The Impacts of Noise Exposure on the Middle Ear Muscle Reflex in a Veteran Population

Naomi F. Bramhall^{a,b}, Kelly M. Reavis^a, M. Patrick Feeney^{a,b}, and Sean D. Kampel^a

^a VA RR&D National Center for Rehabilitative Auditory Research (NCRAR), VA Portland Health Care System, Portland, OR, USA

^b Department of Otolaryngology/Head & Neck Surgery, Oregon Health & Science University, Portland, OR, USA

Emails:

naomi.bramhall@va.gov

kelly.reavis@va.gov

patrick.feeney@va.gov

sean.kampel@va.gov

All correspondence should be addressed to:

Naomi Bramhall, 3710 SW US Veterans Hospital Road (P5 NCRAR), Portland, OR 97239, USA. E-mail: naomi.bramhall@va.gov, Phone: 503-220-8262 x54692

Declarations of interest: None

Abstract

Noise-induced cochlear synaptopathy, the loss of the synaptic connections between inner hair cells and afferent auditory nerve fibers, has been demonstrated in multiple animal models, including non-human primates. However, given that synaptopathy can only be confirmed with post-mortem temporal bone analysis, it has been difficult to determine whether noise-induced synaptopathy occurs in humans. Human studies of noise-induced synaptopathy using physiological indicators identified in animal models (auditory brainstem response [ABR] wave I amplitude, the envelope following response [EFR], and the middle ear muscle reflex [MEMR]) have yielded mixed findings. Differences in the population studied may have contributed to the differing results. For example, due to differences in the intensity level of the noise exposure, noise-induced synaptopathy may be easier to detect in a military Veteran population than in populations with recreational noise exposure. We previously demonstrated a reduction in ABR wave I amplitude and EFR magnitude for young Veterans with normal audiograms reporting high levels of noise exposure compared to non-Veteran controls. In this report, we expand on the previous analysis in the same population to determine if MEMR magnitude is similarly reduced. The results of the statistical analysis, although not conventionally statistically significant, suggest a reduction in mean MEMR magnitude for Veterans reporting high noise exposure compared with non-Veteran controls. In addition, the MEMR appears relatively insensitive to subclinical outer hair cell dysfunction and is not well correlated with ABR and EFR measurements. When combined with our previous ABR and EFR findings in the same population, these results suggest that noise-induced synaptopathy occurs in humans. In addition, the findings indicate that the MEMR may be a good candidate for

non-invasive diagnosis of cochlear synaptopathy/deafferentation and that the MEMR may reflect the integrity of different neural populations than the ABR and EFR.

Keywords: cochlear synaptopathy, hidden hearing loss, middle ear muscle reflex, acoustic reflex, stapedius muscle reflex, noise-induced hearing loss

1. Introduction¹

Noise-induced loss of the synapses between the inner hair cells (IHCs) and the afferent auditory nerve fibers, or cochlear synaptopathy, has been demonstrated in a variety of animal models, including non-human primates (Kujawa & Liberman 2009; Lin et al. 2011; Hickox et al. 2017; Valero et al. 2017). Although temporal bone studies show that age-related cochlear synaptic loss occurs in humans (Viana et al. 2015; Wu et al. 2019), and that synaptic and neuronal loss can occur even when hair cells are still present (Makary et al. 2011; Viana et al. 2015; Wu et al. 2019), quantification of cochlear synapses in living humans is not currently possible. Instead, non-invasive physiological measures have been used as indirect indicators of potential synaptopathy. Wave I amplitude of the auditory brainstem response (ABR) has been the focus of many human studies of synaptopathy because reduction in wave I amplitude was associated with synaptopathy in many of the early animal studies (e.g., Kujawa & Liberman 2009;

¹ Abbreviations: ABR – Auditory brainstem response; AvgDP – Average DPOAE level from 3-8 kHz; BBN – broadband noise; DST – decreased sound tolerance; EFR – Envelope following response; EHF – extended high frequency; LENS-Q – Lifetime exposure to noise and solvents questionnaire; MEMR – Middle ear muscle reflex; MOS – military occupational specialty; NCRAR – National Center for Rehabilitative Auditory Research; peSPL – peak equivalent sound pressure level; SAM – Sinusoidally amplitude modulated; SR – Spontaneous rate; TPP – tympanometric peak pressure; VA – Veterans Affairs; VAPORHCS – VA Portland Health Care System

Furman et al. 2013; Sergeyenko et al. 2013; Fernandez et al. 2015). Several human studies have investigated the relationship between noise exposure and ABR wave I amplitude in young people with normal audiograms, with mixed results. Relationships between noise exposure and ABR wave I amplitude have been demonstrated in Veterans and non-Veterans who report ever using a firearm (Bramhall et al. 2017; Bramhall et al. 2020a), while studies of recreational noise exposure have not demonstrated clear relationships with wave I amplitude (e.g., Fulbright et al. 2017; Grinn et al. 2017; Grose et al. 2017; Prendergast et al. 2017). This suggests that firearm use and other types of military noise exposure may be more likely to lead to synaptopathy than common sources of recreational noise exposure, such as music concert attendance. Another factor that may have contributed to the differing results across studies is that ABR wave I amplitude can be impacted by sex and outer hair cell (OHC) damage. Females tend to have larger ABR wave I amplitudes than males, in part due to differences in head size (Trune et al. 1988; Mitchell et al. 1989). Computational modeling suggests that due to cochlear gain loss, OHC dysfunction leads to a steepening of the ABR wave I amplitude growth function so that at lower stimulus levels, ABR wave I amplitude is reduced, while at higher stimulus levels wave I amplitude is similar or bigger in individuals with OHC loss versus those with normal OHC function (Verhulst et al. 2018). The impact of sex and OHC dysfunction on ABR wave I amplitude complicates the interpretation of this measure as a metric of synaptopathy, especially given that OHC loss may co-exist with synapse loss in many cases (Otte et al. 1978; Felder & Schrott-Fischer 1995). In addition, the results of Bourien et al. (2014) suggest that because the ABR is an onset response, ABR wave I is dominated by high

spontaneous rate (SR)/low threshold auditory nerve fibers and low SR/high threshold fibers have a limited contribution. Given that animal models demonstrate that low SR auditory nerve fibers are the most vulnerable to cochlear synaptopathy (Schmiedt et al. 1996; Furman et al. 2013), ABR wave I amplitude may lack sensitivity as a measure of noise or age-related synaptopathy. These factors have led to the search for an alternative or supplementary physiological indicator of synaptopathy.

