Anatomy and Physiology of Principal Cells of the Medial Nucleus of the Trapezoid Body (MNTB) of the Cat

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Smith, Philip H., Philip X. Joris, and Tom C. T. Yin. Anatomy and physiology of principal cells of the medial nucleus of the trapezoid body (MNTB) of the cat. J. Neurophysiol. 79: 3127–3142, 1998. We have recorded from principal cells of the medial nucleus of the trapezoid body (MNTB) in the cat’s superior olivary complex using either glass micropipettes filled with Neurobiotin or horseradish peroxidase for intracellular recording and subsequent labeling or extracellular metal microelectrodes relying on prepotentials and electrode location. Labeled principal cells had cell bodies that usually gave rise to one or two primary dendrites, which branched profusely in the vicinity of the cell. At the electron microscopic (EM) level, there was a dense synaptic terminal distribution on the cell body and proximal dendrites. Up to half the measured cell surface could be covered with excitatory terminals, whereas inhibitory terminals consistently covered about one-fifth. The distal dendrites were very sparsely innervated. The thick myelinated axon originated from the cell body and innervated nuclei exclusively in the ipsilateral auditory brain stem. These include the lateral superior olive (LSO), ventral nucleus of the lateral lemniscus, medial superior olive, dorsomedial and ventromedial periolivary nuclei, and the MNTB itself. At the EM level the myelinated collaterals gave rise to terminals that contained nonround vesicles and, in the postsynaptic cell as well (Borst and Sakmann 1996; Forsythe and Barnes-Davis 1993a,b; Wu and Kelly 1991) indicates that the mature calyceal input acts primarily on non-N-methyl-D-aspartate glutamatergic receptors to generate a large, fast suprathreshold synaptic response. The rapid repolarization of the synaptic response, allowing these cells to follow their inputs at high rates, and the tendency of these cells to fire once to sustained depolarizing current are, in part, due to a dendrotoxin sensitive, low-threshold potassium conductance. A similar conductance has been reported for the bushy cells that provide the calyceal input to MNTB (Manis and Marx 1991; Oertel 1983; Wu and Oertel 1984). A second fast, high-threshold potassium conductance also is present in MNTB cells serving to rapidly repolarize the action potential (Brew and Forsythe 1995). Thus MNTB is part of an afferent chain with morphological and physiological specializations for temporally precise transmission of signals.
As described above, a rather extensive investigation of both the anatomic and physiological features of the calyceal input and the MNTB cell has been made in brain stem slices. However, several major gaps remain in our knowledge of the function of these cells in vivo. First, the response features of MNTB principal cells to simple auditory stimuli have not been unequivocally established. The only published study of recordings from positively identified—and subsequently labeled—principal cells comes from the rat MNTB (Sommer et al. 1993). In this paper, the authors recorded intracellularly from 11 MNTB principal cells and successfully injected them with horseradish peroxidase (HRP), labeling cell body and dendritic tree as well as much of the axonal field. However, the brief recording times allowed only a very limited measurement of the responses of these cells to auditory stimuli: the characteristic frequency (CF), the frequency at which the threshold intensity was the lowest) of only six cells was determined, and only 10 stimulus trials were used to generate short tone responses to the CF (STCF responses), making it impossible to determine their physiological response type, i.e., primarylike (PL; a response resembling auditory nerve fibers), primarylike-with-notch (PLN; a response resembling the globular bushy cell) or phase-locked (cells of low CF with spikes occurring at a particular phase of a low-frequency stimulus cycle). The remaining in vivo studies of MNTB have used extracellular metal electrodes (Guinan and Li 1990; Guinan et al. 1972a,b; Li and Guinan 1971; Tsuchitani 1994, 1997) and indirect evidence, the presence of a large prepotential, to identify the recorded cells. With metal electrode recordings, many cells in the vicinity of the MNTB exhibit spikes with complex waveforms, so-called prepotential cells. Based on similar recordings from the cochlear nucleus that were proposed to arise from the large auditory nerve endbulb of Held terminal/bushy cell complex (Pfeiffer 1966), Guinan and Li (1990) postulated that these waveforms arose from the calyx/MNTB principal cell complex and consisted of a prepotential in the presynaptic calyx followed by a postsynaptic spike. Responses to short tones from cells displaying such spike waveform were either primarylike, primarylike-with-notch or phase-locked. Subsequently Tsuchitani, (1994, 1997) categorized cells in the vicinity of MNTB as being MNTB principal cells based on the presence of a prepotential and/or a PL or PLN short tone response. Given the large number of fibers in the trapezoid body coursing directly through the MNTB with PL and PLN responses (Smith et al. 1991, 1993b), we believe the presence of a prepotential is essential for positive identification of extracellular recordings from MNTB cells. Furthermore, while it is generally thought that MNTB cells are monaurally driven by the contralateral ear, there are no documented interaural level difference functions in the literature for MNTB cells documenting this characteristic.

A second gap exists regarding the surprising observation in several rodents, bats, and in cat (Adams and Mugnaini 1990; Banks and Smith 1992; Kuwabura and Zook 1992; Kuwabura et al. 1991; Smith 1995; Sommers et al. 1993) that MNTB cells project to the medial superior olive (MSO), a nucleus containing cells that are thought to be comparing the time of arrival of the excitatory inputs from the two ears (Yin et al. 1997). All but one of these studies were in vitro so the auditory response features (CF, STCF response, spontaneous rate) of these MSO-projecting MNTB cells could not be assessed. In the one in vivo study (Sommers et al. 1993), no auditory responses were recorded from the one labeled MNTB cell that projected to MSO. The excitatory input to the MSO comes bilaterally from the spherical bushy cells of the anteroventral cochlear nucleus (AVCN), cells that exhibit enhanced synchronization as compared with their auditory nerve input to low CF tones (Joris et al. 1994a). It is essential to know the character of the inhibitory input to these cells from the MNTB if we are to understand the processing of interaural time disparities in the MSO and interaural level differences in the LSO.

