Single-unit labeling of medial olivocochlear neurons: the cochlear frequency map for efferent axons

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Brown MC. Single-unit labeling of medial olivocochlear neurons: the cochlear frequency map for efferent axons. J Neurophysiol 111: 2177–2186, 2014. First published March 5, 2014; doi:10.1152/jn.00045.2014.—Medial olivocochlear (MOC) neurons are efferent neurons that project axons from the brain to the cochlea. Their action on outer hair cells reduces the gain of the “cochlear amplifier,” which shifts the dynamic range of hearing and reduces the effects of noise masking. The MOC effects in one ear can be elicited by sound in that ipsilateral ear or by sound in the contralateral ear. To study how MOC neurons project onto the cochlea to mediate these effects, single-unit labeling in guinea pigs was used to study the mapping of MOC neurons for neurons responsive to ipsilateral sound vs. those responsive to contralateral sound. MOC neurons were sharpened to sound frequency with a well-defined characteristic frequency (CF). However, their labeled termination spans in the organ of Corti ranged from narrow to broad, innervating between 14 and 69 outer hair cells per axon in a “patchy” pattern. For units responsive to ipsilateral sound, the midpoint of innervation was mapped according to CF in a relationship generally similar to, but with more variability than, that of auditory-nerve fibers. Thus, based on CF mappings, most of the MOC terminations miss outer hair cells involved in the cochlear amplifier for their CF, which are located more basally. Compared with ipsilaterally responsive neurons, contralaterally responsive neurons had an apical offset in termination and a larger span of innervation (an average of 10.41% cochlear distance), suggesting that when contralateral sound activates the MOC reflex, the actions are different than those for ipsilateral sound.

cochlear amplifier; outer hair cell; masking; acoustic protection; descending system

HAIR CELLS OF AUDITORY AND vestibular sense organs receive a prominent efferent innervation from the brainstem (reviewed by Ryugo et al. 2011). One group of efferent neurons, the medial olivocochlear (MOC) neurons, terminates on outer hair cells (OHCs) of the cochlea. Activation of these neurons hyperpolarizes the OHCs (Fuchs 2002) and reduces the vibration of the basilar membrane (Murugasu and Russell 1996; Cooper and Guinan 2006), the responses of inner hair cells (Brown and Nuttall 1984), and the firing of auditory-nerve fibers (Gifford and Guinan 1987). MOC neurons are thought to act by reducing the gain of the “cochlear amplifier,” the process by which the OHC electromotility enhances motion of the receptor organ, the organ of Corti (Dallos 1992). This process, mediated by the OHC protein prestin (Dallos et al. 2008), generates the high sensitivity and sharp tuning of the cochlea. MOC action has several beneficial effects. It shifts the dynamic range of auditory-nerve fibers to higher levels (Wiederhold and Kiang 1970), and it decreases the masking effects of steady background noise (Winslow and Sachs 1988; Kawase et al. 1993; Jennings et al. 2011). Finally, MOC action protects the inner ear from damage due to high-level sound (Reiter and Liberman 1995; Maisoon and Liberman 2000).

To perform these functions, MOC neurons respond to sound (Fex 1962) as part of the MOC reflex (Liberman and Guinan 1998). Individual MOC neurons are sharply tuned to sound frequency, with selectivity measures equal or almost equal to those of the auditory-nerve fibers (Robertson and Guummer 1985; Liberman and Brown 1986; Brown 1989). This high selectivity suggests that the MOC system feeds back onto the cochlea with a frequency-specific mapping. However, the mapping of MOC neurons, including its relationship to that of auditory-nerve fibers, is not known. Only a handful of MOC axons have been reconstructed to all their terminations (Liberman and Brown 1986; Brown 1989). Some of the published axonal terminations represent only portions of axons (Robertson and Guummer 1985). Thus available data do not rule out an MOC termination just basal to the CF position of auditory-nerve fibers, the location of the OHCs involved in the cochlear amplifier (Neely and Kim 1986; Cody 1992; Patuzzi 1996; Pang and Guinan 1997; Shera 2007; Fisher et al. 2012).