More recent animal studies have suggested that there are other non-invasive measures that are sensitive to synaptopathy, such as the envelope following response (EFR) and the wideband middle ear muscle reflex (MEMR) (Shaheen et al. 2015; Valero et al. 2016; Valero et al. 2018). However, because noise exposure can affect OHC function in addition to cochlear synapses, noise exposure-related OHC damage could impact ABR, EFR, and MEMR measures, making the relationship between noise exposure and cochlear synaptopathy more difficult to assess (**Figure 1**). In this respect, both the EFR and MEMR have potential advantages over ABR wave I amplitude. Computational modeling suggests that among individuals with clinically normal hearing, the impact of OHC damage on EFR magnitude is minimal (Encina-Llamas et al. 2019) and we recently demonstrated reduced EFR magnitudes among young Veterans with clinically normal audiograms reporting high levels of previous noise exposure compared to non-Veteran controls, even after statistical adjustment for sex and average distortion product otoacoustic emission (DPOAE) levels (Bramhall et al. 2020a). The MEMR appears to be relatively insensitive to pure tone threshold elevation, with reductions in MEMR threshold for a tonal elicitor only observed when thresholds are poorer than approximately 50 dB HL (Gelfand et al. 1990). Furthermore, a single unit physiology

study in rabbits suggests that the MEMR is generated by the tails of auditory nerve tuning curves (Borg et al. 1990), which should be relatively insensitive to OHC dysfunction. The MEMR is particularly attractive as a potential non-invasive physiological indicator of synaptopathy because the test is more rapid than the ABR or EFR and the MEMR is already part of the standard audiological test battery.

The MEMR is characterized by frequency-specific changes in middle ear impedance in response to suprathreshold ipsilateral or contralateral auditory stimulation and can be measured using a wideband probe stimulus to obtain admittance, power, and absorbance measurements across a wide frequency range (reviewed in Schairer et al. 2013). Unlike ABR wave I amplitude, previous studies of the MEMR have not demonstrated an effect of sex on reflex threshold (Jerger et al. 1972; Osterhammel & Osterhammel 1979). Hall et al. (1982) found increased MEMR magnitude in females compared with males, but only for a 500-1500 Hz noise-band stimulus, not for a 1000 or 4000 Hz signal.

The MEMR is dependent on auditory nerve input and is often absent in individuals with pathologies impacting the auditory nerve such as auditory neuropathy or vestibular schwannoma (Prasher & Cohen 1993; Berlin et al. 2005). It has been suggested that the low SR auditory nerve fibers that appear most vulnerable to noise-induced synaptopathy (Furman et al. 2013) play a key role in driving the MEMR (Liberman & Kiang 1984; Kobler et al. 1992). This suggests that the MEMR may be particularly sensitive to synaptopathy. Valero et al. showed a reduction in MEMR magnitude and MEMR threshold in both anesthetized and unanesthetized mice with confirmed noise-induced synaptopathy when compared to controls (2016; 2018). They

also found that MEMR threshold, but not ABR wave I amplitude, was sensitive to mild synaptopathy (defined as a loss of $\leq 15\%$ of synapses).

A number of human studies have shown relationships between MEMR measurements and noise exposure history. In a sample of adults age 21-54 years with normal audiograms, Shehorn et al. (2020) found that increased self-reported lifetime noise exposure was associated with a reduction in wideband MEMR magnitude. These results suggest that wideband MEMR may be a physiological indicator of synaptopathy in humans. Houghton et al. (1988) observed a reduction in MEMR magnitude in members of the Royal New Zealand Air Force with noise-induced hearing loss (NIHL), defined as an audiometric notch at 4 or 6 kHz, versus control subjects with normal audiograms and less noise exposure. Some of the individuals with NIHL had pure tone thresholds exceeding 50 dB HL, which may have resulted in the reduction of mean reflex amplitudes for that group. However, six of the NIHL subjects had unilateral hearing loss, yet both ipsilateral and contralateral MEMR magnitudes in their normal hearing ear were reduced compared to those in the control group. In addition, a recent retrospective analysis of VA audiological data found that among a sample of 235,091 Veterans with normal audiograms, 31.7% had abnormal clinical contralateral MEMR thresholds, defined as a threshold at 1000 Hz of greater than 95 dB HL (Billings et al. 2018). This indicates that elevated MEMR thresholds are quite common in noise exposed populations, even when audiometric thresholds are normal. These findings suggest a noise exposure-related reduction in MEMR magnitude that is independent of pure tone thresholds, as would be expected to result from synaptopathy. In contrast, Guest et al. (2019) found only weak and inconsistent relationships between clinical

MEMR threshold and noise exposure history in a sample of young adults with normal audiograms, although the noise exposure scores from this sample suggest that the degree of noise exposure may have been more limited than in other studies.

Several studies have found relationships between MEMR measurements (MEMR threshold or magnitude) and predicted perceptual consequences of synaptopathy (Kujawa & Liberman 2015), such as tinnitus and difficulty with complex speech perception. Wojtczak et al. (2017) showed a reduction in wideband MEMR magnitude in adults aged 25-63 years with noise-induced tinnitus and up to a mild hearing loss, even after statistically adjusting for pure tone thresholds. Although ABR wave I amplitude was not measured in the Wojtczak et al. study, other studies have shown a relationship between tinnitus and reduced ABR wave I amplitude (Schaette & McAlpine 2011; Gu et al. 2012; Bramhall et al. 2018; Bramhall et al. 2019), suggesting a link between synaptopathy (or other forms of cochlear deafferentation such as IHC or spiral ganglion cell loss) and tinnitus. In a sample of individuals aged 18-63 years with normal audiograms, Mepani et al. (2020) found that higher clinical and wideband MEMR thresholds were correlated with poorer performance on complex speech perception tasks, such as identification of speech in background noise and time compressed speech. In a sample of adults aged 21-54 years with normal audiograms, Shehorn et al. (2020) showed that lower wideband MEMR magnitudes were associated with poorer performance on a speech-in-noise perception task, particularly when the stimuli were presented at high intensity levels. In contrast, in a sample of young adults (aged 18-39 years) with normal audiograms, Guest et al. (2019) observed a trend of lower clinical MEMR thresholds (for a 4 kHz stimulus) in individuals with tinnitus compared to

controls, although this was not statistically significant. However, they did find a weak, but not statistically significant, correlation between speech-in-noise perception and MEMR threshold for a 4 kHz stimulus, with poorer thresholds associated with higher MEMR thresholds.

