresholds and sluggish responses are more likely to have giant morphology.

The fusiform cells were located in the fusiform cell layer. Cell body locations relative to their best frequencies agreed with a previous description of the frequency map of the cat fusiform cell layer (Spirou et al., 1993). Light microscopic anatomy of these cells and their dendritic trees conformed to the descriptions of labeled cat fusiform cells from previous reports (Rhode et al., 1983b; Smith and Rhode, 1985) and are not illustrated here.

Giant cells were located deep to the fusiform cell layer. Cell body locations relative to their best frequencies agreed with a previous description of the frequency map of the cat fusiform cell layer (Spirou et al., 1993). Light microscopic anatomy of these cells and their dendritic trees conformed to the descriptions of labeled cat fusiform cells from previous reports (Rhode et al., 1983b; Smith and Rhode, 1985) and are not illustrated here.

Giant cells were located deep to the fusiform cell layer. Figure 12 illustrates five giant cells and their locations in the deep DCN. Cells 2 and 4 in Figure 12 were darkly labeled and the majority of the dendritic tree appeared to be labeled. Cells 3 and 5 were moderately well-labeled so that only proximal dendrites could be visualized. We did EM on the cell bodies of three giant cells and one fusiform cell to compare the terminal coverage with previous reports (Smith and Rhode, 1985; Kane et al., 1981). Two of the giant cell bodies (Fig. 13A,B) and the fusiform cell body (Fig. 13D) showed synaptic coverage that was not as dense as the octopus and Oc cell bodies (43%, 44%, 39%); however, one of the giant cells (Fig. 13C) had significant coverage (62%).

The axons of both fusiform and giant cells joined the acoustic stria in the cochlear nucleus and headed dorsally. We occasionally saw collaterals coming off of fusiform cell axons within the deep DCN, as reported previously (Rhode et al., 1983b; Smith and Rhode, 1985), but never saw giant cell collaterals within the ipsilateral DCN. Many of the axons were not darkly labeled while in the CN, so we could have missed such collaterals. We followed 15 fusiform axons and six giant cell axons across the brainstem. The axons of both cell types followed a similar course heading over the restiform body and coursing medially and rostrally toward the midline within the DAS. Figures 14 and 15 illustrate examples of the course of giant and fusiform cell axons. In Figure 14, representative sections show the location of small pieces of one giant cell axon as it heads across the brainstem and into the IC. Figure 15 shows the entire course of a giant cell axon (Fig. 15A) and a fusiform cell axon (Fig. 15B) from two separate experiments in frontal, horizontal, and sagittal planes. Also shown in Figure 15C is the course of a giant and fusiform axon whose cell bodies were in the same cochlear nucleus. On the contralateral side the axons continued rostrally and laterally dorsal to the LSO and MSO then joined the lateral lemniscus just medial to the VNLL and continued dorsally just medial to the dorsal nucleus of the lateral lemniscus (DNLL). The axons of these two cell types never gave off any collaterals within the brainstem to any of the auditory nuclei of the superior olivary complex or the lemniscal nuclei. Four of the fusiform cell axons and two of the giant cell axons faded medial to the DNLL just below the IC, while six fusiform and two giant axons entered the ventral aspect of the IC. Unfortunately, these axons could not be followed past the initial branch points within the IC.

**DISCUSSION**

The data presented here clarify some of the anatomical details of physiologically characterized members of the major classes of projection neurons of the cochlear nucleus whose axons exit via the acoustic stria.