A third gap in our knowledge of the characteristics of these cells is in the anatomy of their synaptic input and output. For the input, early electron microscopic studies (Jean-Baptiste and Morest 1975; Lenn and Reese 1966; Morest 1968, 1973) described the calyceal ending and mentioned that there were other “noncalyceal terminals” on the cell body, but no measures were made of the extent of these terminals, and, because of the unlabeled nature of the cells, a description of the synaptic input to only the most proximal dendrites could be given. For the MNTB output, several light microscopic studies have described the projection pattern of MNTB axons in various species (Banks and Smith 1992; Kuwabura and Zook 1991, 1992; Schofield and Cant 1992; Sommer et al. 1992), but no electron microscopy was done to confirm or describe the terminal morphology. Cant (1984) reported that in the LSO (a major recipient of MNTB axon collaterals), almost three-fourths of the surface of the LSO principal cell body and proximal dendrites are covered with synaptic terminals that are almost exclusively those containing small vesicles many of which are flattened or cylindrical. She proposed that these terminals arose from MNTB axons but admitted that it remained to be demonstrated experimentally (Cant 1984). Thus the ultrastructural identification of the axon terminals of identified MNTB principal cells has never been unequivocally made.

Our goal in these in vivo experiments was to answer the questions described above by characterizing the basic response features of cat MNTB cells, with glass electrodes, and to subsequently label the cell with either HRP or neurobiotin. Labeled cells could be studied at the light and electron microscopic level to examine the morphology of the axonal and dendritic tree as well as the distribution of synaptic inputs and features of the output terminals.

METHODS

Many of the methods used for these experiments have been described in detail elsewhere (Smith and Rhode 1985; Smith et al. 1991, 1993b) and are summarized here. Animals were maintained in an American Association for Accreditation of Laboratory Animal Care (AAALAC)-approved animal care facility, and all methods have been approved by the University of Wisconsin Institutional Animal Care And Use committee.

Surgical procedure

Young adult cats were anesthetized and maintained in an areflexive state with pentobarbital sodium (35 mg/kg). Pinnae were removed, and the external auditory meati cut transversely for inser-
tion of metal ear pieces through which acoustic stimuli, calibrated from 60 Hz to 40 kHz, were delivered. The MNTB was surgically approached from the ventral surface of the brain stem. A small hole was drilled in the basioccipital bone just lateral to the pyramidal tract, and a slit was made in the dura. The small rootlets of cranial nerve VI served as an external landmark for the more deeply located MNTB.

**Acoustic stimuli and data collection**

Calibrated acoustic stimuli generated by a computer-controlled digital stimulus system (Rhode 1976) were delivered from Telex 140 earphones or Radioshack supertweeters. Spike-triggered pulses were sent to a unit event timer and stored for on-line and subsequent analysis. As the electrode was advanced toward the MNTB, a search stimulus of short tone bursts with variable frequency was presented to both ears until an axon or a cell body was encountered.

**Glass electrodes—recording and injection**

Intracellular glass electrodes were filled either with a buffered (pH 7.6), filtered 5% HRP (Sigma) solution in 0.5 M KCl or a 2% Neurobiotin (Vector Labs) solution in 0.5 M KCl. The electrode was lowered over the hole drilled in the skull and advanced in 1-μm steps. Extracellular and intracellular signals were monitored using standard techniques for DC monitoring, amplification, filtering, and display. Recordings from MNTB cells using the glass microelectrodes could only be verified by subsequent location and identification of the labeled cells. However, some features of axonal responses were used to distinguish presumed MNTB axonal responses from those of other axons: 1) because the primary excitatory input to a MNTB cell arises from a globular bushy axon from the contralateral cochlear nucleus, only units driven from the contralateral ear were considered. 2) Because of the interposing synapse between globular bushy axon and MNTB cell, first spike latencies taken from peristimulus time histograms (PSTHs) to STCFs should be slightly longer for a MNTB cell axon than for a bushy cell axon with the same characteristic frequency.

While tones at the unit's CF were presented and the response monitored, entry into the axon, as signaled by a DC shift of from −30 to −60 mV, was accomplished with 100-ms current pulses. After physiological characterization of the cell (see further) HRP or neurobiotin was injected for 2–10 min using 100 ms, 1–5 nA current pulses while CF tones were presented continuously. Current injection was terminated if the response during pauses in the injection changed, which would indicate that the electrode had slipped into a different cell. After data collection, the electrode was withdrawn, the DC shift noted and a 10-nV calibration pulse recorded on the intracellular channel of the tape monitor.

**Metal electrodes—recording**

Commercially available tungsten metal electrodes (Microprobe, 10–20 μM exposed tips, 5 MΩ impedances) were used in separate extracellular experiments. Electrode placement, method of advance, stimulus presentation, and unit response characterization were as described above for glass electrodes. Metal electrode recordings from MNTB cells were distinguished by their prepotentials (Guinan and Li 1990; Guinan et al. 1972a,b; Li and Guinan 1971) and by subsequent histological location of the recording site from lesions made at several points in each penetration (except for 4 cells in 1 animal where histology was not available).

**Tissue processing**

For metal electrode experiments, we perfused the animal with formalin shortly after a lethal dose of pentobarbital sodium was administered after the last penetration. For the HRP/Neurobiotin experiments, the animal was maintained in an areflexive state for 24–36 h after the last penetration using a drip solution of sodium pentobarbital (3% in 5% dextrose) while heart rate, respiration, and withdrawal reflexes were checked every 20 min by one of the experimenters. Wound areas were swabbed at 2-h intervals with lidocaine. After a lethal dose of sodium pentobarbital, the cat then was perfused transcardially with saline followed by two concentrations of phosphate-buffered, calcium-containing glutaraldehyde:paraformaldehyde fixative (0.01:0.01 and 0.02:0.01), and the brain stored either in sucrose buffer (for frozen sectioning) or 1:1 glutaraldehyde:paraformaldehyde fixative (for vibratome sectioning).

For the HRP injections, the following steps were taken. Coronal or horizontal sections were cut at 60- or 70-μm thickness with a vibratome or after freezing and then were reacted using the 3,3'diaminobenzidine (DAB)-nickel/cobalt intensification method (Adams 1981). For light microscopy, sections were mounted on glass slides, counterstained with cresyl violet, and coverslipped. For electron microscopy, vibratomed, HRP-reacted sections were fixed in 2% osmium tetroxide, dehydrated, and flat-embedded in plastic resin. After a camera lucida drawing of the injected axon was made, thin sections were taken, counterstained with uranyl acetate and lead citrate, and observed with a JOEL 100CX electron microscope.

