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Research paper

The middle ear muscle reflex in the diagnosis of cochlear neuropathy

Michelle D. Valero [a, b, *], Kenneth E. Hancock [a, b], M. Charles Liberman [a, b]

[a] Eaton-Peabody Laboratories, Massachusetts Eye and Ear Infirmary, Boston, MA 02114, USA
[b] Department of Otology and Laryngology, Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Cochlear neuropathy, i.e. the loss of auditory nerve fibers (ANFs) without loss of hair cells, may cause hearing deficits without affecting threshold sensitivity, particularly if the subset of ANFs with high thresholds and low spontaneous rates (SRs) is preferentially lost, as appears to be the case in both aging and noise-damaged cochleas. Because low-SR fibers may also be important drivers of the medial olivocochlear reflex (MOCR) and middle-ear muscle reflex (MEMR), these reflexes might be sensitive metrics of cochlear neuropathy. To test this hypothesis, we measured reflex strength and reflex threshold in mice with noise-induced neuropathy, as documented by confocal analysis of immunostained cochlear whole-mounts. To assay the MOCR, we measured contra-noise modulation of ipsilateral distortion-product otoacoustic emissions (DPOAEs) before and after the administration of curare to block the MEMR or curare + strychnine to also block the MOCR. The modulation of DPOAEs was 1) dominated by the MEMR in anesthetized mice, with a smaller contribution from the MOCR, and 2) significantly attenuated in neuropathic mice, but only when the MEMR was intact. We then measured MEMR growth functions by monitoring contra-noise induced changes in the wideband reflectance of chirps presented to the ipsilateral ear. We found 1) that the changes in wideband reflectance were mediated by the MEMR alone, and 2) that MEMR threshold was elevated and its maximum amplitude was attenuated in neuropathic mice. These data suggest that the MEMR may be valuable in the early detection of cochlear neuropathy.

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1. Introduction

Auditory neuropathy is defined clinically by normal OAEs with an absent or grossly abnormal ABR. There are likely numerous etiologies, but in such patients, outer hair cells (OHCs) must be functioning normally, while auditory nerve fibers (ANFs) and/or inner hair cells (IHCs) are presumably absent or dysfunctional. Some patients with auditory neuropathy have normal audiometric thresholds, but extreme difficulty understanding speech even in a quiet environment (Starr et al., 1996). In such cases, the degree of nerve loss/dysfunction must be extreme, and in such patients the MEMR is often absent (Berlin et al., 2005).

Recent work in animals shows that noise exposures that do not permanently damage hair cells can nevertheless cause a permanent partial loss of auditory-nerve peripheral synapses (Kujawa and Liberman, 2009; Liberman et al., 2015). This more moderate type of neuropathy results in a "hidden hearing loss," in which cochlear thresholds, as assayed by either auditory brainstem responses (ABRs) or otoacoustic emissions (OAEs), fully recover, but suprathreshold amplitudes of the ABR wave I, which represent the summed activity of auditory nerve fibers, are permanently reduced. This disconnect between threshold and suprathreshold pathophysiology arises because the noise preferentially damages the subset of auditory nerve fibers with higher thresholds and lower spontaneous firing rates (low- and medium-SR fibers), which constitute ~40% of the auditory nerve population (Furman et al., 2013).

This type of cochlear neuropathy is an important component of age-related hearing loss (Sergeyenko et al., 2013), noise-induced hearing loss (Kujawa and Liberman, 2009), and other types of acquired sensorineural hearing loss (e.g., Ruan et al., 2014). Behavioral audiograms can remain unaffected by neuropathy until it is near-total (~80–90%) thanks to the redundancy of IHC innervation (Schuknecht and Woellner, 1955; Lobarinas et al., 2013). However, more moderate neuropathies, while unlikely to cause perceptual impairment as profound as classic auditory neuropathy, are likely to cause significant deficits in hearing-in-noise, particularly if low-

* Corresponding author. Eaton Peabody Laboratories, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA.
E-mail address: Michelle_Valero@meei.harvard.edu (M.D. Valero).

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SR fibers are preferentially lost, due to the relative insensitivity of low-SR fibers to masking by continuous noise (Costalupes et al., 1984).

Low-SR afferents may also be important drivers of the medial olivocochlear reflex (MOCR: Liberman, 1988, 1991; Ye et al., 2000) and the middle-ear muscle reflex (MEMR: Liberman and Kiang, 1984; Roullier et al., 1986; Kobler et al., 1992). These reflexes can reduce the sound-evoked excitation of inner hair cells by either decreasing the gain of the cochlear amplifier, as is the case for the MOCR, or increasing the impedance of the middle ear, as is the case for the MEMR. The effective stimulus attenuation from either reflex can protect the cochlea from damaging sounds (MEMR: Simmons, 1960; Borg, 1966; MOCR: Rajan, 1995; Reiter and Liberman, 1995). A trauma-induced impairment of the reflexes may initiate a vicious cycle wherein reduced reflex strength worsens cochlear damage, which further reduces the strength of the negative feedback, which may ultimately lead to hair cell loss and permanent threshold shifts (Wang and Ren, 2012). Furthermore, because both MOC and MEM reflexes can enhance signal detection in noise (e.g., Kawase et al., 1993; Pang and Guinan, 1997; respectively), their weakening could exacerbate any hearing-in-noise and word-recognition deficits arising from a loss of low-SR fibers.