**Onset choppers**

Two features indicate that the major role of the onset chopper on the ipsilateral side of the brainstem is to provide a wide-band inhibitory influence on other cell types in the cochlear nucleus. First, the axon collateral system of the Oc cells has terminals with features of inhibitory synapses that innervate both the VCN and DCN. Second, studies have indicated that several cell types within the ipsilateral CN are inhibited by a short latency input, with features consistent with the Oc output. One such cell type is the multipolar cell classified by different authors as type 1, T, or planar (Cant, 1981; Oertel et al., 1990; Doucet and Ryugo, 1997; Albardari, 1999; Doucet et al., 1999). These type 1 cells: 1) have axons that give off collaterals to the DCN before projecting to the IC via the trapezoid body; 2) have axon terminals containing small round vesicles; 3) have sparsely innervated cell bodies; 4) show chopping PSTHs in response to tones; and 5) are narrowly tuned in their frequency response. Smith and Rhode (1989) provided anatomical evidence that the Oc,s synapse on the type 1 multipolar cells in the cat PVCN. In the rodent, Ferragamo et al. (1998) showed that the D stellates, believed to be the rodent equivalent of Oc,s, provide glycergic inhibition to the type 1 multipolars. Pressnitzer et al. (2001) reported that transient choppers in the AVCN, believed to be type 1 multipolars, receive a wide-band inhibition that may arise from Oc,s. Oc projections to the DCN have also been implicated in the wide-band glycergic inhibition demonstrated physiologically in DCN principal cells, as well as in the cells designated type II or vertical (Casparay et al., 1987; Young et al., 1992; Nelken and Young, 1994; Backoff et al., 1997; Joris and Smith, 1998; Spirou et al., 1999; Davis and Young, 2000; Anderson and Young, 2004). Our electron microscopy of Oc terminals in the deep DCN shows that they can synapse on cell bodies or dendrites in this region, which is consistent with a potential influence of the Oc,s on these cell types.

In all but two cases we could follow the axons of these cells dorsally out of the CN and across the brainstem. In one of the two cases the axon heavily innervated the DCN before heading into the stria. At the injection site a large axon branch was also seen heading back down the stria into the tuberculoventral tract to innervate the VCN. In the second case the axon headed into the stria and just before the injection site gave off a collateral that headed back into the cochlear nucleus to innervate the DCN. The observed branching and “looping back” could explain the observation (Kolston et al., 1992) that the commissural stria, at the level where it exits the DCN, contains both small and large diameter glycine-positive axons. The smaller axons might be collaterals looping back. The inability, in these two cases, to follow the main axon out of the cochlear nucleus has at least two possible explanations: 1) these axons only doubled back into the home CN...
Fig. 12. Camera lucida representations of five giant cells in the deep DCN and their locations in sections of the cochlear nucleus. Arrows indicate axons. Asterisks indicate cell body locations in the DCN. Cells and dendritic trees are oriented in the figure as they are in the sections. One of the cells (#1) was lightly labeled so the dendritic tree could not be followed past the primary dendrites. Much of the dendritic tree of the other cells could be seen. Physiology of the five cells is shown in the following panels of Figure 11: 1 (B,C), 2 (A,D), 3 (E), 4 (A,F), 5 (A,G). Scale bars = 1 mm in 3 (applies to all drawings of tissue sections); 100 μm in 4 (applies to all cell drawings).
and did not leave it, or 2) the projection part of the axon did not fill past the injection site. The second option is brought up because it has been our experience that, on rare occasions, when we labeled other cell populations intra-axonally, the axon would only label in one direction from the injection site. This is a rare event but may be explained by the fact that the axon can pull apart at the injection site and form “constriction bulbs” at the two free ends of the break. For example, we have labeled globular bushy cells, whose axons are known to project out of the cochlear nucleus, for which only one labeled constricted end of the axon could be found and followed back to the cell body; while nothing was found in the anterograde direction. Thus, it is possible that some axons pull apart during the recording and simply do not fill past the injection site. Again, this is a rare observation and it should be noted that Arnott et al. (2004) reported two large multipolar cells that were labeled juxtacellularly whose axons did not appear to leave the cochlear nucleus (see below). This method labels the cell at the cell body, so constriction of the axon would not be a plausible explanation for their observation.

While heading across the brainstem to the opposite CN, the majority of our labeled OC axons followed a consistent course. On this course these typical OC axons did not target any of the superior olivary or paraolivary nuclei either ipsilaterally or contralaterally, which is in agreement with the single labeled OC axon reported by Arnott et al. (2004) in the guinea pig. We also noted that there is some diversity in the path taken by these axons across the brainstem, and perhaps the structures innervated by them. Two of our axons did not follow this standard course upon reaching the contralateral side. In one case the axon turned back at the midline and appeared to be returning to the home CN, but faded before its target could be distinguished. A second axon followed the standard course across the ipsilateral brainstem, but then branched within the superior olivary complex contralaterally. Neither branch appeared to turn and head caudally. Unfortunately, both these branches faded, so they could have been headed for any structure, including the opposite cochlear nucleus by a different route.