For the Neurobiotin injections, the following steps were taken. Vibratomed 70-μm section were cut into 0.1 M phosphate buffer, washed, incubated in 0.5% H2O2 in phosphate buffer, then reacted overnight in ABC reagent (Vector ABC kit, in 0.1 M phosphate buffer, pH 7.4 containing 0.3% TritonX, 2% bovine serum albumin, 140 earphones or Radioshack supertweeters. Spike-triggered pulses were sent to a unit event timer and stored for on-line and subsequent analysis. As the electrode was advanced toward the MNTB, a search stimulus of short tone bursts with variable frequency was presented to both ears until an axon or a cell body was encountered.

**Data analysis**

**PHYSIOLOGY.** When a cell or axon was isolated, stimuli were monitored, entry into the axon, as signaled by a DC shift of from peristimulus time histograms (PSTHs) to STCFs should be slightly longer for a MNTB cell axon than for a bushy cell axon with the same characteristic frequency. While tones at the unit’s CF were presented and the response monitored, entry into the axon, as signaled by a DC shift of from −30 to −60 mV, was accomplished with 100-ms current pulses. After physiological characterization of the cell (see further) HRP or neurobiotin was injected for 2–10 min using 100 ms, 1–5 nA current pulses while CF tones were presented continuously. Current injection was terminated if the response during pauses in the injection changed, which would indicate that the electrode had slipped into a different cell. After data collection, the electrode was withdrawn, the DC shift noted and a 10-nV calibration pulse recorded on the intracellular channel of the tape monitor.

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**Data analysis**

**PHYSIOLOGY.** When a cell or axon was isolated, stimuli were presented to each ear separately to determine which ear(s) drove the unit. For all cells, we determined the characteristic frequency (CF, the frequency at which the threshold intensity was the lowest), spontaneous discharge rate, and Q10 (CF/bandwidth at 10 dB above threshold) using an automated threshold tuning curve program. We also measured the STCF at 10- to 20-dB intervals from near threshold to 50–70 dB above threshold using 100 or 200 repetitions of 25-ms tones presented every 100 ms with a 3.9-msec rise/fall time. Amplitude-modulated and click stimuli also were used but the data will not be presented here.

From the STCF response, we derived the mean and standard deviation of first spike latency (see Smith et al. 1991), mean coefficient of variation over the interval 12–18 ms (Young et al. 1988), and the sustained discharge rate and synchronization coefficient (Goldberg and Brown 1969) during the last 15 ms of the tone burst. To correct for spontaneous activity in the computation of the mean and standard deviation of the first spike latency, we eliminated all spikes in the time window from 0 to 2.4 ms after stimulus onset and all trials in which a spike occurred in the last half of this window.

**ANATOMY.** Labeled axons were drawn in the coronal or horizontal plane using a camera lucida with ×63 oil objective. Measurements of the features of terminals on MNTB principal cell bodies and dendrites were quantified using a Summagraphics bit pad. Two graphics tablet interfaced to the same microVax computer used to generate auditory stimuli.
that displayed complex waveforms (Guinan et al. 1972a) and that were subsequently localized to the MNTB by electrode-generated lesions at the recording site. Typical complex waveforms recorded by extracellular metal electrodes in the MNTB are shown in Fig. 1. They usually consisted of a negative-going prepotential, that varied in amplitude from unit to unit, followed ~0.5 ms later by a larger bipolar spike, which was used to trigger the unit event timer. Although sound-driven spikes always were preceded by a prepotential, spontaneously occurring spikes (those not generated by our sound system) unaccompanied by a prepotential were occasionally seen (n = 3 cells; see Fig. 1, bottom 2 panels).

When recording extracellularly with metal electrodes in the vicinity of the MNTB, we used the presence of the prepotential as an indicator that we were recording from a MNTB principal cell. Invariably, ‘‘prepotential’’ cells were driven monaurally from the contralateral ear and did not respond to stimulation of the ipsilateral ear. In all cases, this was confirmed by subjective comparison of the responses (over the audio monitor) to monaural stimulation of the left and right ears as well as binaural stimulation, at 60–70 dB SPL to each ear. In a small number of cases, we verified the lack of binaural input by stimulating the contralateral ear with CF tones at a constant level while varying the ipsilateral SPL over 30–80 dB (Fig. 2). In Fig. 2, the results from four MNTB cells are plotted as interaural level difference (ILD) functions, where ILD is defined as the contralateral minus ipsilateral SPL. For comparison the ILD functions of nine binaural IE cells (cells that were inhibited by contralateral stimulation and excited by ipsilateral stimulation) in the neighboring LSO are shown. This figure clearly illustrates that the response of the MNTB cell is independent of ipsilateral SPL.

With glass electrodes, we have recorded from and injected eight MNTB principal cells. On the basis of features typically used to identify an electrode penetration site (foculcent extracellular reaction product and/or axonal swelling at the point of electrode entry) and the monopolar shape of the action potential, recordings from injected units have thus far been judged as intra-axonal. Extracellular spikes displaying

**RESULTS**

**General**

The recording configurations used to collect the MNTB cell data described in this paper as well as to collect data from the globular bushy cells, which have been reported previously (Joris et al. 1994a; Smith et al. 1993b). MNTB cells were recorded from either intra-axonally with glass electrodes or extracellularly, with metal electrodes in the vicinity of the presynaptic calyx/postsynaptic cell body complex. Bottom: extracellular metal electrode recordings from the MNTB. Waveform showed a prepotential (P.P.) presumed to arise from the calyx, followed by a MNTB cell action potential. Lower 2 traces: sound-driven MNTB action potentials, preceded by a prepotential, are superimposed on a trace, taken in the absence of intentional sound stimuli, showing no prepotential (No P. P.). Scales at top of columns apply to all traces in that column.