MOC neurons consist of three response types (Robertson and Guummer 1985; Liberman and Brown 1986; Brown 1989). About two-thirds of the neurons, Ipsi units, respond only to monaural sound presented to the ipsilateral ear (that ear which receives the MOC terminations). About one-third, Contra units, respond to sound in the contralateral ear, and a small percentage of units, Either Ear units, respond to sound in either ear. A comparison of terminations of the types of neurons has not been made, an important issue since many studies have used contralateral sound to elicit MOC effects with the implicit assumption that they are similar to the effects elicited by ipsilateral or bilateral sound (Veuillet et al. 1991; Chery-Croze et al. 1993; Abdala et al. 2009; Henin et al. 2011). To address these issues, the present study constructs the cochlear frequency mapping for MOC axons and compares the terminations of Ipsi, Contra, and Either-Ear units.

MATERIALS AND METHODS

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. Experiments were carried out within a sound-attenuating and electrically shielded chamber in which the air temperature was heated. Guinea pigs were of the Hartley

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strain and were of either sex; those used for biocytin injections were anesthetized with urethane (1,100 mg/kg) plus fentanyl (0.2 mg/kg) and droperidol (10 mg/kg). Guinea pigs used for horseradish peroxidase (HRP) injections were anesthetized with pentobarbital sodium (Nembutal; 15 mg/kg) plus the above dose of fentanyl and droperidol. The Supplemental doses of anesthesia (~1/3 of the original dose) were administered as needed. After anesthesia, a tracheal cannula was inserted, both pinnae were removed, and the animal’s head was placed in a headholder. A rectal thermometer was used in conjunction with the heated air temperature in the chamber and a heating pad to control the rectal temperature to 38°C. The bulla on the left side was opened posterior to the ear canal and a silver wire was placed on the round window of the cochlea to record the compound action potential, with a reference electrode in the neck muscles. Sound stimuli were produced by half-inch condenser microphones driven as a sound sources a reference electrode in the neck muscles. Sound stimuli were presented to the window of the cochlea to record the compound action potential, with a reference electrode in the neck muscles. Sound stimuli were produced by half-inch condenser microphones driven as a sound source.

MOC neurons (and usually auditory-nerve fibers as well) were labeled in 30 guinea pigs (26 with biocytin and 4 with HRP). These latter four guinea pigs were part of a previous report (Brown 1989). In five other guinea pigs, only auditory-nerve fibers were labeled. In 17 guinea pigs, 1–3 MOC neurons were injected and the same numbers of labeled MOC axons were recovered. Most of these units had resting membrane potentials more negative than −10 mV at the time of injection. In other cases, more units were injected (1–4) than axons recovered (0–3), probably because of the poor quality of some injection parameters (membrane potentials less negative than −10 mV or electrodes passing less injection current). In cases with multiple MOC neurons labeled or attempted to label, the injection parameters as well as the CF and point of innervation along the cochlea (Brown 1989) were used to match a given arbors to the recorded neuron. Postinjection survival times averaged 5 h, 5 min (range 0.5 to 10.5 h). Guinea pigs were then killed by perfusion with 3.5% paraformaldehyde mixed with 0.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3. After dissection from the head, cochleas were postfixed for 1 h and then transferred to phosphate buffer overnight.