In this study, contralateral wideband MEMR, ABR wave I amplitude, EFR, and DPOAEs were measured in young military Veterans and non-Veterans with normal audiograms. The ABR and EFR results have been described previously (Bramhall et al. 2020a). This report focuses on the MEMR results and the relationships between the physiological measures. The hypotheses were that 1) Veterans with high levels of self-reported noise exposure would have reduced MEMR magnitudes and elevated MEMR thresholds compared to non-Veteran controls with minimal noise exposure, 2) MEMR measurements would be relatively insensitive to subclinical OHC dysfunction (i.e. OHC dysfunction in individuals with clinically normal audiograms), as indicated by DPOAE levels, and 3) MEMR magnitude would be correlated with ABR wave I amplitude and EFR magnitude.

2. Material and methods

2.1 Participants

MEMR data from 92 participants (57 Veterans and 35 non-Veterans) aged 19-35 years were analyzed in this study. ABR wave I amplitude and EFR data for many of the participants from this sample were described previously (Bramhall et al. 2020a). Potential participants were recruited from previous studies conducted at the National Center for Rehabilitative Auditory Research (NCRAR), from a database of young Veterans seen at the VA Portland Health Care System (VAPORHCS), and by posting

fliers at the VAPORHCS and at Portland area colleges and universities. All participants completed an audiometric evaluation including tympanometry, air and bone conduction thresholds, and DPOAEs. Inclusion criteria included: normal tympanograms (peak pressure $\pm$ 50 daPa for a 226 Hz tone, compliance between 0.3-1.5 ml), no air-bone gaps > 15 dB and no more than one air-bone gap equal to 15 dB, normal pure tone thresholds (no audiometric thresholds poorer than 20 dB HL from 0.25-8 kHz), no evidence of a noise notch (threshold at 1 or 2 frequencies between 3-6 kHz that is 15 dB or poorer than the adjacent frequencies), normal DPOAEs from 1.5-6 kHz (compared to normative values (Gorga et al. 1997, Table A1)), no significant history of otologic or neurologic disorder (including traumatic brain injury), and no history of firearm use (for non-Veterans only). Individuals reporting decreased tolerance to loud sounds were also excluded because we previously found physiological evidence of hyperactivity early in the auditory pathway among individuals reporting decreased sound tolerance (Bramhall et al. 2020b), which could complicate the interpretation of the MEMR data. After the screening evaluation, all subsequent test measures were completed in a single ear. If only one ear met the study criteria (based on audiometric air and bone conduction thresholds, DPOAEs, and tympanometry), that ear was tested. If both ears qualified for the study, the ear with higher level DPOAEs was tested to minimize the effects of subclinical OHC damage. Among the individuals who qualified in a single ear, the ear that didn't qualify for the study still met the tympanometric and air-bone gap inclusion criteria, with the exception of one participant with high compliance (2.1 ml) and one participant with air-bone gaps, both of whom were excluded from the analysis because middle ear dysfunction in the probe ear could impact contralateral

MEMR measurements. All participants provided written informed consent and were paid for their participation. All study procedures were approved by the VA Portland Health Care System Institutional Review Board.

2.2 Procedures

2.2.1 Audiometry

Pure tone thresholds were assessed in all potential participants from 0.25-8 kHz as part of the screening evaluation. Extended high frequency thresholds from 9-16 kHz were also measured using Sennheiser HDA 200 headphones (Old Lyme, CT), but were not used to determine study inclusion.

2.2.2 Distortion product otoacoustic emissions (DPOAEs)

DPOAE testing was conducted using a custom system that includes an ER-10 B+ probe microphone and EMAN software from Boys Town National Research Hospital (Neely & Liu 1993). DPOAE stimuli were presented at a fixed primary frequency ratio $f_1/f_2=1.2$ and responses were obtained using a primary frequency sweep (DP-gram) from 1-8 kHz in 1/6-octave increments at stimulus frequency levels of $L_1=65$ and $L_2=55$ dB SPL. Responses were compared to the DPOAE levels from a distribution of individuals with abnormal pure tone thresholds (Gorga et al. 1997, Table A1). Only individuals at or above the 90th percentile from 1.5-6 kHz were included in the study. These DPOAE criteria were somewhat relaxed in comparison to our previous study (Bramhall et al. 2017; Bramhall et al. 2018) to improve recruitment numbers. Measurement based stopping rules were employed in which averaging continued until 48 seconds of artifact-free data were collected, until the noise floor was below -30 dB SPL, or the signal-to-noise ratio was ≥ 30 dB.

2.2.3 Wideband middle ear muscle reflex (MEMR)

Contralateral wideband MEMR measurements were obtained using an Interacoustics Wideband Research System consisting of an Interacoustics AT235 tympanometer (Eden Prairie, MN) with modified firmware as described in Liu et al. (2008) and Feeney et al. (2017). A wideband tympanogram with upswept and downswept pressures from +200 to -300 daPa was obtained in the probe ear prior to MEMR testing and tympanometric peak pressure (TPP) was averaged across the two sweep conditions as described in Keefe et al. (2015). MEMRs were measured at average TPP rather than at ambient pressure because MEMRs measured at TPP have been shown to have better test-retest reliability (Feeney et al. 2017) and MEMR measures are less likely to be impacted by measurement error due to individual differences in TPP. The MEMR was measured by presenting two pulses of five clicks to the probe ear, with four pulsed activators (presented to the stimulus ear) interspersed between each pair of clicks, and then calculating the difference in absorbed sound power between the initial baseline click and each subsequent click as described in Keefe et al. (2017). The probe stimulus consisted of wideband clicks (0.2-8 kHz). Elicitor stimuli consisted of broadband noise (BBN, low pass filtered at 8 kHz) ranging from 55-100 dB SPL in 5 dB steps. The duration of the BBN pulse was 116 msec, as described in Feeney et al. (2017). MEMR threshold was calculated as described by Keefe et al. (2017). Change in absorbed power across frequency was calculated at each elicitor level as the sum of the absolute value of the change in absorbed power for frequencies below 2500 Hz, following Keefe et al.'s calculation of ΔL_{W1} . The natural logarithm of change in absorbed power across frequency was used to eliminate heteroskedasticity

and normalize skewed distributions of residuals. This value is referred to as MEMR magnitude in the descriptions of the statistical analysis and results. All other measurements (pure tone thresholds, DPOAEs, ABR, EFR) were obtained in the ear where the elicitor was presented (i.e., the stimulus ear).