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Top: diagrammatic representation of the recording configurations from a medial nucleus of the trapezoid body (MNTB) principal cell or its globular bushy cell input. Globular bushy cell axons were recorded extra or intra-axonally, on the same or opposite side as the MNTB that they innervated, as previously described (Joris et al. 1994a; Smith et al. 1993b). MNTB cells were recorded from either intra-axonally with glass electrodes or extracellularly, with metal electrodes in the vicinity of the presynaptic calyx/postsynaptic cell body complex. Bottom: extracellular metal electrode recordings from the MNTB. Waveform showed a prepotential (P.P.) presumed to arise from the calyx, followed by a MNTB cell action potential. Lower 2 traces: sound-driven MNTB action potentials, preceded by a prepotential, are superimposed on a trace, taken in the absence of intentional sound stimuli, showing no prepotential (No P. P.). Scales at top of columns apply to all traces in that column.

**FIG. 2.** Normalized short tone interaural level difference (ILD) functions for 4 cells localized to the MNTB that displayed prepotentials (encompassed by shaded area below ‘‘MNTB’’) and 9 cells localized to the lateral superior olive (LSO; encompassed by shaded area next to ‘‘LSO’’). Tones at CF were used for all cases shown. For the MNTB recordings, contralateral stimulus was held constant 20–30 dB above threshold while the intensity of the ipsilateral stimulus was varied around 0 ILD. For the LSO cells, we held the ipsilateral level constant at 20–30 dB above threshold and varied the contralateral level.
prepotentials were never seen using glass microelectrodes. Axonal penetration, while short tones at CF were presented, was signaled by a DC shift of 30–60 mV. Four of the injected cells were well labeled with the dendritic tree and the axonal projection field readily distinguishable. The other four were lightly labeled. Although the cell body shape and location as well as primary dendritic tree and primary axon collaterals of these cells were distinguishable, finer details of these could not be examined.

Anatomy

CELL BODIES. Previous extracellular studies of the cat superior olivary complex (Guinan et al. 1972b) indicated that the MNTB is tonotopically organized, with low CF cells placed laterally and higher frequencies situated progressively more medial with isofrequency strips angled dorsomedially to ventrolaterally. We found the absolute location of our labeled MNTB cell bodies within the boundaries of the nucleus hard to quantify. The MNTB boundaries are often difficult to distinguish especially when sections are embedded in plastic but, in general, our data would seem to concur with this map. One labeled cell with a very high CF was situated very medially, embedded in Vth nerve axon bundles. A single, labeled low best-frequency cell was located considerably laterally and our other labeled cells with intermediate CFs were located between these two extremes.

DENDRITIC TREE. Figure 3 illustrates the cell bodies and dendritic trees of our four well-labeled MNTB cells. Typically, the dendritic tree arose from one or two large main dendrites that branched rather profusely within a single compact area much like the dendritic trees of globular bushy cells in the cochlear nucleus, which provide the main excitatory input to these cells. In one instance, however, (Fig. 3, soma at middle left), a single MNTB cell body gave rise to two main dendrites, one with the compact morphology and the other branching occasionally over a fairly extensive area. This cell also displayed the highly unusual feature of two main axons arising from the cell body.

TERMINAL DISTRIBUTION. At the electron microscopic (EM) level globular bushy cell calyceal terminals form the major input onto the MNTB principal cell body. Figure 4 illustrates one such calyx that has been labeled by intraaxonal injection of the globular bushy cell axon giving rise to the calyx, synapsing on an unlabeled MNTB principal cell (Fig. 4, A and B) and another unlabeled calyx synapsing on a MNTB cell labeled by intraaxonal injection of its axon (Fig. 4C). In selected EM sections, we measured the fraction of cell body and dendritic tree surface that was covered by synaptic profiles for three of the well-labeled, plastic-embedded cells (top 3 cells in Fig. 3). For the three cell bodies, 60.9, 66.6, and 68.4% of the total surface was covered with synaptic terminal profiles; 39.6, 43.8, and 48% of the surfaces were covered with terminals containing round vesicles, whereas 21.3, 22.8, and 20.4% of the surface were covered with terminals containing nonround vesicles. The remaining 39.1, 33.4, and 31.6% were terminal-free zones. The total amount of the surface of the proximal dendritic trees, within 100 μm of the cell body were also equally innervated with 65.9% of the total surface covered and 35.5% of the surface covered with terminals containing round vesicles. Synaptic coverage dropped precipitously on the distal dendritic tree (>100 μm from the cell body) where only 15% of the surface was terminal-covered and only 2% covered with round-vesicle containing profiles.

AXON. Our well-injected axons indicate that the projection of an individual MNTB cell axon can be quite extensive innervating a number of auditory nuclei in the ipsilateral brain stem only. Figures 5–8 illustrate examples of projection patterns for labeled axons. The primary sites of innervation of our MNTB cell axons were the ipsilateral LSO and ventral nucleus of the lateral lemniscus (VNLL). In addition, collaterals often were seen with terminal swellings in periolivary regions, medial to the dorsal and ventral aspect of the MSO, which have been designated dorsomedial and ventromedial periolivary regions (DMPO and VMPO) (Spangler et al. 1985, 1987). In some cases, these axons also had collaterals to MSO and the MNTB itself.

Cells whose axons were labeled sufficiently so that they could be followed as far lateral as the LSO (6/8) appeared to innervate this structure. In these cases, the axon usually entered the LSO through either the dorsal or ventral hilus and branched within a fairly confined mediolateral space but rather extensively rostrocaudally (Figs. 5 and 8). In our one example of an injected low-frequency MNTB cell, the axon coursed ventral and lateral to the LSO in the fiber bundle surrounding the LSO until it reached the lateral low-frequency limb where it branched (Fig. 6). In another case, it was unclear whether the axon actually innervated the body of the LSO. This cell had a CF of 27,000 Hz and was cut in the horizontal plane (Fig. 7, left cell). It had a collateral branch that ran ventral to the LSO and then headed dorsally immediately beneath the LSO to branch just ventral to the area where the first signs of the ventral aspect of the LSO would appear in the next-most dorsal section.

