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Dendrites of Medial Olivocochlear (MOC) Neurons in Mouse

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Abstract

Stains for acetylcholinesterase (AChE) and retrograde labeling with Fluorogold (FG) were used to study olivocochlear neurons and their dendritic patterns in mice. The two methods gave similar results for location and number of somata. The total number of medial olivocochlear (MOC) neurons in the ventral nucleus of the trapezoid body (VNTB) is about 170 per side. An additional dozen large olivocochlear neurons are located in the dorsal periolivary nucleus (DPO). Dendrites of all of these neurons are long and extend in all directions from the cell bodies, a pattern that contrasts with the sharp frequency tuning of their responses. For VNTB neurons, there were greater numbers of dendrites directed medially than laterally and those directed medially were longer (on average, 25–50% longer). Dendrite extensions were most pronounced for neurons located in the rostral portion of the VNTB. When each dendrite from a single neuron was represented as a vector, and all the vectors summed, the result was also skewed toward the medial direction. DPO neurons, however, had more symmetric dendrites that projected into more dorsal parts of the trapezoid body, suggesting that this small group of olivocochlear neurons has very different physiological properties. Dendrites of both types of neurons were somewhat elongated rostrally, about 20% longer than those directed caudally. These results can be interpreted as extensions of dendrites of olivocochlear neurons toward their synaptic inputs: medially to meet crossing fibers from the cochlear nucleus that are part of the MOC reflex pathway, and rostrally to meet descending inputs from higher centers.

Keywords

superior olive; cochlear nucleus; efferent; descending pathway; auditory reflex

Dendrites represent an important site for neurons to receive synaptic input. As such, dendrite orientation and pattern can shape a neuron's response properties. For example, octopus cells of the posteroventral cochlear nucleus have long dendrites oriented perpendicular to the incoming auditory nerve fibers (Osen, 1969; Brawer et al., 1974). This orientation allows them to receive input from auditory-nerve fibers that are tuned to a range of frequencies. Octopus cells, which have an onset pattern of discharge in response to a tone burst (Rouiller and Ryugo, 1983; Rhode et al., 1983), are often tuned more broadly than their inputs (Godfrey et al.,

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1975; Palmer et al., 1996). Thus, in the case of octopus cells, dendritic orientation and length form the substrate for a broadened frequency response area, a key response property of these neurons.

Dendritic patterns probably shape the response properties of olivocochlear (OC) neurons. OC neurons form a descending pathway projecting from the brainstem's superior olivary complex to the cochlea. There are two main groups of OC neurons. Medial (M) OC neurons in rodents are located in the medial and ventral portions of the superior olivary complex in subnuclei such as the ventral nucleus of the trapezoid body (VNTB, a nucleus also known as the mediolateral/rostral periolivary nuclei) (White and Warr, 1983; Campbell and Henson, 1988). Lateral olivocochlear (L) OC neurons reside in or near the lateral superior olive. MOC neurons respond to sound and their responses are sharply tuned to sound frequency (Robertson and Gummer, 1985; Liberman and Brown, 1986; Brown, 1989). The sharp tuning suggests that they receive a restricted band of sharply tuned inputs. Much input to MOC neurons is onto their dendrites (Helfert et al., 1988; Thompson and Thompson, 1991a; Benson and Brown, 2006). Although no systematic studies of MOC dendrites have been published, they are reported to be long and sparsely branched, and to radiate in several directions from the soma (Adams, 1983; White and Warr, 1983; Osen et al. 1984; Vetter & Mugnaini, 1991; Brown, 1993; Warr et al., 2002; Sánchez-González et al., 2003).

MOC neurons respond to sound as part of the three-neuron MOC reflex (Brown et al., 2003). The auditory nerve provides the input to the reflex. The second neuron of the reflex lies within the cochlear nucleus, and it projects directly to the efferent neurons, the MOC neurons (Thompson and Thompson, 1991; Ye et al., 2000). MOC neurons are divided into two major groups defined by the ear that excites them (Robertson and Gummer, 1985; Liberman and Brown, 1986). Ipsi neurons respond in the ipsilateral ear (the ear to which the neuron projects) and have cell bodies located on the *opposite* side of the brainstem. To reach the Ipsi neurons, cochlear nucleus projections cross the midline and approach the neurons from their medial side. MOC Contra neurons respond to sound in the contralateral ear (the ear contralateral to the one that receives the neuron's projections) and have cell bodies located on the *same* side of the brainstem. To reach the Contra neurons, cochlear nucleus projections from the opposite side cross the midline and approach these neurons from their medial side as well. Thus, both groups of MOC neurons receive crossing inputs coming from the medial direction. An additional small group of MOC neurons (5–10% of all neurons), Either-Ear neurons, responds almost symmetrically to sound in either ear. The brainstem anatomy of these neurons has not been worked out, but they must receive both crossed and uncrossed inputs.

Additional input to MOC neurons comes from higher centers such as the inferior colliculus (Faye-Lund, 1986; Thompson and Thompson, 1993; Vetter et al., 1993; Mulders and Robertson, 2002; Ota et al., 2004) and auditory cortex (Mulders and Robertson, 2000b). Much of this input, in some cases apparently all of it (Mulders and Robertson, 2000b), ends on the MOC dendrites. These descending inputs may modulate the MOC reflex or even suppress it during visual tasks (Delano et al., 2007). At least some of these inputs approach to MOC neurons from their rostral side (Faye-Lund, 1986). Thus, MOC dendrites directed toward the medial and rostral directions are poised to receive important inputs.