Cochleas were decalcified in refrigerated 0.1 M EDTA for ~5 days. For the biocytin injections, they were then frozen for a few minutes (to increase membrane permeability to allow reagents to penetrate) and then bisected and microdissected into hemi-turns containing the organ of Corti, the osseous spiral lamina, and the spiral ganglion. The tectorial membrane was removed from the organ of Corti. Pieces were incubated in 0.5% hydrogen peroxide, washed in 0.1 M phosphate buffer, and then incubated overnight in an ABC kit at room temperature. Then, they were incubated in diaminobenzidine alone for 15 min, followed by diaminobenzidine containing 0.5% hydrogen peroxide for ~7 min and then washed again in buffer. After processing, the pieces were dehydrated in an ethanol series, then infiltrated in propylene oxide, and finally embedded in epon in a holder of the size and shape of a glass microscope “slide.” The slide was left overnight at room temperature to allow the propylene oxide to evaporate, and then it was cured in a 60° oven overnight. The slide was removed from the holder and examined with a compound microscope. Important pieces were sometimes removed, individually trimmed, and glued to a glass slide, so that a high-power, oil-immersion lens could be focused on the specimen. For the HRP cases, cochleas were embedded in a polymerized gelatin/albumin mixture and sectioned as described previously (Brown 1989). Brainstems were processed similarly to the cochlear sections and some auditory-nerve fibers were recovered in the cochlear nucleus, but the central axon of only one MOC neuron was recovered and only as far as the vestibular nerve root (not to its cell body of origin).

The database consists of 35 axons that were filled well enough to determine the basal-most and apical-most positions of their innervations. In 32 of these axons, the OHCs innervated and counts of endings were determined, but in the other three axons (2 Ipsi and 1 Contra units) the number of OHCs could not be determined because of missing tissue (1 axon) or fading of the reaction product with increasing distance from the injection site in the apical direction (2 axons). Points of innervation along the organ of Corti were drawn using a compound microscope with a camera lucida attachment. From the drawings, the span of the MOC axon innervation from the most basal to the most apical hair cell innervated was obtained. The midpoint position of this span along the cochlear spiral, divided by the organ of Corti’s total length (measured from drawings done at low magnification), was defined as the “innervation midpoint.” Within the axon span, the center of gravity was determined by calculating a weighted sum of all endings on all three rows of OHCs numbered from the basal-most to the apical-most position and dividing by the hair cell number. This measure is expressed as a fraction of the distance from the basal-most to apical-most innervated OHC (e.g., see Fig. 8A) or as the position of the fraction along the cochlea divided total cochlear distance (e.g., see Fig. 7C). Statistical tests were computed using Kaleidagraph software using the 0.05 significance level.
RESULTS

Tuning curves and morphology of labeled axons. Tuning curves from the 35 labeled MOC neurons were sharply tuned to sound frequency (Fig. 1) and encompassed a wide range of CFs (1.19–16.7 kHz). Thresholds at CF averaged 32.7 dB (SD 16.8, range 9.5–81.1) and were not correlated with CF or response type (data not shown). This large range has been previously reported (Liberman and Brown 1986; Brown 1989).

The OHCs act as the cochlear amplifier (Dallos 1992; Dallos et al. 2008), and they were the target of the labeled axons of the database (25 Ipsi units, 8 Contra units, and 2 Either-Ear units). A photomicrograph of a portion of a labeled axon is shown in Fig. 2. The high density of labeling makes it possible to resolve innervation of individual OHCs (see counts in Fig. 2 legend). Conversely, the low background in the immediately adjacent areas of the organ of Corti makes it clear that there is no additional labeling (this axon produced 2 other patches of innervation that were located apically outside the field of view). For another axon, the complete reconstruction of its termination is shown in the drawing of Fig. 3. This axon had 2 patches of innervated OHCs (dots), a small basal patch of 5 and a larger apical patch of 25. Other than OHCs, the database contained only two examples of other terminations: a single terminal branch from one axon (from a neuron with a low CF of 1.53 kHz) that ended in nearby Hensen’s cells and a single terminal branch from another axon that ended in the inner hair cell region.