Of the cells with axons labeled darkly enough to reliably follow through the body of the MSO (6/8) three showed collaterals with terminals in the MSO (Figs. 6–8). Although our population is small, this innervation appeared to be frequency dependent in that the three cells with collaterals to MSO had CFs of 635, 6,300, and 13,000 Hz, whereas those without obvious collaterals had CFs of 13,500, 17,680, and 27,000 Hz. Innervation of MSO by MNTB collaterals was much less elaborate than that seen in the LSO with fewer branches innervating a smaller region of the nucleus. The cells that innervated MSO did so in a tonotopic fashion with the low-frequency MNTB cell sending collaterals to the dorsal most aspect of the MSO, the intermediate CF MNTB cell innervating the proposed midfrequency region of the MSO, and the highest CF unit innervating the MSO very ventrally. It is impossible to know, however, whether the CF of the MSO region innervated exactly matched the CF of the MNTB cell innervation.

Cells with well-labeled axons that could be followed for a considerable distance rostrally (5/8) all sent a collateral to the VNLL (Figs. 6–8). This collateral would typically head rostrally, under the VNLL, and then head dorsally, directly beneath or rostral to the VNLL in the lateral lemniscus, sending multiple branches into the body of the VNLL.

We made measurements of the axon along its path at the...
light microscopic level. As described in a previous publication (Smith et al. 1993b), these represent measurements of the axoplasm and do not include the myelin sheath and may be complicated by a number of factors. In the vicinity of the cell body, the axon diameter appeared to be quite large. Before beginning to give off its major collaterals, the axon diameter was usually between 4 and 6 μm. After beginning to branch, the major collaterals that, for example, headed laterally to LSO or rostrally toward VNLL were typically 3–4.5 μm and maintained this diameter until approaching near to the nucleus. Subsequent secondary branching of one of these main collaterals, for example when the major collateral projecting to the LSO began giving off major branches soon after entering the hilus, were usually 1.5–3 μm.

At the electron microscopic level, the main MNTB axon typically myelinated 30–40 μm from the cell body. The swellings along and at the ends of myelinated collateral branches were terminals containing nonround synaptic vesicles. Figure 9 illustrates two such labeled terminals on the primary dendrite and cell body of a principal cell in the LSO and represents the first direct evidence of the terminal configuration of the MNTB principal cell axon.

**Physiology**

The primary excitatory inputs to MNTB cells are the large somatic calyceal terminals that arise from GBC axons. Thus it would seem appropriate to make comparisons of MNTB

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**FIG. 3.** Camera lucida drawings of 4 labeled MNTB cells. Three cells on the left are drawn in the horizontal plane, whereas the cell on the right is drawn in the coronal plane. *, swelling where the electrode penetrated the axon and the axon distal to the recording site is much thinner than the axon between the injection site and the cell body. Scale bar at top right applies to all cells.
cell responses with those of GBCs that we have previously reported (Smith et al. 1991).

The CFs of our MNTB cell population ranged from 300 Hz to 36 kHz and thresholds at CF from 6 to 63 dB SPL (mean = 21 dB SPL). This compares with 300 Hz to 31 kHz and -1 to 53 dB for GBCs. Short tone responses were, in general, similar to GBC responses with some noticeable variations. Figure 10 shows PSTHs from representative MNTB cells over the entire frequency range and may be compared with the globular bushy cell response (Fig. 1 of Smith et al. 1991). More than 1 kHz the onset component of the MNTB response often would be well timed at higher stimulus intensity levels giving the response a PLN appearance. In some instances, however, the onset portion did not become well timed at any level (Fig. 10, bottom left and 4th trace in right column). We also occasionally noted what appeared to be a slight “sag” in the sustained portion of the short tone response (Fig. 10, bottom left and middle trace in right column).

Figure 11 shows the spontaneous spike rates (top) as well as the latency (middle), and standard deviation (bottom) of the short tone-induced first spike of the extracellularly (○) and intraaxonally (□) recorded MNTB cell population compared with the same data from our labeled globular bushy cell population. A number of comparisons may be made between populations. First, only a few of the MNTB cells had CFs <3 kHz (2/45) while this CF was represented much more extensively in our GBC population (~4% for MNTB cells vs. 12/35 (34%) for GBCs). Those two MNTB cells with CFs <3 kHz had spontaneous rates of 0 spikes/s as did most of the GBCs population with such CFs (8/12 with 0 spikes/s and 11/12 <5 spikes/s). More than 3 kHz, the spontaneous rates tended to be higher for both MNTB cells and GBC populations (mean = 27 spikes/s vs. 12.4 spikes/s, respectively). If the boundaries chosen in the auditory nerve by Liberman (1978) are used for distinguishing high, medium, and low spontaneous rates (SRs) (18 and 1 spikes/s, respectively), the percentages of high, medium, and low SRs in MNTB cells were 55, 27.5, and 17.5%, respectively, compared with 23, 46, and 31% for GBCs and 65, 20, and 15% for auditory nerve (AN).

As with GBCs, the latencies of MNTB cells decreased with increasing CF and, as might be expected given a requisite synaptic delay between GBC axons and MNTB cell axons, the MNTB cell latencies tended to be longer than GBC latencies for cells with similar CFs (Fig. 11 B). Finally, as expected from the sharp onset response of many MNTB cells, the timing of the first spike is quite precise (mean first spike standard deviation = 0.76 ms) although not as precise as the GBC input (0.34 ms, Fig. 11 C).

DISCUSSION

Summary

We labeled eight MNTB cells intraaxonally after obtaining their physiological response properties. In agreement with previous qualitative anatomic descriptions (Jean-Baptiste and Morest 1975; Lenn and Reese 1966; Morest 1968, 1973), our measurements show that calyceal coverage can take up one-fourth to one-half of the total surface of the cell.
FIG. 5. Camera lucida reconstruction of a high (17.7 kHz) characteristic frequency principal cell, in the right MNTB, sectioned in the coronal plane (see also Fig. 3). **Top left**: higher power drawing of the cell body, dendritic tree and initial portion of the axon. *, penetration site of the electrode. **Bottom left**: low power reconstruction of the same cell and its axon collateral field. Location of some of the innervated nuclei appear out of place on this 2-dimensional drawing because of the large rostro-caudal distance traversed by the axon. Axon gave off numerous collaterals innervating an appropriate frequency region of the LSO [given the characteristic frequencies (CF)], MNTB, dorsomedial and ventromedial periolivary regions (DMPO and VMPO), and ventromedial region of the ventral nucleus of the lateral lemniscus (VNLL) before fading in the LL. **Bottom right**: short tone response of this cell at its CF (see also Fig. 10).
body and primary dendrites. In addition, we have shown that nonexcitatory inputs also concentrate on the cell body and consistently cover around one-fifth of the surface, but the source of this input or means of activation is unknown. Our data also show that inputs with both excitatory and inhibitory features are rare on the distal dendritic tree. We also have shown for the first time at the ultrastructural level that the terminals of these MNTB axons contain nonround vesicles and, when observed in the LSO, terminate on the somata and primary dendrites of LSO principal cells.