2.2.4 Auditory brainstem response (ABR)

ABR testing was completed in 76 of the 92 study participants using an Intelligent Hearing Systems SmartEP system (Miami, FL) and Etymotic Research gold foil ER3-26A tiptrode electrodes (Elk Grove Village, IL) placed in the ear canal as described in Bramhall et al. (2020a). Waveforms were generated using alternating polarity 4 kHz toneburst stimuli presented at 3 levels (90, 100, and 110 dB peSPL). A stimulus repetition rate of 11.1/sec was used and at least 2 replications of each waveform were obtained. Electrode impedance was less than or equal to 5.0 k Ω in all but four participants, for whom impedances were no greater than 7.0 k Ω . Positive peaks and the following negative trough for wave I was initially identified with an automated Python-based peak picking program (adapted from Buran 2015). Peaks and troughs were then evaluated by two experienced audiologists and reassigned if both were in agreement. Wave I amplitude was defined as the difference between the voltage at the positive peak and the voltage at the following negative trough. ABR wave I amplitude was unscorable for one participant at 90 dB peSPL and was not collected for one participant at 110 dB peSPL due to tester error, resulting in data for 75 participants at these stimulus levels.

2.2.5 Envelope following response (EFR)

EFRs were recorded in 77 of the 92 study participants using Intelligent Hearing Systems (Miami, FL) SmartEP-CAM as described in Bramhall et al. (2020a). Electrode impedance was less than or equal to 5.0 k Ω in all but four participants, who had impedances no greater than 8.5 k Ω . Stimuli consisted of 80 dB SPL 4 kHz tones sinusoidally amplitude modulated (SAM) at 110 Hz. Continuous electroencephalography was epoched and processed in MATLAB (MathWorks, Natick, MA) as described in Bramhall et al. (2020a). The EFR magnitude at 110 Hz was calculated as a ratio of the signal and noise voltages (i.e. a signal-to-noise ratio [SNR]), expressed in dB.

2.2.6 Hearing questionnaire

The presence of tinnitus was assessed by asking all participants the question, “Do you have constant or frequent ringing in the ears?”. Non-Veterans who responded “yes” were excluded from the analysis to limit the likelihood of individuals with auditory dysfunction being included in the control group. Relationships between ABR, EFR, and MEMR measurements and report of tinnitus will be addressed in a separate paper.

2.2.7 Assessment of noise exposure history

As described in Bramhall et al. (2020a), the Lifetime Exposure to Noise and Solvents Questionnaire was used to assess lifetime noise exposure in all study participants (LENSQ; Bramhall et al. 2017; Gordon et al. 2017; Griest-Hines et al. 2021). Two Veterans did not report firearm use on the LENS-Q, which was most likely a reporting error because military Service members typically complete firearms training during basic training. Non-Veterans who reported ever using a firearm were excluded from the study because firearm use is a risk factor for synaptopathy (Bramhall et al. 2017). The LENS-Q was scored as described in Griest-Hines et al. (2021), except that

military occupational specialty (MOS), a descriptor of the job performed during military service, was not included in the LENS-Q score. The Veterans Benefits Administration has rated each MOS according to the probability of experiencing hazardous noise exposure while performing job duties (low, medium, or high; VBA 2010). Veteran participants were divided into two groups (Veteran High Noise and Veteran Medium Noise) based on overall LENS-Q score and reported MOS. The Veteran High Noise group consisted of Veterans with an MOS rated as having a high probability of hazardous noise exposure or a LENS-Q score in the top 25th percentile for all Veterans in the sample. The remaining Veterans were assigned to the Veteran Medium Noise group.

2.3 Statistical analysis

The primary goal of the statistical analysis was to estimate the population mean MEMR magnitude by noise exposure group. Empirical growth plots were constructed to view MEMR magnitude by elicitor level. MEMR magnitudes are expected to naturally vary among individuals and across elicitor levels within ears of the same individual. Because the data are multilevel, with change in MEMR magnitude at each elicitor level nested within participants, we used a liner mixed model (Singer & Willett 2003) to analyze the relationship between noise exposure group and MEMR magnitude for elicitor levels ranging from 55 to 100 dB SPL (collected in 5 dB steps). Finally, to account for natural variation in the growth function, subject-specific elicitor level effects along with a subject random intercept were included. Mixed-effects models allow for the estimation of the average intercept by noise exposure group and elicitor level via fixed effects and for individual deviation from the average via estimation of random effects.

The random effects covariance matrix was modeled as unstructured. A multilevel model of the population mean MEMR magnitudes was used as shown below (Eq. 1):

$$\begin{aligned}\mu_i = & \beta_0 + \beta_1 \cdot \textit{High Noise Group}_i + \beta_2 \cdot \textit{Middle Noise Group}_i + \beta_3 \cdot \textit{Level}_i + \beta_4 \\ & \cdot \textit{High Noise Group}_i \cdot \textit{Level}_i + \beta_5 \cdot \textit{Middle Noise Group}_i \cdot \textit{Level}_i \\ & + \theta_{s[i]}^{\textit{intercept}} + \theta_{s[i]}^{\textit{level}} \cdot \textit{Level}_i\end{aligned}$$

Each observation in the data set is indexed by i . $\textit{Level}_i$ is the elicitor level producing the i th MEMR magnitude. $\textit{High Noise Group}_i$ is an indicator variable taking on a value of one if the noise group attached to the i th observation is equal to “Veteran High Noise” and zero otherwise. Similarly, $\textit{Middle Noise Group}_i$ is an indicator variable taking on a value of one if the noise group attached to the i th observation is equal to “Veteran Middle Noise” and zero otherwise. β is the fixed effect parameter and θ_s is a vector of subject-specific random effects for intercept and elicitor level.

We modeled the effects of noise exposure group on MEMR magnitude, so that the average group difference in MEMR magnitude is the contrast between the model-based population average MEMR magnitude for the Veteran groups (Veteran High Noise and Veteran Middle Noise) minus the model-based population average MEMR magnitude for the reference group (Non-Veteran Control). We estimated the 95% confidence intervals (CI) around the estimates of the average difference in MEMR magnitude.