We used stains for acetylcholinesterase (AChE) and retrograde labeling with Fluorogold (FG) to identify OC neurons in the mouse. The AChE stain method stains OC somata, dendrites, and axons (Churchill and Schuknecht, 1959; Warr, 1975; White & Warr, 1983; Osen et al., 1984; Thompson and Thompson, 1986; Vetter & Mugnaini, 1992). The mouse was chosen because of the availability of genetically modified animals and other interesting strains for hearing research (Ollo and Schwartz, 1979; Vetter et al., 1999; Liberman et al., 2002; Brown and Vetter, 2006). Interest in this species also arises because its short life span renders it

attractive for studies of the effects of aging (e.g., Zettel et al., 2007). Yet there has been only one previous study of the central distribution of OC neurons (Campbell and Henson, 1988) and the strain of mouse used was not identified. Work on the cochlear terminations of OC neurons in CBA/CaJ mice shows a typical mammalian plan with some exceptions (Maison et al., 2003). We now present new findings on the OC dendritic patterns that have implications for the responses of these neurons.

EXPERIMENTAL PROCEDURES

Animals

A total of twenty-five mice of CBA/CaJ strain were used (13 for AChE stains, 9 for FG labeling, and 3 for horseradish peroxidase (HRP) labeling). The mice were 2 – 4 months old. All experimental procedures on animals were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, and were performed under approved protocols at the Massachusetts Eye & Ear Infirmary.

AChE Stains

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) and sacrificed by intracardiac perfusion with physiological saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.2. After post-fixing for approximately 1 h, brainstems were dissected and immersed in the same fixative for another 1 h, then immersed in 30% sucrose overnight. Transverse or sagittal sections were cut at 80 μ m on a freezing microtome. Stains for AChE were by the Koelle indirect method (Koelle and Friedenwald, 1949) and modified similar to Osen et al. (1984). First, sections were incubated for 30 min in acetylthiocholine medium (0.072 g ethopropazine, 1.156 g acetylthiocholine iodide, 0.750 g glycine, 0.5 g copper sulfate, and 6.8 g sodium acetate in 1000 ml distilled water, titrated to pH 5.0), rinsed in distilled water, incubated for 1 min in 4% sodium sulfide solution (pH 7.8), rinsed, incubated for 30 s in 1% silver nitrate, and rinsed again. The sections were dried, then dehydrated and counterstained with neutral red.

Retrograde Labeling

Mice were anesthetized as above. Access to the cochlea on the left side was achieved by removing the tympanic membrane. A small hole was drilled through the lateral wall at about the midpoint of the cochlea and a pipette was used to inject 1–5 μ l of 30% HRP in Tris buffer (pH 7.3) or 4% FG (Fluorochrome, Denver, CO, USA) in saline. Previous attempts at infusing tracer through the opened round window while applying suction at the oval window failed to produce consistent labeling. Mice recovered for 1 day (HRP) or 8 – 11 days (FG) after injection and then were re-anesthetized and sacrificed by intracardiac perfusion with physiological saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.2. The fixation and sucrose procedures were as described above. Transverse sections were cut at 80 μ m on a freezing microtome. For HRP visualization, sections were processed with tetramethylbenzidine (Mesulam, 1982). For FG visualization, sections were examined with a fluorescence microscope, or processed using standard immunohistochemistry with an anti-FG antibody (Fluorochrome, Denver, CO, USA) at a dilution of (1/100,000).

MOC Angle Measurements

Stained or labeled neurons were drawn using a light microscope with 10x and 60x objectives. The sample of reconstructable dendrites is composed mainly of dendrites not intertwined with other labeled elements or separated from other labeled elements by being at the margins of the labeled areas. The drawings were scanned into a computer and Image J used to compute the MOC neuron's dendrite lengths and angles. Dendrites that split into two processes within 10

μm of the soma were considered two separate dendrites. Dendrite angle (to tip) was measured by drawing a line from soma to dendrite tip (Fig. 6A, inset), using the angle of this line with respect to the dorsal direction. For transverse sections, the dorsal/ventral direction was defined as parallel to the midline of the section. For sagittal sections, the rostral/caudal direction was defined by a line connecting two points on the ventral surface of the brainstem at the limits of the MOC neuron rostro-caudal distribution. Dendrite length (Fig. 6, Fig. 7) was taken as the length of the line from soma to tip. Total summed length (Fig. 8) was the path length of all dendrite branches traced from the drawings. Initial angle (Fig. 11) was measured by drawing a line down the dendrite's shaft beginning just distal to the tapering of the soma and extending for 10 μm . Dendrite diameter was measured over this same region. T-tests were used as tests of statistical significance unless stated otherwise.

RESULTS

Distribution and Number of OC Neurons

Within the superior olivary complex of AChE-stained material, some neurons were stained a dense black color, giving them a Golgi-like appearance (Fig. 1). These neurons were stained the darkest of all neurons in the brainstem and were particularly impressive because of little background staining within the SOC. Two major groups of AChE-stained neurons were separated according to location. The first group was composed of large neurons located in the ventral nucleus of the trapezoid body (VNTB), which we presume to be Medial (M) OC neurons as described previously in mouse (Osen et al., 1984; Campbell and Henson, 1988). The second group was composed of smaller neurons presumed to be Lateral (L) OC neurons located within LSO (LOC intrinsic neurons). There were also two minor groups of OC neurons: LOC shell neurons, closely associated with the LSO on its margins and within its dorsal hilus, and DPO neurons, large, darkly stained neurons located in the dorsal periolivary nucleus (DPO) just dorso-medial to the LSO. The DPO neurons seemed to be distinct from shell neurons because their distribution extended medially as far as the dorsomedial periolivary zone (according to the terminology of Osen et al., 1984), which is close to the medial nucleus of the trapezoid body (Fig 2). Also, they were large, multipolar neurons (rather than fusiform shaped as most shell neurons), their dendrites could be traced considerable distances but were not associated with the LSO or its margins, and retrograde labeling (see below) indicated that the majority were on the side of the brainstem opposite the cochlea innervated (unlike the mainly ipsilateral shell neurons seen by Vetter and Mugnaini, 1992). However, DPO neurons did extend laterally up to the LSO hilus and this lateral portion of their distribution is very close to the LSO, merging with shell neurons here. Our data suggest that DPO neurons belong in the MOC group, but because of some uncertainty, analysis of DPO neurons is kept separate from the main group of MOC neurons in the VNTB.