Rather than a stereotypical number of OHCs innervated by single MOC axons, there was large variability. The total number of OHCs innervated per axon ranged from 14 to 69 (avg. 38.6, SD 14.0, n = 32 axons). High-CF axons (Figs. 3 and 4, A and B) tended to innervate fewer hair cells than the low-CF axons (Figs. 4, C and D). The dependence on CF is plotted for all the axons in Fig. 5A. There was little difference between Ipsi units (avg. 38.6 OHCs innervated per axon, SD 14.0) and Contra units (avg. 39.4 OHC/axon, SD 13.6) although the greater number of low-CF Contra units may make this a biased comparison. The two Either-Ear units innervated somewhat fewer hair cells (avg. 33.5 OHC/axon). The MOC endings were large (Fig. 2) and were usually formed at the bases of the hair cells. Sometimes multiple endings were formed on individual OHCs (see counts in legends of Figs. 2 and 3). On average, the number of endings per axon was 46.8 (SD 17.2, range 16–87). The number of endings per axon is also a decreasing function of CF (Fig. 5B).

Fig. 1. Tuning curves from labeled medial olivocochlear (MOC) neurons. Response type and characteristic frequency (CF) are indicated next to each tuning curve. Each neuron was from a different preparation. SPL, sound pressure level; Ipsi, ipsilateral.

Fig. 2. Photomicrograph of the termination of a labeled MOC branch in the organ of Corti, from the second turn of the cochlea. A total of 44 outer hair cells (OHCs) were innervated by this branch (15 in row 1, 23 in row 2, and 6 in row 3), and it formed a total of 64 endings (26 in row 1, 30 in row 2, and 8 in row 3). Two other branches of this axon terminated out of the field of view in smaller patches apically. This labeled axon was from an MOC Ipsi unit with a CF of 4.21 kHz.
Row 1 OHCs play an important role in the cochlear amplifier (Liberman and Dodds 1984), and MOC axons preferentially innervated this row (Fig. 6). For the total of 1,273 innervated hair cells in the database, 47.8% were in row 1. On a per axon basis, the average number of innervated OHCs in row 1 was significantly larger than the average number in row 2 (18.5 vs. 12.9 per axon, t-test, P = 0.01), and the average number in row 2 was significantly larger than the average number in row 3 (12.9 vs. 7.2, t-test, P = 0.0001). The highest numbers of row 1 OHCs were innervated by axons with CFs <5 kHz (30–50 per axon, Fig. 6A), whereas these axons only innervated 0–15 in row 3 (Fig. 6C). The small number of OHCs innervated in row 3 is observed in the example axon terminating in the second cochlear turn (Fig. 2) compared with the more even coverage of the hair cell rows by the example axon terminating in the lower first (basal) turn (Fig. 3). Although Ipsi units innervated fewer row 1 OHCs per axon than Contra units, this difference was not significant (avg. 16.3 vs. 24.5, t-test, P = 0.06).

Mappings. The mapping of CF to region of cochlear termination for MOC neurons was constructed from physiological and anatomical data like that contained in Figs. 1, 3, and 4. For the axon of Fig. 3, the midpoint of innervation (middle arrow), normalized by the total cochlear distance, was located at a position 22.40% from the basal end of the cochlea. Its CF was 14.0 kHz and it was an Ipsi unit. The midpoints for the axons of Fig. 4 are indicated (arrows), and the physiological data are adjacent to the drawings. The mapping for all axons of the database (Fig. 7A) shows a tonotopic relationship, with low-CF axons terminating apically and high-CF axons terminating more basally. Contra units terminated slightly more apically than Ipsi units for a given CF. The best-fit lines for the two unit types (see Fig. 7A legend) predict that, at a CF equal to 4 kHz, there is a difference of 4.32% distance (~1/3 octave in guinea pigs: Tsuji and Liberman 1997). The two Either-Ear units in the database followed the mapping for Ipsi units. Both Ipsi and Contra units showed considerable spread of data from the lines. One cluster of data points from units with CFs ~4 kHz, all Ipsi units, illustrates this variability: the most basally terminating axon had a midpoint of 40.16% and the most apically terminating axon had a midpoint of 54.68%.