In previous analyses of the relationship between noise exposure history and physiological indicators of synaptopathy (Bramhall et al. 2017; Bramhall et al. 2020a), we statistically adjusted for OHC function by including DPOAE level as a predictor in our regression model (e.g., AvgDP). However, noise exposure can impact both OHC

function and cochlear synaptic integrity, as indicated in **Figure 1**. Because DPOAE levels, an indicator of OHC function, can be impacted by noise exposure, if we add AvgDP as a predictor in our model, this will adjust out a portion of the noise exposure effect on MEMR magnitude. This problem is referred to as post-treatment bias (Montgomery et al. 2018). For measurements of EFR magnitude and ABR wave I amplitude in response to high intensity level stimuli, computational modeling suggests that the impacts of subclinical OHC dysfunction will be minimal (Verhulst et al. 2018; Encina-Llamas et al. 2019) and adjustment for OHC function may not be necessary. However, the impact of subclinical OHC dysfunction on the MEMR has not been explored either experimentally or with computational modeling. Given concerns that OHC function may impact MEMR magnitude, we assessed the relationship between average DPOAE level from 3-8 kHz (AvgDP) and MEMR magnitude. This required expanding the model shown in Eq. 1 to include the fixed effects of AvgDP and the interaction between AvgDP and noise exposure group. This mixed-effects model allowed for estimation of the mean MEMR magnitude at AvgDP levels set to -5, 0, and 5 dB SPL and at each elicitor level. Individual deviation from the averages were estimated via random effects, while controlling for noise exposure group.

All data management and analyses were conducted using SAS (SAS Institute Inc., Cary, NC, USA), version 9.4.

3. Results

3.1 Noise exposure group characteristics

A summary of the participant characteristics for each noise exposure group is shown in **Table 1**. Mean age is similar across the three groups. The Non-Veteran

Control and Veteran Medium Noise groups are well-balanced in terms of sex, while the Veteran High Noise group is dominated by males. The Non-Veteran Control group has the lowest mean LENS-Q score (indicating less noise exposure) and the Veteran High Noise group has the highest mean LENS-Q score. Because LENS-Q score is on a log scale, a one point difference in LENS-Q score represents a 10-fold difference in noise exposure. Therefore, the Veteran High Noise group has a mean reported noise exposure history that is more than 10 times that of the Veteran Medium Noise group. Twenty-six of the 35 participants in the Veteran High Noise group reported an MOS that is rated as having a high probability of hazardous noise exposure. Twenty Veterans (5 Medium Noise and 15 High Noise) reported tinnitus in the test ear. Mean pure tone averages (PTAs), for both the conventional frequencies and the extended high frequencies (EHFs), differ by less than 5 dB across groups. Audiometric thresholds from 0.25 to 16 kHz are plotted by noise exposure group in **Figure 2**. The largest contrasts in mean pure tone thresholds between the Veteran High Noise and Non-Veteran Control groups are at 6, 12.5, 14, and 16 kHz (5.4, 6.8, 8.6, and 5.6 dB, respectively), with poorer mean thresholds for the Veteran High Noise group. All other group contrasts are less than 4 dB. Mean AvgDP is 1.8 dB lower for the Veteran High Noise group than for the Non-Veteran Control group. Individual and group mean DPOAE levels are plotted in **Figure 3**. Mean DPOAE levels from 1-8 kHz differ by less than 5 dB across groups, with the biggest difference at 8 kHz, where the Veteran High Noise group has a mean DPOAE level that is 4.2 dB less than that of the Non-Veteran Control group.

3.2 MEMR by noise exposure group

Mean MEMR thresholds, MEMR growth functions, and MEMR spectra are plotted by noise group in **Figure 4**. Five study participants had no MEMR at the maximum elicitor level (100 dB SPL), one from the Non-Veteran Control group and four from the Veteran High Noise group. For the purpose of plotting MEMR threshold, these participants were assigned a threshold of 105 dB SPL. Mean MEMR thresholds (green triangles) are higher for the Veteran High Noise group than for the other noise exposure groups (**Figure 4A**). MEMR growth functions (**Figure 4B**) show lower mean MEMR magnitude for the Veteran High Noise group compared with the Non-Veteran Control group for elicitor levels of 70 dB SPL and higher. Likewise, mean MEMR spectra for a 100 dB SPL elicitor (**Figure 4C**) indicate a smaller negative change in absorbed power from 710-1400 Hz and a smaller positive change from 2000-2800 Hz for the Veteran High Noise group compared with the Non-Veteran Control group, indicating a weaker MEMR.

3.3 Modeled relationship between noise exposure group and MEMR magnitude

The modeled contrast in mean MEMR magnitude between the Non-Veteran Control and Veteran High Noise groups increases with elicitor level, with lower MEMR magnitudes for the Veteran High Noise group (**Figure 5**). At the highest elicitor level (100 dB SPL), the model results show a 0.29 dB reduction in mean MEMR magnitude for the Veteran High Noise group compared to the Non-Veteran Control group. To put this into context, the modeled mean MEMR magnitude for the Non-Veteran Control group in response to a 100 dB SPL elicitor is 1.14 dB (95% confidence interval = 0.82 to 1.46 dB). Using the parameter estimate of 0.29 dB as our best approximation of the true difference between groups, this represents a magnitude reduction of ~25% for the

Veteran High Noise group. Note however, that for a 100 dB SPL elicitor, the confidence intervals are consistent with a true difference between groups of anywhere from a 0.74 dB decrease in MEMR magnitude for the Veteran High Noise group compared with the Non-Veteran Control group to a 0.16 dB increase. The modeled mean MEMR magnitude contrasts between the Non-Veteran Control and the Veteran Medium Noise groups are close to zero at all elicitor levels and the confidence intervals range from approximately -0.5 to 0.5 dB. Modeled mean MEMR magnitude contrasts between the Veteran groups and the Non-Veteran Control group for each elicitor level are shown in **Table 2**.

3.4 Modeled relationship between average DPOAE level and MEMR magnitude

To investigate the effect of OHC dysfunction on MEMR magnitude, we modeled the relationship between AvgDP and MEMR magnitude as a function of MEMR elicitor level. One might expect that if there was an association between OHC function and MEMR magnitude, it would be most apparent at lower elicitor levels where cochlear amplification is greatest and minimal at higher elicitor levels (> 80 dB SPL), where the basilar membrane response is more linear (Ruggero et al. 1997; Robles & Ruggero 2001). The model included an interaction effect between AvgDP and noise exposure group so that the relationship between AvgDP and MEMR magnitude could vary by noise exposure group. The model results are plotted in **Figure 6** and the slopes defining the relationship between AvgDP and MEMR magnitude for each noise exposure group and elicitor level can be found in **Table 3**. The results show an increase in MEMR magnitude as AvgDP increases only for the two Veteran groups, although the lower bound of the estimated slopes crosses zero, so there is not enough evidence to

overturn the null hypothesis of no association between AvgDP and MEMR magnitude. In contrast, MEMR magnitude shows little variation with AvgDP at the lower elicitor levels (55-75 dB SPL) in the Non-Veteran Control group and increases slightly with increased AvgDP at the higher elicitor levels. For the Non-Veteran Control group, an increase of 1 dB in AvgDP is associated with -0.8 to 0.6% growth in change in absorbed power across frequency for elicitor levels from 55-75 dB SPL.