As has been previously described in cat and other species (Banks and Smith 1992; Kuwabara and Zook 1991, 1992; Schofield and Cant 1992; Sommer et al. 1992; Spangler et al. 1985), MNTB cells send their axonal projections only to areas of the ipsilateral brain stem, namely (in cat) the ventral nucleus of the lateral lemniscus, the lateral and medial superior olives, the dorsomedial and ventromedial periolivary nuclei, and the MNTB. The only previous in vivo intracellular study (Sommer et al. 1992) indicated that the rat LSO receives MNTB inputs with best frequencies from 47 kHz down to \( \approx 14 \text{ kHz} \). Our labeled cells show that, in the cat, this frequency range extends down to \( \approx 635 \text{ Hz} \). In addition, despite an admittedly small sample, our labeled cell data provides evidence that the MNTB projection to the MSO may be frequency specific—cells with CFs below \( <13 \text{ kHz} \) innervate the MSO, cells with CFs \( >13 \text{ kHz} \) do not.

Physiologically our short tone data from cells, positively identified by intracellular staining, show that MNTB cell responses are like those of globular bushy cells with similar best frequencies. We also have included physiological data from cells in the MNTB recorded extracellularly that displayed prepotentials. Prior to this, the only evidence that the complex spikes seen in the MNTB arose from the MNTB principal cell and its calyx was provided by Guinan and Li (1990). By stimulating either LSO or DMPO while recording a complex spike unit in the MNTB, they could antidromically activate a spike, designated the ‘‘A’’ spike. This A spike resembled the portion of the complex waveform, designated the ‘‘C2’’ component, that was believed to be the postsynaptic MNTB cell spike. When the complex spike was activated by stimulation of the trapezoid body at the midline, thus driving the globular bushy axon/calyceal synapse and presumably activating the MNTB principal cells, the antidromic A spike was refractory and could not be driven for \( \sim 1 \text{ ms} \), implying that the A spike and C2 component were both generated by the MNTB cell. We felt that this evidence, together with our positive identification of MNTB principal cells with physiology comparable to the complex waveform units, was sufficient to permit our inclusion of the extracellular data.

**Globular bushy cell input—a comparison of responses**

The data available from our MNTB cell sample show the results of what is apparently a good transmission of temporal information across the synapse. Globular bushy cells carry a variety of accurately timed information about low- as well as high-frequency auditory stimuli. Our previous reports (Joris et al. 1994a; Smith et al. 1991) have shown that low-frequency globular bushy cells can phase lock to the stimulus waveform of a short tone at their CF with remarkable precision (as measured by the synchronization coefficient) and often will entrain (generate a spike for every cycle of the stimulus waveform). Because our sample of MNTB cells with CFs \( <1 \text{ kHz} \) is so small, it is difficult to compare GBC and MNTB cell populations in terms of phase locking capabilities. Nevertheless the positively identified MNTB cell with a CF \( <1 \text{ kHz} \) showed ‘‘enhanced’’ synchrony, i.e., its maximum synchrony (0.89) was high relative to that of most auditory nerve fibers at that frequency. Also, maximum

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**FIG. 6.** Right: camera lucida reconstruction of a low \( (635 \text{ Hz}) \) characteristic frequency MNTB cell, located in the right MNTB, sectioned in the coronal plane. Cell body was lightly stained and only the main dendrite and 2nd-order branches could be seen so the dendritic tree is not illustrated. Axon sent collaterals into the low-frequency, lateral limb of the LSO as well as into the dorsal, low-frequency region of the medial superior olive (MSO) at the 2 rostrocaudal locations illustrated. Branches were also seen in the VMPO. A number of collateral branches appeared to fade before termination, so this cell may have innervated other areas as well. Two profiles of the MSO represented are from 2 sections that contained collateral branches in this nucleus at 2 rostrocaudal locations. LSO profile represents the outline of the LSO in the section that contained the bulk of the collateral branches in the lateral limb of the LSO. Thus only these parts of the axon collateral branches are placed accurately in the drawing relative to the illustrated locations of the LSO and MSO. Scale bar = 500 \( \mu \text{m} \). Inset, bottom left: short tone response of this cell at its characteristic frequency (see also Fig. 10).
synchronization for the one MNTB cell in our sample with a CF between 1 and 3 kHz was at the lower edge of the range observed in the auditory nerve. These observations are consistent with synchronization in GBCs, which also show enhanced synchronization at CFs < 1 kHz and poor synchronization at intermediate CFs (1 kHz < CF < 3 kHz). GBCs with CF > 3 kHz show enhanced synchronization to tones in the low-frequency tail of the tuning curve (Joris et al. 1994b), but we did not test this in MNTB cells. As seen in Fig. 10, the onset component of the MNTB cell responses was well timed, although as a population not as well timed as globular bushy cells (Fig. 11C). Finally, GBC and MNTB cells show a slight improvement in envelope phase-locking compared with the auditory nerve, and overall the two populations are indistinguishable in their envelope phase-locking properties (Joris and Yin, unpublished data).