Our labeled axons entered the opposite cochlear nucleus by heading under the spinal trigeminal nucleus and tract and entering the ventral division of the cochlear nucleus. After making gross injections, Cant and Gaston (1982) noted that many of the labeled commissural axons use this pathway. They also noted that some of the axons appear to enter the opposite cochlear nucleus dorsally via the acoustic stria. In support of this finding, we recorded one axon in the stria (see Fig. 1, bottom right, PSTH) that was driven by the contralateral ear and had an OC response pattern with short latency.

The primary projection of the OC axon contralaterally is to the CN, where, like the ipsilateral projection, it appears to be providing a wide-band inhibition over the entire CN to several of the major cell groups (Cant and Gaston, 1982; Schofield and Cant, 1996; Babalian et al., 1999, 2002; Alibardi, 2000; Shore et al., 2003). These may include both type 1 and 2 multipolar, globular and spherical bushy, octopus, and fusiform cells. As we (Joris and Smith, 1998) and others have discussed previously, the contralateral inhibition at the level of the cochlear nucleus has certain features that are consistent with known properties of OC neurons, namely, a stronger inhibition to noise than to tones and a short latency. Despite these findings of binaural effects at the level of the CN, the functional role of this contralateral inhibition remains obscure. For example, despite the ability of the OC cell category to display excellent envelope phase-locking, the inhibitory response and did not leave it, or 2) the projection part of the axon did not fill past the injection site. The second option is brought up because it has been our experience that, on rare occasions, when we labeled other cell populations intra-axonally, the axon would only label in one direction from the injection site. This is a rare event but may be explained by the fact that the axon can pull apart at the injection site and form “constriction bulbs” at the two free ends of the break. For example, we have labeled globular bushy cells, whose axons are known to project out of the cochlear nucleus, for which only one labeled constricted end of the axon could be found and followed back to the cell body; while nothing was found in the anterograde direction. Thus, it is possible that some axons pull apart during the recording and simply do not fill past the injection site. Again, this is a rare observation and it should be noted that Arnott et al. (2004) reported two large multipolar cells that were labeled juxtacellularly whose axons did not appear to leave the cochlear nucleus (see below). This method labels the cell at the cell body, so constriction of the axon would not be a plausible explanation for their observation.

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of their postsynaptic targets to this well-timed input is sluggish and temporally unremarkable (Joris and Smith, 1998). Second, where contralateral auditory stimulation has been presented in the presence of ipsilateral auditory stimulation, the reduction in firing due to the contralateral inhibition is easily overcome by ipsilateral excitation (Young and Brownell, 1976; Joris and Smith, 1998; Recio, pers. commun.).

**On-Ls**

A classification scheme was recently developed (Winter and Palmer, 1995) for distinguishing two forms of onset cells in the guinea pig: $O_L$s and $O_S$s. For both sets of cells the steady state response at 20 dB above threshold is less than 50 sp/sec and the onset to steady-state ratio greater than 10. To subdivide these onset cell types, features of the onset response were considered. Units were classified as $O_L$ if, in response to tones, there was a single onset peak followed by a pause in firing and then a resumption of firing at a low level. Units were classified as $O_S$ if there were multiple onset peaks and no pause before any sustained activity. Recent work in this same lab (Arnott et al., 2004) has shown that juxtacellularly labeled multipolar cells with $O_L$ response features had axons that did not appear to project out of the CN. Based on the physiological criteria described above, one of our labeled stellates could be classified as $O_L$ (Fig. 2, cell 6). This cell had some anatomical features resembling the $O_L$ cell population,
but others that did not. Unfortunately, since we have only a single example of this cell type, the question remains as to whether there is a distinct set of $O_C$ and $O_L$ multipolar cells that perform different functions.