The pattern of AChE staining described above is very similar to the pattern of OC neurons retrogradely labeled by cochlear injections of FG. In Figure 3, the left panels are an atlas of transverse sections showing retrograde labeling following an injection of FG into the left cochlea. The right panels are an atlas from a different animal showing AChE-stained neurons, which presumably project to either the left or right cochleas. Both techniques show that MOC neurons are located mainly in the VNTB throughout its entire rostro-caudal extent, from the caudal-most point near the facial motor nucleus to the rostral most point near the beginning of the ventral nucleus of the lateral lemniscus (Campbell and Henson, 1988). FG labeling indicates that the neurons projecting to the left cochlea are bilaterally distributed in both the VNTB and DPO, but that there are more of them on the right side of the brain, the side opposite to the injected cochlea. The DPO neurons are distributed in a rostro-caudal course parallel to the LSO and just rostral to it, although not as far rostral as the VNTB neurons. LOC intrinsic neurons

are distributed in a shorter rostro-caudal extent, defined by the limits of the LSO, and appear only on the same side of the brain as the injected cochlea.

Counts of OC neurons in retrograde labeling and AChE staining are also similar. For VNTB neurons (Fig. 4A), the average totals per cochlear FG injection were 166.7, for AChE stains were 171.4, and for HRP were 148. In FG injections, about two-thirds (69.3%) of these neurons were on the side of the brainstem opposite to the injected cochlea and HRP data (63.3%) are similar. For DPO neurons (Fig. 4B), the average totals were 12.3 (FG) and 13.3 (AChE) but HRP labeling was found in only two neurons in the three mice. Similar to VNTB labeling, the DPO neurons labeled by FG were mainly (75.0%) on the opposite side. In contrast, LOC intrinsic neurons were entirely on the same side of the brain; their average totals varied greatly among our techniques (avg. 112.1 neurons in 9 FG injections, avg. 129.3 neurons in 3 HRP injections, or avg. 214.9 neurons in 9 AChE-stained brainstem halves). The FG counts had a wide range (12 – 192 neurons per injection), perhaps indicating that the low numbers reflect labeling of only a portion of the population. LOC shell neurons were uncommon (averaging 10 neurons per injection (6 FG injections), of which 80% were ipsilateral and 20% were contralateral) and averaging 13.1 neurons/brainstem side in AChE stains. They were found mainly in the dorsal hilus of the LSO, on its lateral edge and just rostral to it. Their dendrites often took a course along the margin of the LSO, perpendicular to dendrites of intrinsic LOC neurons. If at least one of neuron's dendrites was associated with the LSO or its capsule it was considered to be a shell neuron (Sanchez-Gonzalez et al., 2003).

Somata of FG-labeled VNTB neurons were large, averaging $161.2 \mu\text{m}^2$ in area (SEM $5.1 \mu\text{m}^2$, $n=79$). In AChE stains, though, VNTB neurons were about 18% smaller, averaging $132.7 \mu\text{m}^2$ (SEM $2.2 \mu\text{m}^2$, $n=237$). Perhaps this difference arises because FG labeling formed a smooth filling of the soma whereas the AChE stain was more crystalline in nature and soma edges were less well defined. There was no obvious dependence of area on cell position as a function of rostro-caudal position in the VNTB (data not shown). Somata of FG-labeled neurons in DPO were of similar size to VNTB neurons, averaging $151.7 \mu\text{m}^2$ (SEM $7.9 \mu\text{m}^2$, $n=11$) and here also AChE-stains produced smaller soma areas, averaging $124.5 \mu\text{m}^2$ (SEM $3.9 \mu\text{m}^2$, $n=52$). LOC intrinsic neurons were much smaller, averaging $86.3 \mu\text{m}^2$ (SEM $2.5 \mu\text{m}^2$, $n=91$ FG-labeled LOC neurons). LOC shell neurons, however, were comparable in size to MOC neurons, averaging $160.0 \mu\text{m}^2$ (SEM $21.8 \mu\text{m}^2$, $n=5$) for FG labeling neurons and averaging $147.9 \mu\text{m}^2$ (SEM $7.5 \mu\text{m}^2$, $n=32$) for AChE staining.

Axons of both VNTB and DPO neurons were stained for AChE (Fig. 1) and could be seen emanating from either the soma or a proximal dendrite of individual neurons. The axons of VNTB and DPO neurons were similar in diameter. The axons ran dorsally from the superior olivary complex towards the facial genu near the midline. At this point, some stained fibers crossed the midline whereas others joined crossing stained fibers to form the olivocochlear bundle that ran laterally in the vestibular nerve root and gave off branches to the cochlear nucleus before exiting the brain (Fig. 2, right panels).

Non-OC neurons were sometimes labeled by these techniques: FG labeling of somata was sometimes seen in the parvocellular region ventral and anterior to the motor nucleus of the fifth cranial nerve (Azeredo et al., 1999); these labeled neurons were likely to be tensor tympani motoneurons (Thompson et al., 1998) and were not further considered. There was light AChE staining of other nearby neurons (presumed facial and stapedius motoneurons, vestibular efferent neurons) known to be cholinergic (Sherriff and Henderson 1994).