As described in RESULTS, the percentage of MNTB cells we recorded from, with CFs < 3 kHz, was significantly lower (4%) than the percentage of globular bushy cells (34%) we previously recorded from with similar CFs. We feel that, even though the frequency map of Guinan et al. (1972b) indicates that low frequencies are less well represented than higher frequencies in MNTB, 4% is probably not a true reflection of the actual number of MNTB cells with CFs < 3 kHz but is probably biased by our surgical approach and electrode placement. In these experiments, the electrode was inserted into the brain stem just lateral to the pyramidal tract. In some instances, the electrode entered the brain near the rootlets of the abducens nerve perpendicular to the surface of the brain. Using this approach the more laterally situated, lower frequency regions of the MNTB are not traversed. Even when we angled the electrode so that it diverged from the midline with increasing depth the lateral aspect of the MNTB was often still not reliably sampled. This would probably explain the disparity in mean spontaneous rates compared with the auditory nerve, and overall the two populations are indistinguishable in their envelope phase-locking properties (Joris and Yin, unpublished data).

FIG. 7. Camera lucida drawings of 2 MNTB cells in the horizontal plane illustrating the extent of the axon collateral fields. Cell body of the cell on the left (CB) was located in the left MNTB. CF of the cell was 27 kHz. Dendritic tree of this cell has not been drawn in this figure, to eliminate confusion of dendrites with axon collateral branches, but is illustrated in Fig. 3 (top left cell). Destination of the main axon branches are indicated (to LSO, to VNLL). Other collaterals are in MNTB and VMPO. Cell body of the cell on the right (CB) was located in the right MNTB. CF of the cell was 13 kHz. Destination of main collaterals are indicated (to LSO, to VNLL). Collateral branches between the cell body and the to-LSO label are primarily in the MSO. Enlarged version of the cell body and dendritic tree of this cell are illustrated in Fig. 3 (bottom left). Scale bar = 1 mm.
Globular bushy cell calyceal and other inputs

In our previous report (Smith et al. 1991), we noted that the labeled calyx of a globular bushy cell axon covered ~24% of its postsynaptic MNTB cell body surface, while the total (labeled and unlabeled) round vesicle coverage of the cell body was 26.4%. In this case, the great majority of the excitatory input was the labeled calyx, providing evidence that this cell received only one calyx. In the present study, the total round vesicle terminal coverage for three of our labeled MNTB cell bodies was 39.6, 43.8, and 48%. What does this say about the excitatory synaptic input to the MNTB cell body? 1) In the four cases, we have examined in detail the extent of somatic coverage by excitatory terminals has varied considerably, ranging from slightly more than one-fourth to slightly less than one-half. Thus if each MNTB cell is receiving only one calyx, then there may be considerable variation in amount of surface of MNTB cells covered by one globular bushy calyceal terminal. 2) Alternatively, some MNTB cells receive two calyces or 3) some MNTB cells receive one calyx and a lot of other noncalyceal round vesicle inputs. These could potentially arise from the small “precalycean” terminals of other globular bushy cell axons that make smaller terminal endings in the MNTB (Morest 1968; Smith et al. 1991). The axons of other projection neurons of the cochlear nucleus, with round vesicle containing terminals, do not venture into the MNTB so they are unlikely sources for additional excitatory inputs. These include the spherical bushy cells (Smith et al. 1993b), the stellate cells the axons of which exit the cochlear nucleus via the trapezoid body and that respond in a chopper fashion to short tones (Smith et al. 1993a), the octopus cells of the posteroverentral cochlear nucleus (PVCN) and the giant and fusiform cells of the dorsal cochlear nucleus (DCN) (Joris et al. 1992). Little information is available on the other potential sources of excitatory inputs to MNTB in the brain stem. Labeled MSO principal cells in the guinea pig (Smith 1995) never gave off collaterals to MNTB, and there is only one report of an occasional projection to MNTB from labeled rat LSO principal cell the axon of which crossed the midline (Banks and Smith 1992).

We also measured the nonround vesicle coverage of the same four cell bodies. The nonround vesicle coverage of the cell receiving the labeled calyx reported previously (Smith et al. 1991) was 21.6%, 21.3, 22.8, and 20.4% for the three cells in the present study. Thus it would appear that the presumably inhibitory “nonround vesicle” input to the soma is quite heavy and, at least in terms of surface coverage, is constant from cell to cell. Anatomically, it has been shown in cat and guinea pig that glycine immunoreactive terminals are rather sparse in the MNTB (Adams and Magnaini 1990; Helfert et al. 1989; Wenthold et al. 1987) as is glycine receptor labeling (Zarbin et al. 1981) while γ-aminobutyric acid (GABA) immunoreactive terminals are quite numerous (Adams and Magnaini 1990). This agrees with our observations here and similar observations in the rat (Banks and
Smith 1992) that the MNTB cells, which are the major source of glycinergic innervation to the auditory brain stem, only give off occasional sparsely branching collaterals within the home nucleus. Little evidence is available as to the source of any of the GABAergic terminals nor is there evidence as to whether they are activated by auditory stimuli. Kuwabura et al. (1991) described axon collaterals of cells in the rodent ventral nucleus of the trapezoid body that could branch in MNTB, but it is not known what transmitter these cells manufacture. In vitro physiology in the mouse has shown that bath application of either GABA or glycine decreases the input resistance in only about half of the mouse MNTB cells tested (Wu and Kelly 1995). Further in vitro data indicated that shock stimulation of the trapezoid body at the midline or ipsilateral to the MNTB in the rat (Banks and Smith 1992) but not the mouse (Wu and Kelly 1991) would occasionally evoke inhibitory postsynaptic potentials (IPSPs) that could be blocked by the glycine antagonist, strychnine. Our in vivo data on the response of MNTB cells to binaural stimulation as the sound to the contralateral ear was held constant while the sound to the ipsilateral ear was increased (Fig. 2) showed no inhibitory affects on the contralaterally driven output. In other in vivo experiments, Guinan and Li (1990) also could not demonstrate any sort of inhibitory affect on the calyceal driven MNTB cell output either by auditory stimulation to either ear or by shock stimulation of the trapezoid body. Some of our MNTB short tone responses show an apparent sag in the sustained response (Fig. 10), but it would be premature to propose an inhibitory input as the cause. Thus inhibitory inputs on MNTB cells appear to be present and functional but there is no evidence of activation by simple auditory stimuli.