To gain insight into the relationship of MOC terminations to the site of the cochlear amplifier, auditory-nerve fibers were labeled (Fig. 4, arrowheads). Their terminals contact a single inner hair cell, which is a “pinpoint” mapping compared with the broader swath of MOC axon terminations. The overall data set for nerve fibers consisted of 39 fibers, which were labeled either via the spiral-ganglion recording site in the basal turn (n = 27, CFs 13.3–17.8 kHz, e.g., Fig. 4, A and B) or at a modiolar recording site (n = 12, CFs of 1.4–6.2 kHz, e.g., Fig. 4, C and D). The nerve-fiber data had less spread from the best-fit line (Fig. 7B, dashed line, R = 0.99) compared with the
MOC mapping. In this characteristic, as well as in slope and intercept, the nerve fiber mapping is similar to that of an earlier study in guinea pig (Tsuji and Liberman 1997; Fig. 7B, dotted line). With the nerve-fiber line as a reference, the best-fit line for all types of MOC neurons (Fig. 7B, solid line) is just apical (0.8% cochlear distance) at 15 kHz and further apical (8.0% cochlear distance) at 1 kHz. The best-fit lines for the nerve-fiber data (from the present study) and MOC data can be compared using statistical tests (Kleinbaum et al. 2008). The null hypotheses of parallel lines and equal intercepts are rejected ($P = 0.016$, and $P < 0.001$, respectively), that is, the two lines are significantly different. However, another test that used only data from Ipsi units (Fig. 7A, dashed line) indicates parallel lines ($P = 0.646$) and equal intercepts (although just at the edge of being significantly different, $P = 0.053$). Thus Ipsi MOC units and nerve fibers have similar mappings, but Contra units have a different mapping.

The relationship between nerve fibers and MOC axons of similar CFs was examined in individual cochleas where both types of neurons were labeled (Fig. 4). Twenty-two cases had pairs with similar CFs (within 1 octave) using labeled nerve fibers (e.g., Fig. 4) or a visible recording site (e.g., Fig. 3) from which a radial line was drawn (9 other cochleas did not have pairs or labeled MOC neurons close in CF to the recording site and were not adjusted). It was assumed that the labeled nerve fiber position anchored at that point the nerve-fiber CF mapping for that cochlea. Then, the difference between this anchor point and the CF mapping for nerve fibers (see Fig. 7B legend) was used to adjust the MOC innervation position. The average adjustment was 0.65% (range $-3.49$ to 6.34%) and using this adjusted position did not significantly affect the MOC mapping (see Fig. 7B legend). The nerve-fiber terminals in the organ of Corti also indicate the position of OHCs involved in the cochlear amplifier for their CF, which starts at this position and extends basally (Neely and Kim 1986; Cody 1992; Patuzzi 1996; Pang and Guinan 1997; Shera 2007; Fisher et al. 2012). Where there were labeled pairs, the cochlear amplifier position can be calculated accurately for the CF of the MOC neurons (gray shading on Fig. 4). In Fig. 4, only one MOC neuron terminations had extensive overlap with the predicted position of the cochlear amplifier (Fig. 4C), but the other three neuron terminations had no overlap because the amplifier was located basally (Fig. 4, A, B, and D).