Dendrites of OC Neurons

General Course—Dendrites radiated from the stained VNTB and DPO neurons in most directions. The relatively unbranched dendrites ran in neuropil (Fig. 1, Fig. 2, Fig. 5) that

presumably contains fibers of the trapezoid body, always avoiding two nearby nuclei (LSO, medial nucleus of the trapezoid body, MNTB), and almost always avoiding another nearby nucleus (SPN, superior paraolivary nucleus). Dendrites of VNTB neurons were particularly apparent coursing medially from the somata, where they were mainly directed toward the more ventral parts of the trapezoid body, ventral to the MNTB. Some dendrites of these neurons were directed more dorso-medially between the MNTB and the SPN (Fig. 2B, upper arrow). For more rostrally located neurons (e.g., Fig. 2C, Fig. 5), dendrites were confined to the VNTB and the adjacent trapezoid body, which hugs the medial edge of the VNTB for a large rostro-caudal extent. These rostral dendrites do not invade the pontine reticular nucleus or the longitudinal fasciculus. In contrast to VNTB dendrites, dendrites of DPO neurons extended medially into the most dorsal part of the TB, usually dorsal to the MNTB. This is a more dorsal orientation than most VNTB dendrites. At more rostral positions where DPO neurons no longer occur, though, VNTB dendrites can shift dorsally (Fig. 5C). Dendrites often had swellings at their tips (e.g., Fig. 9, top), although not all dendrites had swellings. Swellings were seen more often in AChE material than in FG material where the reaction product sometimes became very light toward the ends of the dendrites. In contrast, HRP labeling did not appear to fill MOC dendrites to their tips.

Measurements of individual MOC dendrites—Although most dendrites became intertwined with other stained dendrites or exited the plane of section, some could be traced in their entirety from soma to tips. We consider here all dendrites that could be reconstructed in four cases stained for AChE (two sectioned transversely and two sectioned sagittally) as well as 9 cases of FG labeling (all sectioned transversely). AChE-stained dendrites of VNTB neurons ranged in length from 40 to 330 μm (avg. length 153.3 μm for 118 dendrites) and dendrites of DPO neurons ranged from 53 to 252 μm (avg. length 132.0 μm for 18 dendrites). Dendrites labeled with FG were shorter in VNTB (range 17 to 239 μm , avg. 114.7 μm for 120 dendrites) and in DPO (range 15 to 238 μm , avg. 114.1 μm for 32 dendrites). These FG labeled dendrites were sometimes fainter toward the tips so fading of the reaction product could not be ruled out. For neurons from material sectioned in the transverse plane, the lengths of the reconstructed dendrites are plotted in polar format in Figure 6 against the angle formed by a line connecting the soma to the tip of the dendrite (Fig. 6A, inset). The dorsal direction was defined as an angle of 0°. Similar data are plotted for neurons from material sectioned in the sagittal plane (Fig. 7).

As can be seen from the polar plots (Fig. 6, Fig. 7), MOC dendrite length depends on dendrite orientation. In transverse sections, dendrites oriented toward the medial direction are somewhat longer than those toward the lateral direction (Fig. 6). Both FG-labeled and AChE-stained dendrites showed this effect. For AChE stains of VNTB, the average length of the dendrites oriented toward the medial half (angles between 0° and 180°) was 178.4 μm (SEM 8.1 μm , $n=71$), whereas the average length of the dendrites oriented laterally was 115.3 μm (SEM 6.6 μm , $n=47$). Overall, the medial-half dendrites were thus on average about 50% longer than lateral-half dendrites. For FG labeling in VNTB, the lengths of those dendrites oriented toward the medial half averaged 124.4 μm (SEM 4.7 μm , $n=75$), whereas those dendrites oriented laterally averaged 98.4 μm (SEM 7.0 μm , $n=45$), a difference of about 25%. Neurons in DPO had dendrites that were more symmetric (Fig. 6E). In AChE stains, DPO medially directed dendrites averaged 154.7 μm in length whereas laterally directed dendrites averaged 120.5 μm . In FG labeling, medially directed, FG labeled dendrites averaged 125.5 μm in length whereas laterally directed dendrites averaged 106.0 μm .

Dendrite lengths were the most asymmetric for neurons in the more rostral part of the VNTB. To illustrate this finding, VNTB neurons were separated into caudal, middle, or rostral groups, according to their position relative to the LSO. These groups corresponded approximately to the caudal 20%, middle 40%, and rostral 40% of the VNTB based on the number of sections

containing each group. A pronounced medial extension of dendrites was seen in rostral MOC neurons (Fig. 6D), where the 44 dendrites pointing medially averaged 188.2 μm in length but the 15 dendrites pointing laterally averaged only 129.0 μm . There was also some tendency for neurons caudal to the LSO to have longer dendrites medially: 11 dendrites pointing medially averaged 165.75 μm whereas the 14 dendrites pointing laterally averaged 123.3 μm . The middle subgroup of MOC neurons, which lies ventral to the LSO, deviates from the pattern of other neurons because this subgroup tended to have longer dendrites projecting medially and laterally and shorter dendrites dorsally and ventrally (Fig. 6C), giving some of them a “flattened” appearance (Fig. 2B). These neurons (Fig. 1) are constrained ventrally by the ventral surface of the brainstem and dorsally by overlying nuclei (LSO and SPN), which are almost never invaded by the dendrites.