**Calyceal and postsynaptic physiology**

In addition to the anatomic specializations, some physiological specializations also apparently have developed both pre- and postsynaptically to aid in the secure and rapid transfer of accurately timed information. On the basis of whole cell patch recordings from calyceal terminals, the calyx possesses a large, high-voltage-activated calcium conductance with fast activation and deactivation kinetics (Borst et al. 1995) that would be required for rapid synaptic transmission. In addition, preliminary reports (Forsythe 1994) showed...
that, in current clamp, the presynaptic terminal can spike at high rates in response to depolarizing current pulses because of a fast delayed rectifier potassium current that allows rapid spike repolarization. Such a rapid response capability also might be requisite for rapid synaptic transmission. The synaptic current generated postsynaptically by the calyx is fast and very large, averaging ~5 nA (Borst and Sakmann 1996), but this current amplitude diminished significantly during repetitive stimulation such that the calyceal synapse could not make the MNTB cell fire reliably to a 100-Hz shock stimulation of the bushy cell input. It would appear, however, that such poor postsynaptic following is probably a function of the immature preparation used (8- to 10-day-old rats) because shock rates of the bushy cell input generating MNTB cell spikes were reliably followed to well over 500 Hz in a mature mouse slice preparation (Wu and Kelly 1993). Postsynaptically the MNTB cell has specialized potassium conductances (Banks and Smith 1992). One has been designated $I_{TBA}$, which might allow for rapid spike repolarization, the other $I_{DTX}$, which might permit only a single spike to occur in response to the huge calyceal input (Brew and Forsythe 1995).

**MNTB cell output**

Our anatomic data on the projections of MNTB principal cells correspond well with Spangler et al. (1985). With one exception, all axons that could be followed to LSO innervated that structure in a fashion that was consistent with the proposed frequency map of the LSO (Guinan et al. 1972b). Our one example of a low-frequency MNTB cell sending a projection to the low frequency limb supports the contention (Finlayson and Caspary 1991; Joris and Yin 1995) that some low-frequency LSO cells are inhibited by contralateral stimulation.

The terminals we examined at the EM level had nonround vesicles and terminated on large dendritic profiles or the cell bodies of principal cells. Cant (1984) reported that almost three-fourths of the surface of the LSO principal cell body and proximal dendrites are covered with synaptic terminals that are almost exclusively those containing small vesicles many of which are flattened or cylindrical. Our labeled synaptic terminals from identified MNTB principal cells correspond to the description both in location and vesicle content (Cant 1984) and provide the first direct evidence that these terminals are derived from axons of MNTB cells. In the rat, Moore and Caspary (1983) showed that the contralaterally evoked inhibition of ipsilaterally evoked excitation could be blocked by application of the glycine antagonist strychnine. Similar results were obtained in the slice preparation, by Wu and Kelly (1991), with IPSPs evoked by electrical activation of MNTB. Thus our anatomic data, showing that the axon terminals of MNTB cells to the LSO have nonround synaptic
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IPSPs, evoked by MNTB shock, in MSO principal cells were blocked by strychnine (Grothe and Sanes 1993, 1994; Smith 1995), confirming the glycinergic nature of the input. The function of such an actively driven inhibitory input to the MSO from MNTB remains speculative. Several possibilities include a MNTB input the CF of which is slightly off the CF of the innervated MSO cell could serve to suppress coincident excitatory inputs at non-CF frequencies; mediation of precedence-like effects; and changing the MSO cells resting membrane potential during contralateral ear stimulation.

All MNTB neurons with darkly labeled axons had a collateral that could be followed rostrally to VNLL. Unfortunately nothing is known about the distribution of synaptic terminals on cells in the VNLL or their ultrastructure nor is there any information on why VNLL cells might require a contralaterally driven inhibitory input when much of the excitatory input to this nucleus is also from the contralateral side. Collaterals also were sent to the two nuclei medial to the MSO, the DMPO and VMPO. In the cat, GBCs often send an excitatory collateral to synapse on cells in the DMPO (Smith et al. 1991), so, as with the VNLL, we again note a region receiving both a contralateral excitatory input from cochlear nucleus and an inhibitory input from MNTB, a nucleus that is driven by contralateral excitatory input. The rodent superior paraovaliy nucleus (SPN) is a large periolivary nucleus dorsomedial to the MSO that is considered the homolog of the cat DMPO. It receives input from both cochlear nuclei (Thompson and Thompson 1991) as well as a strong topographic input from MNTB principal cells (Banks and Smith 1992), and its cells project primarily either to the inferior colliculus or cochlear nucleus (Schofield 1991). Although some extracellular recordings were done in vivo on cells in the gerbil SPN and cat DMPO (Guinan et al. 1972a; Spitzer and Semple 1995), the physiology of a large population of cells in this nucleus has never been explored in great detail and consequently the possible effects of the MNTB input, on auditory response properties of cells here, are not known.

We also occasionally noted a collateral being given off within the MNTB and, as noted above, inhibitory synaptic events can be elicited in rat MNTB cells by shocking their axons, provide an important piece of data linking the presumed inhibitory function of MNTB onto ipsilateral LSO to the anatomic data of Cant (1984) showing flattened vesicles on the soma and proximal dendrites of LSO cells.

For axons that could be followed through MSO the ones with the three lowest CFs sparsely innervated the nucleus in appropriate frequency regions, whereas the three with the higher best frequencies did not. Clark (1969) and Schwartz (1980) have reported the presence of terminals with flattened vesicles on principal cells in cat MSO. The innervation of MSO by MNTB cells also has been noted anatomically in rat, bat, mouse, gerbil, and guinea pig (Banks and Smith 1992; Kuwabura and Zook 1991, 1992; Smith 1995), and

![Graphs and data](image-url)

**FIG. 11.** Comparison of the spontaneous activity (top), 1st spike latency (middle), and 1st spike standard deviation (bottom) between labeled globular bushy cells, that we have previously reported (Smith et al. 1991) and MNTB cells. Labeled MNTB cells (MNTB) were recorded from intraxonally. Unlabeled MNTB cells recordings using metal electrodes (PP) were identified based on the presence of a prepotential (see Fig. 1) and subsequent location of an electrode generated lesion site within the confines of the MNTB. Globular bushy cells axons were either recorded on the side ipsilateral or contralateral to their parent cell body.

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