The mappings of MOC innervation presented so far were based on midpoint, and such a measure may give a misleading view of the effects of MOC action if there is a disproportionate number of endings toward one edge of the MOC axon termination. For example, the axon shown in Fig. 3 had most of its endings in the large patch at the apical edge of its span (toward the right in Fig. 3). To quantify this asymmetry, the center of gravity (see MATERIALS AND METHODS) was computed for each axon; for the axon of Fig. 3 the center of gravity (Fig. 3, right arrow) was the fraction 0.729 of the distance from the basal end of its innervation span. The cochlear mapping for this measure, expressed as a percent distance along the cochlear spiral, is plotted in Fig. 7C. The best-fit lines (dashed) vs. the earlier-described midpoint of innervation (solid) are almost the same. The center of gravity (expressed as a fraction) also had no obvious pattern across CF (Fig. 8A). The center of gravity did not differ significantly ($t$-test, $P = 0.85$) between Ipsi units (avg. 0.490, SD 0.148) and Contra units (avg. 0.503, SD 0.150).
**Innervation spans.** The labeled MOC axons had large variability in their spans of termination, encompassing spans that were small (Fig. 4A), intermediate (Figs. 3 and Fig. 4D), and large (Fig. 4B and C). For all the labeled axons (Fig. 8B), spans ranged from 150 to 4910 μm, which corresponds to between 0.79 and 23.80% of the total cochlear distance. Axons with small spans were not due to fading of the reaction product along their course. Also, axons with small spans were not potentially confused with other axons, since 8 of 10 axons with spans of <2% cochlear distance were the only axons labeled in those particular cochleas. There was a trend for the lower CF units to have larger spans (Figs. 8B and 9). For example, units with CFs <6 kHz had spans that averaged 7.43% (SD 6.43, n = 15), whereas units with CFs >6 kHz have spans that averaged 4.45% (SD 4.93, n = 20). This difference was not statistically significant (t-test, P = 0.13), and there is a low R value to a linear fit to the points (data not shown) because of the large variability. The spans for Ipsi units were significantly smaller than those for Contra units (t-test, P = 0.01). For Ipsi units, the average span was 4.52% cochlear distance (SD 3.88, range 0.79 to 17.03) and for Contra units, the average span was 10.41% (SD 8.59, range 1.41 to 23.80). For the two Either-Ear units, the spans were 1.84 and 2.40%, more like Ipsi units than Contra units.

**DISCUSSION**

Anatomical terminations of MOC neurons. The sample of labeled MOC axon terminations documented here is large enough to compare the cochlear frequency mapping to auditory-nerve fibers and show that it is similar in many ways. While the mapping of individual nerve fibers is pinpoint and precise
(Liberman 1982; Tsuji and Liberman 1997), the mapping of MOC axons consists of broader swaths that have higher variability. Contra MOC units had apical offsets and broader innervation spans relative to Ipsi units even though they innervated about the same number of hair cells. Either-Ear units have not previously been labeled; although only two of these uncommon units were labeled here, both had anatomical characteristics like those of Ipsi units. The relatively broad pattern of innervation contrasts with the sharply tuned responses of MOC neurons (Fig. 1).

Single-unit recordings tend to sample large neurons (Stone 1973), and the following calculation suggests that the axons sampled in the present study have average numbers of endings. The average number of MOC endings observed in the present study (avg. 46.8/axon), multiplied by the number of MOC neurons projecting to one cochlea (n = 615; Robertson et al. 1987; Brown et al. 2013, although larger n’s were found by Aschoff and Ostwald 1987), yields a total of 28,782 MOC endings per cochlea. Dividing by the number of OHC in the guinea pig cochlea (2,400; Coleman 1976) yields ~12 endings per OHC, which is higher than the number of efferent endings per OHC (8–10) reported previously in the guinea pig (Engstrom 1960). Thus the present data appear to have oversampled axons that form the largest numbers of endings, which may be those with the largest diameters.