Our data also indicate that there were more dendrites pointing medially than laterally. Combining AChE and FG data for VNTB neurons, there were 146 dendrites oriented toward the medial half versus 92 dendrites oriented toward the lateral half. The Rayleigh test for randomness of a circular distribution (Batschelet, 1981) was significant for these data ($r = 0.16$, $Z_{\text{observed}} = 6.42$, $Z_{\text{critical}} = 2.99$ for $\alpha = 0.05$), indicating that they are a skewed, non-random distribution. In contrast, there were equal numbers of DPO neuron dendrites pointing medially vs. laterally (25 each). Here, the Rayleigh test is not significant ($r = 0.04$, $Z_{\text{observed}} = 0.09$, $Z_{\text{critical}} = 2.98$ for $\alpha = 0.05$), indicating a uniform distribution.

In sagittal sections, MOC dendrites also have length-dependent orientation, being longer in the rostral direction than in the caudal direction (Fig 7) but not more numerous. Only AChE material is available in this plane of section. For those VNTB dendrites directed toward the rostral direction (angles between 180° and 360°), the average length was 135.4 μm (SEM 7.2 μm , $n=31$), about 25% longer than those dendrites pointing caudally (average length 110.0 μm , SEM 9.1 μm , $n=31$). This effect was most exaggerated for neurons located rostrally (Fig. 7D). One effect of the rostral extension is seen in transverse sections, in which dendrites of MOC neurons are routinely found in several sections rostral to the rostral-most neurons. In contrast, DPO dendrites showed a more symmetric pattern, being almost the same average length rostrally (avg. 138.4 μm) as caudally (avg. 131.7 μm) although there was a greater range of lengths rostrally.

Another orientation dependence of VNTB dendrites is the amount of branching. Reconstructed dendrites were counted as a single segment if unbranched, 3 segments if the primary dendrite split into two secondary dendrites, etc. Branchlets were not counted if they were less than 20 μm in length. The relatively unbranched dendrites made “dendrograms” (Sholl, 1956) fairly uninteresting. Combining FG and AChE data, dendrites averaged 3.2 segments (range 1 – 10) for VNTB neurons and averaged 2.9 (range 1 – 7) for DPO neurons, a difference that was not significant. For VNTB neurons, those dendrites directed toward the medial half were significantly more branched (avg. of 3.7 segments compared to those directed toward the lateral half (avg. 2.5 segments). This greater number of branches was one factor in the greater total length when all path lengths of every dendrite branch was summed (Fig. 8). In contrast, DPO dendrite branching was not different in the medial direction (avg. 2.9) vs. the lateral direction (avg. 2.9).

So far, we have been considering dendrite angle to the tip of the reconstructed dendrite, which was plotted in Figure 6–Figure 8. This angle to the tip was predicted by the initial angle (Fig. 11) taken by the dendrite as it came off the cell body, since most dendrites did not change direction much from their initial part near the cell body to the tip. Both VNTB and DPO neurons showed this relationship separately (data not shown). These initial angle data allowed us to use an expanded sample to determine that there were only small differences in the numbers of dendrites projecting medially ($n=218$) vs. laterally ($n=181$), or rostrally ($n=73$) vs. caudally

($n=82$). In contrast, there was little predictive value of another initial property of the dendrite: the initial diameter of the dendrite (avg. $2.46\ \mu\text{m}$ for 31 dendrites) did not predict length to tip or the total dendrite length, a different result from measurements of thalamocortical neuron dendrites (Ohara and Havton, 1994).

For FG-labeled MOC neurons, it was possible to compare those neurons located on the same and opposite sides of the brainstem relative to the injected cochlea, and important comparison because the major ear that drives the neuron's response to sound is different in these two groups (Liberman and Brown, 1986). MOC neurons on the two sides of the brainstem were similar in morphology. The similarities included the average number of dendrites formed per neuron, the average dendrite lengths, and the patterns of the data in polar plots of length vs. angle.

Reconstruction of all dendrites from single neurons—Our data indicate that dendrites are asymmetric not only at the population level but at the level of single neurons. In a few instances, all dendrites of single neurons could be reconstructed. One example of a reconstructed neuron in the transverse plane is shown in Figure 9 and another example in the sagittal plane is shown in Figure 10. All dendrites could be reconstructed for a total of 29 neurons in AChE stains and 17 neurons labeled with FG, all sectioned in the transverse plane. These neurons formed between 2 and 5 dendrites (avg. 3.52). For the reconstructed neurons a simple index of overall symmetry was to treat each dendrite as a vector as before (Fig. 6A, inset), and compute a vector summation of all vectors from a single neuron. The result was an overall dendrite orientation vector for each neuron (each plotted as a single point on Figure 12). Points close to the origin indicate neurons with dendrites symmetrically oriented around the soma whereas points farther away indicate dendrites skewed to one direction. There are examples of single neurons with vectors in all directions; however, the population is clearly skewed toward the medial direction (33 points in the medial half and only 7 points in the lateral half of the plot). The Rayleigh test (Batschelet, 1981) is significant ($r = 0.466$, $Z_{\text{observed}} = 8.67$, $Z_{\text{critical}} = 2.98$ for $\alpha = 0.05$), indicating a skewed, non-random distribution. DPO neurons had a more symmetric pattern (3 points in the medial half and 3 points in the lateral half of the plot) with the Rayleigh test statistic not significant ($r = 0.322$, $Z_{\text{observed}} = 0.6234$, $Z_{\text{critical}} = 2.865$ for $\alpha = 0.05$).