3.5 Relationship between MEMR, ABR, and EFR measures

Relationships between the three physiological indicators of synaptopathy (MEMR magnitude, ABR wave I amplitude, and EFR magnitude) are plotted in **Figure 7**. For these plots, ABR wave I amplitude at 90 dB peSPL was chosen because modeled group differences between the Non-Veteran Control and Veteran High Noise groups were largest at this stimulus level (Bramhall et al. 2020a). EFR magnitude for a modulation depth of 40% was used because shallower modulation depths have been predicted to be more sensitive to loss of synapses with low SR auditory nerve fibers (Bharadwaj et al. 2014). Finally, MEMR magnitude for a 100 dB SPL elicitor was selected because the model results from section 3.3 suggest the biggest MEMR magnitude differences between the Non-Veteran Control and Veteran High Noise groups at this level. ABR wave I amplitude and EFR magnitude were positively correlated (Pearson correlation coefficient = 0.40, p-value = 0.0002), while MEMR magnitude was not significantly correlated with ABR wave I amplitude or EFR magnitude.

4. Discussion

4.1 Noise exposure-related reductions in MEMR magnitude are consistent with ABR and EFR results

The model results suggest a reduction in mean MEMR magnitude for the Veteran High Noise compared to the Non-Veteran Control group that increases with elicitor level, with a mean decrease for a 100 dB SPL elicitor of 25%. Although this contrast is not conventionally statistically significant because the confidence interval overlaps zero, the parameter estimate is consistent with the reduction in MEMR strength observed in a mouse model of noise-induced synaptopathy (Valero et al. 2018) and with previous findings of reduced ABR wave I amplitude and EFR magnitude for the Veteran High Noise group compared with the Non-Veteran Control group in the same sample (Bramhall et al. 2020a). Taken together, noise exposure-related reductions across these three different physiological indicators of synaptopathy suggest that noise-induced cochlear synaptopathy occurs in humans, at least in populations with a history of military noise exposure.

4.2 MEMR magnitude does not appear to be impacted by subclinical OHC dysfunction

The lack of an association between AvgDP and MEMR magnitude in the Non-Veteran Control group, particularly at the lower elicitor levels where cochlear amplification is greatest, suggests that the relationship between AvgDP and MEMR magnitude in the Veteran groups is related to noise exposure effects on both AvgDP and the MEMR, rather than an inherent association between OHC function and MEMR magnitude. This suggests that subclinical OHC dysfunction does not lead to a reduction in MEMR magnitude. This finding is consistent with the results of Borg et al. (1990),

which indicate that the MEMR is generated by the tails of auditory nerve tuning curves, making MEMR measurements relatively insensitive to OHC damage.

4.3 MEMR measurements differ from ABR and EFR measurements

Despite the variety of confounding factors (e.g., head size and anatomy, sex, OHC function) that can induce variability in ABR wave I amplitude and EFR magnitude, in this sample, low ABR wave I amplitudes are associated with weaker EFRs. This suggests that both ABR wave I amplitude and EFR magnitude are measuring a common element of the auditory system, namely auditory nerve function. It is surprising that MEMR measurements do not appear to have a strong relationship with ABR and EFR measurements. One possible explanation for this finding is that MEMR magnitude is more specific to loss of low SR auditory nerve fibers, while ABR wave I amplitude and EFR magnitude measurements are more sensitive to loss of high SR fibers. Individuals from the Veteran High Noise group may tend to have a combination of both low and high SR loss. This view is supported by 1) the results of Paul et al. (2017), which suggest that tinnitus, reported by 43% of the High Noise Veterans in the current study, may be associated with loss of high SR fibers and 2) by the reduced EFR magnitudes for the Veteran High Noise group compared to controls (Bramhall et al. 2020a), even though computational modeling suggests that the EFR is dominated by high SR fibers (Encina-Llamas et al. 2019). This could explain why model results show a reduction in mean MEMR magnitude, ABR wave I amplitude, and EFR magnitude for the Veteran High Noise group compared to the Non-Veteran Control group, but the MEMR measurements are not well correlated with the ABR and EFR measurements. Alternatively, this result may be related to differences in the frequency content of the

stimulus for each measurement. The ABR and EFR measurements both used 4 kHz stimuli, while the MEMR was measured with a broadband stimulus. However, at the high stimulus levels used to collect the ABR and EFR measurements, frequency specificity is expected to be limited.

4.4 Comparison to other human MEMR studies

The observed reduction in modeled mean MEMR magnitude for High Noise Veterans compared with Non-Veteran Controls is consistent with the results of Shehorn et al. (2020), who showed a correlation between self-reported lifetime noise exposure history and MEMR magnitude among adults aged 21-54 years with normal audiograms, half of whom reported hearing difficulties. Specifically, greater reported noise exposure was associated with lower MEMR magnitudes. In contrast, Guest et al. (2019) measured MEMR thresholds in young adults (aged 18-40 years) with normal audiograms, half of whom reported speech-in-noise perception difficulty, and observed only very weak relationships between MEMR threshold and self-reported lifetime noise exposure, with no consistent trend across sex and frequency. However, in that study, MEMR thresholds were measured with a clinical system using a 226 Hz probe tone, which provides limited information in comparison to a wideband probe considering that changes in absorbance occur over a large frequency range. A single frequency probe tone does not allow for individual differences in the MEMR spectrum and can result in mischaracterization of MEMR threshold (e.g., Bharadwaj et al. 2019, Figure 5). It is also possible that differences in the study population contributed to the differing findings. The study population for the present study is more likely to include a large range of noise exposure histories than the Guest et al. study, given the inclusion of military Veterans,

and the Shehorn et al. study, which used the same noise exposure questionnaire as the Guest et al. study, included participants with a larger range of noise exposure scores. Sampling from populations with more noise exposure may make it easier to detect a relationship between self-reported noise exposure and the MEMR.