**Octopus cells**

Although our sample is small, we provide the first examples of physiologically characterized octopus cells whose cell body and axonal projection pattern were recovered and analyzed. Four responded to tones at their CF with a well-timed onset response that could be classified as $O_I$. One had a less well-timed onset response but the CF of this cell was too high to deliver stimuli at levels well above threshold, which generally improves the timing of the first spike. The cell bodies were all located in the OCA. Electron microscopy of one of the labeled cell bodies and proximal dendrites showed dense synaptic coverage, a feature that has been previously described (Kane, 1973). The axons headed out of the cochlear nucleus and across the brainstem to innervate the contralateral VNLL and

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**Fig. 15.** Giant and fusiform cell axon course. A: Frontal (top), horizontal (middle), and sagittal (bottom) views of the course of a giant cell axon across the brainstem to the contralateral inferior colliculus. B: Frontal (top), horizontal (middle), and sagittal (top) views of the course of a fusiform cell axon across the brainstem to the contralateral inferior colliculus. C: Frontal (top) and horizontal (bottom) views of the course of a fusiform and giant axon across the same brainstem. Cochlear nucleus containing the cell body is labeled “ipsi.” NLL, nuclei of the lateral lemniscus. Other abbreviations as in Figure 5. Scale bar = 2 mm in B (applies to all drawings).
the terminals here could form multiple synapses on a single cell body. As a population, the octopus axons innervate several periolivary nuclei, but individual members may innervate none, one, or more of these nuclei.

In vitro experiments on octopus cells by Oertel and colleagues (Golding et al., 1995, 1999; Gardner et al., 2001; Oertel et al., 2000) have shown that octopus cells have very low input resistances, short time constants, and rapid glutamate receptor kinetics that make them well suited for detecting coincident arrivals of synaptic inputs from many small auditory nerve terminals and responding only when they arrive in precise register. This is supported by the responses of these cells to click trains and amplitude-modulated stimuli (Godfrey et al., 1975; Rhode, 1994), which we have also observed and will report in a subsequent article.

It is interesting to note the anatomical and physiological similarities between these octopus cells and globular bushy cells in the AVCN. Slice data have shown that both cell types have nonlinear membrane features that allows them to generate only a single spike in response to constant depolarizing current (Oertel, 1983; Golding et al., 1995), and to follow short, synchronous synaptic inputs at high rates. Anatomically, the axons of both cell types are also similar (see Cant and Benson, 2003, for review). Neither axon provides substantial innervation within the cochlear nucleus (Smith and Rhode, 1987, this study). Axons of both cell types are large and project out of the cochlear nucleus to consistently influence the principal cells of one brainstem nucleus: the VNLL by octopus cells, and the MNTB by bushy cells. The endings of these axons in the MNTB and VNLL are large and excitatory and generate multiple terminations directly onto cell bodies. Extracellular recordings from these postsynaptic cells show prepotentials that represent the activity within the large axon terminal. The terminal recipient cells in the MNTB and VNLL are inhibitory and have been shown to have nonlinear membrane properties similar to their CN input, firing only once to depolarizing current pulses (Banks and Smith, 1992; Wu, 1999; Zhao and Wu, 2001).

**Fusiform and giant cells**

While one morphological class of DCN projection neurons—the fusiform (or pyramidal) cells—has been particularly well studied, the other morphological class is probably the least studied projection neuron of the entire CN. In contrast to other CN principal cells, giant cells have not been associated with a distinct response pattern. Extensive extracellular studies in the decerebrate cat by Young and colleagues (reviewed by Young and Davis, 2002) have not revealed any dichotomous differences that may be associated with the two types of DCN principal neurons, and these authors have therefore concluded that the two types of neurons do not differ in their physiology. This is remarkable because these cells are so different in their morphology and location in the layered DCN, and thus presumably also in their inputs.

We provide the first in vivo data from morphologically identified giant cells. The data are limited and fragmented because the recording times were generally short and because it was difficult to find stimuli to drive the cells. Giant cells tended to show high thresholds, high spontaneous activity that could be inhibited by tones over a wide frequency range, sluggish and irregular responses, and a bias to very high frequencies. The responses of only one case (Fig. 11H) showed the key features displayed by the majority of fusiform cells, i.e., P/B PSTHs, a strongly nonmonotonic rate curve to STCF, and an excitatory response to broadband noise. On the other hand, some of the labeled cells which lacked the key features just mentioned turned out to be fusiform cells (Fig. 10G–J), although they did have the expected better tonal thresholds. Thus, our data do not provide physiological signatures allowing unequivocal distinction between the two cell types, but they nevertheless strongly suggest that the two populations differ in their physiology: the archetypal P/B pattern and its associated properties predominates in fusiform cells, but not in giant cells.