DISCUSSION

Number and Distribution of OC neurons in the mouse

Our study confirms that mouse has an OC system similar to that of most other mammals, having lateral and medial groups. Our results demonstrate that MOC neurons in mouse are mainly located in a single nucleus, a plan observed in many species. This group extends the full rostro/caudal extent of the VNTB and is most numerous rostrally (Campbell and Henson, 1988). Using either AChE stains or FG labeling, the per-cochlea number of MOC neurons in VNTB averages about 170, and, for DPO neurons, averages about a dozen. These are comparable to numbers to MOC neurons in hamster (103 neurons, Sánchez-González et al., 2003) and gerbil (173 neurons, Helfert and Schwartz, 1987) but smaller than other species such as the rat (583 neurons, Aschoff and Ostwald, 1987; reviewed by Sánchez-González et al. (2003). Compared to larger species such as the cat, which has around 550 MOC neurons (Warr et al., 2002), this is a “scaled down” number of MOC neurons. The number of auditory-nerve fibers in mouse (12,578; Ehret, 1979) is also scaled down from the number in cat (about 50,000; Nadol, 1988). Our study also demonstrates a similarity of AChE stains and retrograde labeling, at least for MOC and DPO neurons, in terms of distribution patterns and numbers of neurons. The AChE stain is thus a good marker for these neurons in mouse, a conclusion previously made for rats and mice by Osen et al. (1984) and for cat by (Warr, 1975). It is not clear why DPO neurons have not been labeled in HRP studies of rats or mice (White and Warr, 1983; Campbell and

Henson, 1988; present results); they are labeled by fast blue, diamidino yellow (Aschoff and Ostwald, 1987), or biocytin (Brown, 1993).

Our results suggest that DPO neurons belong to the MOC group. In favor of this view is their distribution that extends medially to the MNTB, much of which would be medial to a line through the medial superior olive, which was originally used to separate MOC from LOC neurons (Warr and Guinan, 1979). However, this group extends laterally almost to the LSO hilus, an area where shell neurons have been observed in other species. Secondly in favor of an MOC designation, DPO neurons occur on both sides of the brainstem, with the majority on the opposite side (Fig. 3B). This pattern is like other MOC neurons and unlike the mainly ipsilateral LOC neurons, in particular, the shell neurons that are mostly on the same side of the brain as the innervated cochlea (Vetter and Mugnaini, 1992; Azeredo et al. 1999; Sánchez-González et al., 2003). DPO neuron axons in our material were observed to be as thick in diameter as VNTB axons. The diameters of shell neurons are not known for sure; however, if they correspond to bidirectional fibers supplying the inner hair cell region (Warr and Boche, 2003), then their diameters should be less than MOC axons (Brown, 1987). This might further suggest DPO neurons are MOC neurons rather than LOC shell neurons. In other species, a group of large, presumably MOC neurons is found in this general location in gerbil (Helfert et al., 1988), guinea pig (Aschoff and Ostwald, 1987) and cat (Warr et al., 2002). The relative numbers of neurons in this group relative to the number in the VNTB varies greatly among with species. For example, the chinchilla lacks the VNTB group and most MOC neurons are found dorsally within a nucleus designated dorsomedial peri-olivary nucleus (Azeredo et al., 1999). Most other species, however, have the great majority of MOC neurons in the VNTB. In mouse, the group of neurons in the dorsal location spans between the medial limb of the LSO and the lateral portion of the MNTB, perhaps encompassing both DPO and/or dorsomedial peri-olivary nucleus if such a distinction exists in mouse.

Our data are consistent with the idea that AChE is also a good marker for LOC neurons. The large variation in number of retrogradely labeled LOC neurons does not allow us to make firm conclusions on the exact number or the correlations, though. Small difference might be expected if there are small numbers of LOC neurons that are dopaminergic (Mulders and Robertson, 2004). Campbell and Henson (1988) also reported variability and chose to report their largest numbers with the idea that these were the best injections of tracer. If these neurons are completely stained by AChE stains and the largest numbers of retrograde labeling are representative, then there are several hundred LOC intrinsic neurons per side in mouse.

MOC Dendrites

Present results indicate that MOC dendrites extend for substantial distances, confirming earlier studies of MOC neurons (Adams, 1983; White and Warr, 1983; Osen et al., 1984; Vetter and Mugnaini, 1992; Warr et al., 2002; Sánchez-González et al. (2003). MOC neurons share this property with other VNTB neurons (Schofield and Cant, 1991). We also found that MOC dendrites are sparsely branched, as noted in previous studies (Osen et al., 1984; Vetter and Mugnaini, 1992; Sánchez-González et al. (2003). Although their dendrites are longer, their overall appearance and amount of branching appears to be similar to radiate multipolar neurons of the cochlear nucleus (Doucet and Ryugo, 1997). Our study is the first to quantify MOC numbers of dendrites and their lengths and demonstrate that they depend on orientation. Dendrites directed medially are more numerous, longer, and a bit more branched than those directed laterally, and the vector derived from summing individual dendrite vectors from a single neuron usually points medially. MOC neurons receive inputs from PVCN neurons that project crossing axons cross in the trapezoid body (Thompson and Thompson, 1991). Medial dendrites of MOC neurons could be directed toward these inputs. This crossing input is larger than uncrossed input from the PVCN, which approaches the neurons from their lateral sides.

Input to MOC neurons also arises from neurons in the marginal shell of AVCN and this input is mainly crossed also (Ye et al., 2000); its route of projection has not been established. MOC neurons receive important synaptic input onto their dendrites. Light microscopy demonstrates that cochlear nucleus endings are present on the dendrites as well as the somata (Thompson and Thompson, 1991). Other inputs are also present onto the dendrites (Mulders and Robertson, 2005). Electron microscopic studies indicate synapses present on both the dendrites and somata (Helfert et al., 1988; Benson & Brown). The type of synaptic terminal with small, round vesicles may be from cochlear nucleus neurons (Benson and Brown, 2006).