4.5 Clinical utility of the MEMR

MEMR thresholds are currently part of the standard audiometric test battery and can be measured within a few minutes. MEMRs are typically measured clinically using a 226 Hz probe tone. However, using a wideband probe such as a chirp or click for MEMR measurements results in lower reflex thresholds (12 dB lower for a BBN activator, Feeney et al. 2017) and information about impedance changes across frequency. MEMRs for a tonal elicitor are relatively insensitive to pure tone threshold elevation up to 50 dB HL (Gelfand et al. 1990) and the model results presented here suggest that subclinical OHC dysfunction has little impact on MEMR magnitude measured using a broadband elicitor. This indicates that the MEMR may be an ideal test measure for synaptopathy in individuals with mild to moderate hearing loss. In the future, MEMR magnitude could be evaluated clinically by comparing to normative reference intervals. However, interpretation of a weak or absent MEMR is complicated by the fact that the MEMR pathway includes multiple components in addition to the auditory nerve, including the cochlea, cochlear nucleus, superior olivary complex, facial nerve nucleus, facial nerve, stapedius muscle, and eardrum. Dysfunction at any of these points could impact the MEMR. Integrity of only a subset of these components (the eardrum, middle ear and cochlea) are assessed as part of the standard audiometric

battery. Therefore, diagnosis of deafferentation may require additional confirmatory testing with another measure such as ABR wave I amplitude.

4.6 Limitations

As with ABR wave I amplitude and EFR magnitude, the MEMR can't differentiate between synapse loss and other types of cochlear deafferentation (i.e., IHC or spiral ganglion cell loss). However, data from animal models and human temporal bone studies suggest that age- and noise exposure-related IHC loss is minimal and that synapse loss occurs prior to loss of spiral ganglion cells (Kujawa & Liberman 2009; Sergeyenko et al. 2013; Viana et al. 2015; Wu et al. 2019; Fernandez et al. 2020). Therefore, we feel noise-induced synaptopathy is the most compelling explanation for the observed relationships between self-reported noise exposure and physiological indicators of cochlear deafferentation (MEMR magnitude, ABR wave I amplitude, and EFR magnitude).

5. Conclusions

The statistical analysis described in this report estimates a 25% reduction in mean MEMR magnitude (in response to a 100 dB SPL elicitor) for Veterans reporting high noise exposure compared with non-Veteran controls. Although the confidence intervals overlap zero, the modelled group contrast in mean MEMR magnitude is consistent with previously demonstrated reductions in ABR wave I amplitude and EFR magnitude in the same Veteran population. Reductions in ABR, EFR, and MEMR measurements are consistent with animal studies of noise-induced synaptopathy and suggest that noise-induced synaptopathy may occur among individuals with a significant history of military noise exposure. In addition, the results indicate that subclinical OHC

dysfunction has limited impact on MEMR measurements. This increases the likelihood that the MEMR will be a useful diagnostic indicator of cochlear synaptopathy/deafferentation. Finally, the demonstrated correlations between ABR and EFR measurements, but not MEMR measurements, may indicate that the MEMR is more specific to loss of low SR auditory nerve fibers than other physiological indicators of deafferentation.

Acknowledgements

This work was supported by the Department of Veterans Affairs, Veterans Health Administration, Rehabilitation Research and Development Service - Award #C2104-W (to N.F.B.) and #C2361-C (to NCRAR). Research audiologist support was also provided by the Department of Defense Hearing Center of Excellence and zCore Business Solutions, Inc.. The opinions and assertions presented are private views of the authors and are not to be construed as official or as necessarily reflecting the views of the VA or the Department of Defense.

References

- Berlin, C. I., Hood, L. J., Morlet, T., Wilensky, D., St John, P., Montgomery, E., Thibodaux, M. (2005). Absent or elevated middle ear muscle reflexes in the presence of normal otoacoustic emissions: a universal finding in 136 cases of auditory neuropathy/dys-synchrony. *J Am Acad Audiol*, 16, 546-553.
- Bharadwaj, H. M., Mai, A. R., Simpson, J. M., Choi, I., Heinz, M. G., Shinn-Cunningham, B. G. (2019). Non-Invasive Assays of Cochlear Synaptopathy - Candidates and Considerations. *Neuroscience*, 407, 53-66.
- Bharadwaj, H. M., Verhulst, S., Shaheen, L., Liberman, M. C., Shinn-Cunningham, B. G. (2014). Cochlear neuropathy and the coding of supra-threshold sound. *Front Syst Neurosci*, 8, 26.
- Billings, C. J., Dillard, L. K., Hoskins, Z. B., Penman, T. M., Reavis, K. M. (2018). A Large-Scale Examination of Veterans with Normal Pure-Tone Hearing Thresholds within the Department of Veterans Affairs. *J Am Acad Audiol*, 29, 928-935.
- Borg, E., Counter, S. A., Engstrom, B., Linde, G., Marklund, K. (1990). Stapedius reflex thresholds in relation to tails of auditory nerve fiber frequency tuning curves. *Brain Res*, 506, 79-84.
- Bourien, J., Tang, Y., Batrel, C., Huet, A., Lenoir, M., Ladrech, S., Desmadryl, G., Nouvian, R., Puel, J. L., Wang, J. (2014). Contribution of auditory nerve fibers to compound action potential of the auditory nerve. *J Neurophysiol*, 112, 1025-1039.
- Bramhall, N. B., McMillan, G. P., Kampel, S. D. (2020a). Envelope following response measurements in young veterans are consistent with noise-induced cochlear synaptopathy. *OSF Preprints*. Available from <https://doi.org/10.31219/osf.io/aze56>
- Bramhall, N. F., Theodoroff, S. M., Kampel, S. D. (2020b). Measures of Synaptopathy Linked with Tinnitus and Hyperacusis. In *Assoc. Res. Otolaryngology. Abs.: p. 327*. San Jose, CA.
- Bramhall, N. F., McMillan, G. P., Gallun, F. J., Konrad-Martin, D. (2019). Auditory brainstem response demonstrates that reduced peripheral auditory input is associated with self-report of tinnitus. *J Acoust Soc Am*, 146, 3849.
- Bramhall, N. F., Konrad-Martin, D., McMillan, G. P. (2018). Tinnitus and Auditory Perception After a History of Noise Exposure: Relationship to Auditory Brainstem Response Measures. *Ear Hear*, 39, 881-894.
- Bramhall, N. F., Konrad-Martin, D., McMillan, G. P., Griest, S. E. (2017). Auditory Brainstem Response Altered in Humans With Noise Exposure Despite Normal Outer Hair Cell Function. *Ear Hear*, 38, e1-e12.
- Buran, B. (2015). Auditory-wave-analysis: v1.1. Retrieved April 14, 2017, from <http://zenodo.org/record/17365#.VoMCrjbUi70>.
- Encina-Llamas, G., Harte, J. M., Dau, T., Shinn-Cunningham, B., Epp, B. (2019). Investigating the Effect of Cochlear Synaptopathy on Envelope Following Responses Using a Model of the Auditory Nerve. *J Assoc Res Otolaryngol*, 20, 363-382.
- Feeney, M. P., Keefe, D. H., Hunter, L. L., Fitzpatrick, D. F., Garinis, A. C., Putterman, D. B., McMillan, G. P. (2017). Normative Wideband Reflectance, Equivalent