**MOC terminations vs. the site of the cochlear amplifier.** The present results, showing preferential MOC termination on OHCs in row 1, are consistent with the importance of this row in the function of the cochlear amplifier. The importance of row 1 was originally shown in noise-exposed preparations in which lesions of stereocilia on row 1 hair cells, with virtually no other damage to OHCs, were accompanied by large losses in auditory-nerve sensitivity near CF (Liberman and Dodds 1984). Present data indicate a trend in innervation between the OHC rows (1 > 2 > 3) and suggest a similar trend for contribution to the amplifier function. MOC innervation trends between the rows and along the cochlear length have been reported previously (Ishii and Balough 1968; Guinan et al. 1984; Brown 1987; Liberman et al. 1990). The CF-to-position mapping of MOC neurons described in the present study is one of many such tonotopic mappings in the auditory pathway (Clopton et al. 1974; Ryan et al. 1982; Friauf 1992; Muniak et al. 2013). Present results suggest that the MOC mapping is similar to the auditory-nerve fiber mapping, either indistinguishable from it (for MOC Ipsi units) or just apical to it (for MOC Contra units, although these data are more limited). Previous studies of MOC labeling in cat and guinea pig, most of which are Ipsi units, are consistent with present results (Robertson and Gummer 1985; Liberman and Brown 1986). Thus the Ipsi units form a feedback system that is in frequency alignment with their CFs and those of auditory-nerve fibers. The more apical offset of Contra units is reminiscent of earlier projection data from injections made into the superior olivary complex (Guinan et al. 1984). Without any other information, such data suggest that MOC neuron effects would be
largest at places tuned to their CFs or slightly below. With the use of contralateral sound to activate MOC neurons, largest effects on auditory-nerve fibers are indeed found for frequencies around CF (Warren and Liberman 1989). Similarly, contralateral sound is most effective for frequencies close to the frequency of an otoacoustic emission in the ipsilateral ear (Veuillet et al. 1991; Chery-Croze et al. 1993; Lilaonitkul and Guinan 2012), although caution is needed here because the spatial extent of the emission generators is not clearly known and almost certainly depends on sound level and the type of emission. In the present study, in contrast, there is the direct measurement of the position of MOC innervation compared with the CF place for auditory-nerve fibers.

The region of OHCs that is most active in the amplifier, however, is ~650 μm (¼ octave) basal to the CF place for nerve fibers of the basal turn (gray shading on Fig. 9), as shown by lesion and perturbation studies (Cody 1992; Fisher et al. 2012). Although those studies are limited to the cochlear basal turn, a basal offset in all cochlear regions is indicated by modeling studies (Neely and Kim 1986; de Boer and Nuttall 2000), by calculated gain functions from basilar membrane measurements (Shera 2007), and by extensive studies of two-tone suppression in which the nerve-fiber response to a probe at CF is suppressed by a second tone that presumably “jams” the cochlear amplifier (Sachs and Kiang 1968; Schmidt 1982; Prijs 1989; Geisler et al. 1990; Kanis and de Boer 1994; Nobili and Mammano 1996; reviewed by Patuzzi 1996). However, there is much less known about extent of the cochlear amplifier in the apical cochlear regions (and MOC labeling data there are also lacking). Given that the MOC and nerve-fiber mappings are close, and that the cochlear amplifier extends basally, the MOC terminations of a given CF do not generally reach basally to the site of the cochlear amplifier for that CF (Fig. 9). Only about half (17 of 34) of the MOC axon spans in the present study infringe on this region, and almost all of the terminations extend apical to this region. Similar conclusions are seen for individual cochleas, where auditory nerve fibers were used to compute the location of the cochlear amplifier and where the location of the amplifier for the CF of the labeled MOC axons was usually basal to the MOC termination (Fig. 4, A, B, and D).

It seems unlikely that terminations of MOC axons were missed in the present study, because of the excellent signal-to-noise of the labeling (Fig. 2), because all branches and endings were recovered without evidence of fading for almost all axons, and because the important basal part of the terminations is nearest the injection site (Fig. 3) where the reaction product is the darkest.