MOC dendrites were also somewhat longer in the rostral direction compared to the caudal direction. The rostral extension is towards descending inputs from sources located rostrally. For example, inputs from the inferior colliculus travel in the lateral lemniscus caudally toward the MOC neurons (Faye-Lund, 1986; Thompson and Thompson, 1991) and MOC neurons on the same side extend dendrites rostrally to meet this input. However, descending input from the inferior colliculus of the opposite side approaches MOC neurons medially as it crosses in the trapezoid body (Thompson and Thompson, 1991; Vetter et al., 1993).

DPO neurons had dendrites that differed in important ways from VNTB neurons. First, DPO dendrites project into more dorsal parts of the trapezoid body compared to the more ventral dendrites of VNTB neurons. This finding suggests that the two types of dendrites receive differing types of input, because there is a segregation of fibers within trapezoid body according to diameter and cell type of origin in the cochlear nucleus. At least in cat (Brownell, 1975; Smith et al., 1993), thick axons of presumed globular bushy cells are located ventrally, medium-diameter axons of presumed spherical bushy cells are located dorsally, and thin axons of presumed stellate/multipolar cells are located very ventrally or in the middle, between the two types of bushy cell axons. Secondly, DPO dendrites show less asymmetry of their dendrites (Fig. 6E) and less asymmetry for single neurons as measured by our vector analysis (Fig. 12). These data might suggest that DPO neurons receive as much uncrossed as crossed input, perhaps thus responding more equally to input from either ear. In fact, an Either-Ear class of MOC neurons consists of about 5 – 10% of the MOC units recorded in cats (Liberman and Brown, 1986) and guinea pigs (Robertson and Gummer, 1985; Brown, 1989). Such neurons also differ from other MOC neurons by having higher spontaneous rates and having more evidence of binaural interaction (Liberman and Brown, 1986; Brown, 1989), perhaps a result from input via the dorsal component of the trapezoid body. Recordings of OC neurons are not available in mouse, but a search for neurons with symmetric dendrites in cats or guinea pigs might support this idea by yielding a similar small percentage of OC neurons. Guinea pigs and cats do have large OC neurons located in dorsal positions (Aschoff and Ostwald, 1987; Warr et al., 2002) that could correspond to the DPO neurons observed here in mouse. It is unlikely that the DPO neurons correspond exclusively to those OC neurons projecting bilaterally, since those neurons also occur in the VNTB, at least in guinea pig (Robertson et al., 1987).

There were no clear morphological differences between labeled VNTB or DPO neurons located ipsilateral vs. contralateral to the injected cochlea. This locational difference probably corresponds to the dominant-ear response pattern, with Ipsi units located on the opposite side and Contra units located on the same side relative to the cochlea to which they project (Brown and Liberman, 1986). Both groups would be expected to receive crossing inputs that arrive from the medial direction. Except for the dominant ear that drives the unit, the physiological characteristics of Ipsi and Contra units are reported to be similar, in line with our morphological results.

MOC dendrites travel for long distances from the soma. This long distance offers huge regions for input from the variety of sources reported to project to these neurons: the cochlear nucleus (Thompson and Thompson, 1991; Ye et al., 2000), inferior colliculus (Faye-Lund, 1986;

Thompson and Thompson, 1993; Vetter et al., 1993; Mulders and Robertson, 2002; Ota et al., 2004), locus coeruleus and serotonergic/catecholaminergic sources (Thompson and Thompson, 1995; Woods and Azeredo, 1999; Mulders and Robertson, 2000a; Horvath et al., 2003), and auditory cortex (Mulders and Robertson, 2000b). In spite of these long dendrites in all directions, MOC neurons have sharp frequency tuning (Robertson and Gummer, 1985; Liberman and Brown, 1986; Brown, 1989). At least some MOC dendrites would be expected to cut across any potential tonotopic axis, which may be in the medial-to-lateral dimension in the VNTB region (Guinan et al., 1972; Brown 1993). One idea is that frequency-specific input from the cochlear nucleus predominantly targets one component of the MOC neuron, such as just the soma, or just the medially projecting dendrites. Other areas of the neuron might receive other types of inputs. There are differences in synaptic profiles between the soma and proximal dendrites of MOC neurons (Helfert et al., 1988; Benson and Brown, 2006). Most notably, the type of synaptic terminal proposed to arise from the cochlear nucleus and mediate the sharp tuning becomes less frequent on proximal dendrites compared to the somata (Benson and Brown, 2006). However, distal dendrites have not been examined nor has there been a comparison between medial vs. lateral dendrites. Perhaps the longer medial dendrites observed in the present study result from the need to accommodate crossing inputs from the cochlear nucleus – future studies will be needed to test this type of hypothesis.

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List of Abbreviations

AChE, acetylcholinesterase
AVCN, anteroventral cochlear nucleus
DCN, dorsal cochlear nucleus
DPO, dorsal periolivary nucleus
FG, Fluorogold
HRP, horseradish peroxidase
LOC, lateral olivocochlear
LSO, lateral superior olive
MNTB, medial nucleus of the trapezoid body
MOC, medial olivocochlear
OC, olivocochlear
PVCN, posteroventral cochlear nucleus
SPN, superior paraolivary nucleus
TB, trapezoid body
VII, facial motor nucleus
VNTB, ventral nucleus of the trapezoid body

Figure 1.

Dark AChE staining (black) in presumed OC neurons in the superior olivary complex in a photomicrograph of a transverse section through the left side of the mouse brainstem. Most MOC neurons are in the ventral nucleus of the trapezoid body (VNTB) and extend dendrites medially toward the trapezoid body (TB). A few stained neurons are located more dorsally, in the dorsal periolivary nucleus (DPO) and extend dendrites in all directions. Stained MOC axons are visible projecting dorsally, which eventually form the olivocochlear bundle. More lightly-stained presumed LOC neurons are seen within (intrinsic neurons) and on the margins of (shell neurons) the LSO. LOC dendrites and axons are not well stained. Neutral red counterstain. Scale bar: 100 μ m.