EM of the cell body of one fusiform cell showed fairly sparse synaptic coverage, in agreement with previous data on these cells (Smith and Rhode, 1985). EM of three of our labeled giant cells indicates that they may receive a sparse somatic innervation but can receive innervation that is as dense as that seen on octopus or O2 cell bodies. Kane et al. (1981) also reported a variable innervation density between members of the giant cell population. Our sample is too small and our data too fragmented to determine whether giant cell terminal density is correlated with any anatomical or physiological parameter of this population. The axons of both cell types leave the cochlear nucleus via the DAS. Some fusiform axons show collaterals within the DCN, but we saw no giant cell axon collaterals. Unfortunately, these axons were often lightly labeled, so we cannot say with certainty that they did not have collaterals and did not influence other cells in the CN. The main fusiform or giant cell axon takes a similar course on their way into the lateral lemniscus. Along this course many of the axons were darkly labeled, yet we never saw any collaterals given off to the brainstem auditory nuclei in the olivary complex or the nuclei of lateral lemniscus, so it is clear that the DCN output has no direct effect on neurons interposed between the CN and the inferior colliculus. Both sets of axons entered the IC but faded before the branching pattern could be distinguished, so we were unable to carefully study individual axonal innervation patterns or determine whether any of the axons continued to the thalamus. Malmierca et al. (2002) have shown in the rat that at least some of the giant cells and perhaps some of the fusiform cells in the DCN project to the medial division of the auditory thalamus. Whether this is a subpopulation of the giant and fusiform cells and whether these same cells also synapse in the IC is unknown.

What might be the function of these projection neurons of the DCN? Several studies approached this question by lesioning output of the DCN to look at its effect on behavior or the response of downstream targets (Bengry et al., 1977; Casseday and Neff, 1975; Jenkins and Masterton, 1982; Masterton and Granger, 1988). Sutherland et al. (1998a,b) and May (2000) reported that such a lesion disrupted sound orientation behavior. Young and colleagues (Young et al., 1995; Davis et al., 1996; Kanold and Young, 2001) have shown that the DCN projection neurons respond not only to auditory stimuli but to somatosensory inputs from muscle proprioceptors in and around the pinna as well. Such evidence has led to speculation that the DCN output may be involved in coordinating pinna orientation and localization cues found in the different spectra of sounds located at different points in space (see review, Young and Davis, 2002).
At present, it is not known whether the fusiform and giant cells perform the same or different functions. Oliver et al. (1997) showed that innervation in the IC from the DCN overlaps with contralateral LSO innervation. Although both sets of DCN principal cells are presumed to be generating this innervation pattern, there is no evidence that fusiform and giant axons influence the same or different cells in the region. Ramachandran et al. (1999) showed that some IC cells, designated type O, had response features similar to fusiform and giant cells and speculated that some of these cells were getting these response features from their DCN inputs. Davis and colleagues (Davis, 2002; Davis et al., 2003) provided evidence that a subclass of the type O category, the low rate type Os, receive direct input from cells using the DAS and speculated that this pathway may be part of a circuit that is specialized to detect spectral features. Again, none of these studies were able to distinguish whether giant cell axons, fusiform axons, or both, contributed to the observations. One piece of anatomical data may provide an initial indication that the two DCN output cell types have different functions. This is based on a unique synaptic input to the fusiform cells that shows plasticity. Fusiform cells have both a spiny apical dendritic tree extending into the superficial DCN layers and a set of nonspiny basal dendrites extending into the deep DCN. Like the fusiform basal dendrites, giant cell dendrites are aspiny and confined primarily to the deep DCN, where the primary inputs are from auditory nerve fibers. In the superficial DCN layers, fusiform apical dendrites receive parallel fiber inputs from granule cells that have been shown to display activity-dependent plasticity, while the auditory nerve input does not (Fujino and Oertel, 2003). Thus, even if the fusiform and giant cell axons projected to exactly the same sites/cells, one input (the fusiforms) might have a response that could vary, depending on the conditions, while the other (giants) might lack this ability.

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