Neurotrophin overexpression can repair noise-induced neuropathy in transgenic mice (Wan et al., 2014), but a major impediment to the application of neurotrophin therapies to humans is the inability to diagnose this subtotal primary neural degeneration. The amplitude of ABR wave I is a useful indicator of neuropathy in animals and has been shown to scale well with synaptic loss as documented in post-mortem histopathology (Kujawa and Liberman, 2009; Furman et al., 2013). However, although group differences in the ABR wave-I amplitude can be detected in humans (e.g., Schaette and McAlpine, 2011), they are highly variable in the clinic, which may limit their diagnostic utility (Gorga et al., 1988; Nikiforidis et al., 1993). The envelope-following response may be a more robust diagnostic tool (Plack et al., 2014; Shaheen et al., 2015), but a battery of short-duration tests may be most useful in differential diagnoses.

If the MOC and MEM reflexes are driven by the low- and medium-SR fibers that are selectively destroyed in cochlear neuropathy, then some measure of reflex strength might serve as a useful, non-invasive, and objective assay to aid in the detection of hidden hearing loss in humans. In the present study, we tested this hypothesis by measuring MOC and MEM reflexes in anesthetized mice with noise-induced cochlear neuropathy.

2. Materials and methods

2.1. Animals and groups

Male CBA/CaJ mice were used in this study. Group 1 (n = 12) mice were received at 6 wks. At 7 wks, half the animals were exposed to neuropathic noise. Cochlear function tests (see below) were measured 2 wks post exposure, and at least 2 days after that, contra-noise modulation of DPOAEs was measured. The cochleas were then harvested from a subset of exposed and control mice for histological analysis. Group 2 (n = 8) mice were received at 10 wks. At 16 wks, half were exposed to neuropathic noise. Cochlear function and contra-noise modulation of wideband acoustic reflectance was measured at 24 h, and 1, 2, 4, and 8 wks post exposure. The cochleas were then harvested for histological analysis. When required, curare alone or curare with strychnine was injected intramuscularly at 4 mg/kg and 10 mg/kg, respectively, after tracheostomy and mechanical ventilation. For all physiological measurements, mice were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) and placed inside an electrically and acoustically shielded room maintained at 30 °C. Heart rate was monitored via the ABR electrodes. Booster injections (1/3 of the original dose) were given when whisking-related noise was observed on the ABR trace, which was typically 30–45 min following the last injection. Curarized animals were given boosters at 30 min intervals. All procedures were approved by the Animal Care and Use Committee at the Massachusetts Eye and Ear Infirmary.

2.2. Noise exposure

Awake mice were placed unrestrained inside a mesh container within a reverberant chamber and were presented with spectrally flattened, octave-band noise (8–16 kHz) for two hours. The SPLs were 97.5 and 99 dB SPL for Group 1 and Group 2, respectively.

2.3. Stimulus calibration

Acoustic systems were regularly calibrated in a small custom coupler by comparing the output voltage of the probe tube microphone to that of a calibrated ¼ condenser microphone (Larsen-Davis Type 2530). For each subject and each placement of the acoustic system, the ear-canal SPL was calibrated with a moderate-level chirp stimulus to determine the transducer voltage required to produce the target SPLs.

2.4. Cochlear function tests

For DPOAEs, f_1 and f_2 primary tones ($f_2/f_1 = 1.2$) were presented with f_2 varied between 5.6 and 45.2 kHz in half-octave steps and $L_1-L_2 = 10$ dB. At each f_2, L_2 was varied between 10 and 80 dB SPL in 10-dB increments. DPOAE threshold was defined as the L_2-level eliciting a DPOAE of magnitude 5 dB SPL. Stimuli were generated with 24-bit digital I–O cards (National Instruments PXI-4461) in a PXI-1042Q chassis, amplified by an SA-1 speaker driver (Tucker–Davis Technologies, Inc.), and delivered from two electrostatic drivers (CUI CDMG15008-03A) in our custom acoustic system. An electret microphone (Knowles FG-23329-P07) at the end of a small probe tube was used to monitor ear-canal sound pressure. For ABRs, tone pips (5-msec duration, 0.5-msec ramp) were presented in alternating polarity at 40 Hz using the acoustic assembly described above. At each test frequency (5.6–45.2 kHz in 1/2-octave steps), stimulus levels were incremented from 10 to 80 dB SPL. Responses were measured from needle electrodes in vertex-to-pinna configuration with the ground just above the tail. A Grass pre-amplifier (Model P511) amplified (10,000X) and band-pass filtered (0.3–3 kHz) the ABR waveforms. 1024 artifact-free waveforms (512 of each polarity) were averaged to produce the final ABR trace. Threshold was defined by visual inspection of the stacked waveforms. Wave-I amplitude was defined as the difference between the maximum of the peak and the minimum of the subsequent trough.

2.5. Modulation of DPOAEs by contralateral noise (group 1)

DPOAE-eliciting stimuli were presented to the ipsilateral ear at $f_2 = 32$ kHz ($f_2/f_1 = 1.2$). First, DPOAEs were measured in a 13 × 13 level-matrix (1-dB resolution) with primary tones presented continuously for 0.5 sec at each L_1-L_2 combination. Then, a 6 × 6 level matrix, centered at the appropriate L_1-L_2 combination (see Results), was measured in 1-dB resolution. For the second matrix, primary tones were presented continuously for 7 sec at each L_1-L_2 combination, and the contralateral noise was presented during the 6th second (5-msec ramp). The contralateral noise was spectrally flattened, 2 octave-band noise centered at f_2 and presented at 95 dB SPL. A 5-sec period of silence was interposed between each level step.