How can these data explain the biggest effects at CF with few projections to the position of the amplifier? This question

Fig. 8. A: fractional center of gravity of MOC terminal arbors (see Fig. 3 and MATERIALS AND METHODS) as a function of CF. B: innervation span for MOC axons as a function of CF. Span (in % cochlear distance) is measured from the most basal to the most apically innervated OHC (see Fig. 3).
does not appear to be fully explained by existing knowledge, but part of the answer requires consideration of sound level. Cochlear amplifier measurements are usually performed at low sound levels where the contribution of the amplifier is maximal (Patuzzi 1996). In contrast, the MOC system acts mostly at moderate and high sound levels. MOC firing rates do not become significant until high sound levels (Liberman 1988a, 1988b; Brown et al. 1998), and high rates of stimulation of the OC bundle are needed for peripheral effects (Wiederhold and Kiang 1970; Brown and Nuttall 1984). At these high sound levels, the pattern of MOC firing shifts such that maximal rates are in response to frequencies just below MOC neuron CF (Liberman 1988b), and this offset, while variable, averages about a one-quarter octave (Fig. 12C in Warren and Liberman 1989). Thus, if frequency for maximal MOC firing rate was plotted instead of CF, the points of Fig. 9 would shift toward the left so that a larger portion would have involvement with the cochlear amplifier. With this consideration, the MOC firing at high sound levels is a better match for the position of the cochlear amplifier. An untested idea is that those MOC terminations that have the most deviation from the region of the amplifier (such as Contra units) might have the most downward shift in frequency from CF to frequency of maximal firing. These shifts might also reduce some of the variability in termination observed for MOC axons. However, another part of the answer to the alignment problem may be that MOC neurons have actions that use mechanisms other than altering the gain of the cochlear amplifier (Guinan and Stankovic 1996). These mechanisms might be important in the MOC function to protect the cochlea from overstimulation, which occurs at the highest sound levels where the cochlear amplifier contribution is minimal.

Functional effects of large MOC spans. The present study demonstrates that some MOC axons terminate in wide spans along the organ of Corti. The largest span of the present study, a Contra unit, was almost 24% of the total cochlear length, which would correspond to approximately two octaves of sound frequency. Like those studied here in the guinea pig, MOC terminations in other species can also be broad. In the cat, 23–84 OHCs were innervated per MOC axon and the spans were up to 2.8 mm, just under an octave span (Liberman and Brown 1986). In mice, axonal spans are at least as broad, 8.8–35% of the total cochlear length (Wilson et al. 1991) and 35% of the cochlear length (Brown et al. 1991). Significant termination span is the anatomical substrate for a single point along the cochlea to be affected by MOC neurons tuned to a variety of CFs. In fact, in human experiments, an otoacoustic emission can be affected by a range of frequencies presented to the contralateral ear (Veuillet et al. 1991; Cherry-Croze et al. 1993; Lilaonitkul and Guinan 2009a), and the range extends over several octaves (Lilaonitkul and Guinan 2012). Although the spatial extent of the emission generators is not known, these data suggest wide effects of Contra MOC neurons. A second mechanism explaining wide effects of these reflex elicitors is the variability in MOC projection, so that innervation midpoints or centers of gravity do not fall right on the best-fit line (Fig. 7). This variability would tend to broaden the functional effects of the overall MOC system. In the present study, Contra units had innervation spans that were on average about twice as great as those of Ipsi units. These large spans offer a mechanism for the greater spatial summation of effects from contralateral elicitors vs. ipsilateral elicitors as bandwidth increases (Lilaonitkul and Guinan 2009b). This increase recruits additional, off-CF MOC neurons, and these Contra neurons have spans wide enough to encompass the CF position (whereas many Ipsi neurons do not). Overall, such ipsilateral/contralateral differences in the MOC reflex suggest differences in the functional roles of the two types of MOC neurons leading to the OHCs, even in the way the cochlear amplifier is controlled depending on whether sound is presented to the ipsilateral vs. the contralateral ear.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: M.C.B. conception and design of research; M.C.B. performed experiments; M.C.B. analyzed data; M.C.B. interpreted results of experiments; M.C.B. prepared figures; M.C.B. drafted manuscript; M.C.B. edited and revised manuscript; M.C.B. approved final version of manuscript.

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