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DENDRITES OF MEDIAL OLIVOCOCHLEAR 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.

Key words: superior olive, cochlear nucleus, efferent, descending pathway, auditory reflex.

Dendrites represent an important site for neurons to receive synaptic input. As such, dendritic orientation and pattern can shape a neuron's response properties. For example, octopus cells of the posteroventral cochlear nucleus (PVCN) 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 (Rhode et al., 1983; Rouiller and Ryugo, 1984), are often tuned more broadly than their inputs (Godfrey et al., 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 olivocochlear (MOC) 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 medioventral/rostral periolivary nuclei) (White and Warr, 1983; Campbell and Henson, 1988; Motts et al., 2008). Lateral olivocochlear (LOC) 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, 1991; 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 and Mugnaini, 1992; 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 to sound 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

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Abbreviations: AChE, acetylcholinesterase; AVCN, anteroventral 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 parolivary nucleus; TB, trapezoid body; VNTB, ventral nucleus of the trapezoid body.

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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 (5–15% of all neurons), Either-Ear neurons, respond almost symmetrically to sound in either ear. The brainstem anatomy of theses 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 enhance it during visual tasks (Delano et al., 2007). At least some of these inputs approach 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 and Warr, 1983; Osen et al., 1984; Thompson and Thompson, 1986; Vetter and 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 lifespan 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 25 mice of CBA/CaJ strain were used (13 for AChE stains, nine for FG labeling, and three 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 and Ear Infirmary. A minimum number of animals was used. Humane preoperative and postoperative care minimized their suffering.

AChE stains

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) and killed 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 μm 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 μl 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 killed 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 μm 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) at a dilution of (1/100,000).

MOC angle measurements

Stained or labeled neurons were drawn using a light microscope with 10× and 60× 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 (National Institutes of Health) 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 (Figs. 6, 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.