Figure 2.

Medial extensions of dendrites of MOC neurons. Camera lucida drawings of the left-side superior olivary complex, showing (A) all OC somata superimposed from four serial ACh-E stained sections, (B) the large somata and dendrites contained in just one of the sections shown in A, and (C) MOC somata and dendrites from a more rostral section containing only VNTB. In B and C, arrows show the extensive MOC dendrites running medially and dorso-medially toward the trapezoid body (TB). In panel B, DPO neurons and their dendrites face the most dorsal part of the TB. Scale bar: 100 μ m.

Figure 3.

The similarity in positions of OC neurons retrogradely labeled with Fluorogold (FG, left panels) and stained for AChE (right panels). In these atlases, the individual drawings show the somata within two superimposed 80- μ m thick transverse sections; each dot indicates one neuron (except for neurons within the LSO, which are too numerous to be indicated individually). The FG atlas shows the OC neurons retrogradely labeled from a single injected cochlea (on the left side) whereas the AChE atlas shows presumed left-projecting as well as right-projecting OC neurons. The OC bundle of stained axons is indicated on the AChE atlas. VII: facial motor nucleus; DCN, PVCN, AVCN: dorsal, posteroventral, anteroventral cochlear nucleus; DPO: dorsal periolivary nucleus; LSO: lateral superior olive, MNTB: medial nucleus of the trapezoid body; VNTB: ventral nucleus of the trapezoid body. Scale bar: 1 mm. The number of MOC neurons in VNTB (A) is around 150 per side regardless of labeling method, whereas the number of DPO neurons (B) depends on labeling method. Columns show averages and error bars show SEMs. Number of cases counted were AChE: 9 brainstem halves counted in 6 mice; FG: 9 brainstems; HRP: 3 brainstems. FG and HRP counts show numbers on the same (white) and opposite (black) sides of the brainstem relative to the cochlea injected with tracer; AChE counts show number in one half of the brainstem (gray).

Figure 4.

Figure 5. Micrograph showing individual MOC neurons with dendrites (arrows) extending medially up to 300 μm as labeled by FG (top) or stained for AChE (bottom). Neurons are located in the rostral VNTB on the left side of the brainstem; medial is toward the right. Scale bar: 100 μm .

Figure 6.

Polar plots showing that dendrites are longer in the medial direction for VNTB neurons but not for DPO neurons. Inset: Each dendrite was approximated by a line from soma to tip. Length of this line was used as dendrite length and angle of this line from dorsal was used for dendrite angle. Polar plots show dendrite angles (in degrees) and lengths (in μm) for 152 FG-labeled dendrites (open symbols) from 6 mice brainstems and 136 AChE-stained dendrites (filled symbols) from 2 mice brainstems sectioned in the transverse plane. A: all dendrites from neurons located in VNTB and DPO; B–E: neurons from subgroups separated according to their position. The VNTB caudal group was defined as neurons caudal to the LSO, the middle group as neurons in sections containing the LSO, and the rostral group as neurons rostral to the LSO; these groups had no distinct separation at their boundaries. Numbers of observations of dendrites does not represent the numbers of neurons in each group; for example the group of neurons ventral to the LSO is a dense cluster whose dendrites were intertwined and difficult to separate so fewer points are on this plot (panel B).

Figure 7.

Polar plots showing that dendrites are longer in the rostral direction for all neurons. Plots show dendrite angles and lengths for 110 AChE-stained dendrites from 2 mice brainstems sectioned in the sagittal plane. A: all dendrites from neurons located in VNTB and DPO; B–E: neurons from subgroups separated according to their position as described in legend of Fig. 6.

Figure 8.

Polar plots showing that the summed path length of all dendrite branches for an individual dendrite is also larger in the medial direction. Data are plotted separately according to section plane for VNTB neurons (A,C) and for DPO neurons (B,D). Shown are data from FG-labeled dendrites (open symbols) and AChE-stained dendrites (filled symbols).

Figure 9.

Reconstruction of a single AChE-stained MOC neuron in the VNTB showing preferential extension of the dendrites in the medial direction. All dendrites were contained within the single transverse section (inset, dots show other stained neurons). Part of the axon is also shown. Scale bar: 50 μ m. Inset: Position of labeled neuron in the VNTB in the transverse section. Inset scale bar: 0.5 mm.

Figure 10. Reconstruction of a single AChE-stained MOC neuron in the rostral VNTB showing preferential extension of the dendrites in the rostral direction. All dendrites were contained within the single sagittal section. No axon was distinguishable. Scale bar: 50 μ m. Inset: Position of labeled neuron in the sagittal section. Inset scale bar: 0.5 mm.

Figure 11.

Scatter plot showing dendrite angle to tip (as measured in Fig. 6, inset) is predicted by the dendrite's initial angle. The initial angle was measured as the dendrite emanates from the cell body (see Methods). Data are from transversely sectioned material including both VNTB and DPO neurons; open symbols plot FG data and closed symbols plot AChE data.

Figure 12.

Single neuron vector angles and magnitudes plotted for 17 FG-labeled and 29 AChE-stained neurons that were reconstructed to all their dendrites in the transverse plane. To compute a vector for a single neuron, each of the neuron's dendrites was treated as a separate individual vector with its own length and angle (Fig. 6, inset), then all vectors of a single neuron were summed. The resultant single neuron vector is plotted on the figure as a point. Points near the origin indicate a pattern of equal-length dendrites radiating symmetrically in all directions; points in the right half of the plot indicate a pattern of more dendrites and/or longer dendrites in the medial direction.