- Admittance at the Tympanic Membrane, and Acoustic Stapedius Reflex Threshold in Adults. *Ear Hear*, 38, e142-e160.
- Felder, E., Schrott-Fischer, A. (1995). Quantitative evaluation of myelinated nerve fibres and hair cells in cochleae of humans with age-related high-tone hearing loss. *Hear Res*, 91, 19-32.
- Fernandez, K. A., Guo, D., Micucci, S., De Gruttola, V., Liberman, M. C., Kujawa, S. G. (2020). Noise-induced Cochlear Synaptopathy with and Without Sensory Cell Loss. *Neuroscience*, 427, 43-57.
- Fernandez, K. A., Jeffers, P. W., Lall, K., Liberman, M. C., Kujawa, S. G. (2015). Aging after noise exposure: acceleration of cochlear synaptopathy in "recovered" ears. *J Neurosci*, 35, 7509-7520.
- Fulbright, A. N. C., Le Prell, C. G., Griffiths, S. K., Lobarinas, E. (2017). Effects of Recreational Noise on Threshold and Suprathreshold Measures of Auditory Function. *Semin Hear*, 38, 298-318.
- Furman, A. C., Kujawa, S. G., Liberman, M. C. (2013). Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. *J Neurophysiol*, 110, 577-586.
- Gelfand, S. A., Schwander, T., Silman, S. (1990). Acoustic reflex thresholds in normal and cochlear-impaired ears: effects of no-response rates on 90th percentiles in a large sample. *J Speech Hear Disord*, 55, 198-205.
- Gordon, J. S., Griest, S. E., Thielman, E. J., Carlson, K. F., Helt, W. J., Lewis, M. S., Blankenship, C., Austin, D., Theodoroff, S. M., Henry, J. A. (2017). Audiologic characteristics in a sample of recently-separated military Veterans: The Noise Outcomes in Servicemembers Epidemiology Study (NOISE Study). *Hear Res*, 349, 21-30.
- Gorga, M. P., Neely, S. T., Ohlrich, B., Hoover, B., Redner, J., Peters, J. (1997). From laboratory to clinic: a large scale study of distortion product otoacoustic emissions in ears with normal hearing and ears with hearing loss. *Ear Hear*, 18, 440-455.
- Griest-Hines, S. E., Bramhall, N. F., Reavis, K. M., Theodoroff, S. M., Henry, J. A. (2021). Development and Initial Validation of the Lifetime Exposure to Noise and Solvents Questionnaire in U.S. Service Members and Veterans. *Am J Audiol*, 1-15.
- Grinn, S. K., Wiseman, K. B., Baker, J. A., Le Prell, C. G. (2017). Hidden Hearing Loss? No Effect of Common Recreational Noise Exposure on Cochlear Nerve Response Amplitude in Humans. *Front Neurosci*, 11, 465.
- Grose, J. H., Buss, E., Hall, J. W., 3rd. (2017). Loud Music Exposure and Cochlear Synaptopathy in Young Adults: Isolated Auditory Brainstem Response Effects but No Perceptual Consequences. *Trends Hear*, 21, 2331216517737417.
- Gu, J. W., Herrmann, B. S., Levine, R. A., Melcher, J. R. (2012). Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J Assoc Res Otolaryngol*, 13, 819-833.
- Guest, H., Munro, K. J., Plack, C. J. (2019). Acoustic Middle-Ear-Muscle-Reflex Thresholds in Humans with Normal Audiograms: No Relations to Tinnitus, Speech Perception in Noise, or Noise Exposure. *Neuroscience*, 407, 75-82.

- Hall, J. W. (1982). Acoustic reflex amplitude. I. Effect of age and sex. *Audiology*, 21, 294-309.
- Hickox, A. E., Larsen, E., Heinz, M. G., Shinobu, L., Whitton, J. P. (2017). Translational issues in cochlear synaptopathy. *Hear Res*, 349, 164-171.
- Houghton, J. M., Greville, K. A., Keith, W. J. (1988). Acoustic reflex amplitude and noise-induced hearing loss. *Audiology*, 27, 42-48.
- Jerger, J., Jerger, S., Mauldin, L. (1972). Studies in impedance audiometry. I. Normal and sensorineural ears. *Arch Otolaryngol*, 96, 513-523.
- Keefe, D. H., Feeney, M. P., Hunter, L. L., Fitzpatrick, D. F. (2017). Aural acoustic stapedius-muscle reflex threshold procedures to test human infants and adults. *J Assoc Res Otolaryngol*, 18, 65-88.
- Keefe, D. H., Hunter, L. L., Feeney, M. P., Fitzpatrick, D. F. (2015). Procedures for ambient-pressure and tympanometric tests of aural acoustic reflectance and admittance in human infants and adults. *J Acoust Soc Am*, 138, 3625-3653.
- Kobler, J. B., Guinan, J. J., Jr., Vacher, S. R., Norris, B. E. (1992). Acoustic reflex frequency selectivity in single stapedius motoneurons of the cat. *J Neurophysiol*, 68, 807-817.
- Kujawa, S. G., Liberman, M. C. (2009). Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J Neurosci*, 29, 14077-14085.
- Kujawa, S. G., Liberman, M. C. (2015). Synaptopathy in the noise-exposed and aging cochlea: Primary neural degeneration in acquired sensorineural hearing loss. *Hear Res*, 330, 191-199.
- Liberman, M. C., Kiang, N. Y. (1984). Single-neuron labeling and chronic cochlear pathology. IV. Stereocilia damage and alterations in rate- and phase-level functions. *Hear Res*, 16, 75-90.
- Lin, H. W., Furman, A. C., Kujawa, S. G., Liberman, M. C. (2011). Primary neural degeneration in the Guinea pig cochlea after reversible noise-induced threshold shift. *J Assoc Res Otolaryngol*, 12, 605-616.
- Liu, Y. W., Sanford, C. A., Ellison, J. C., Fitzpatrick, D. F., Gorga, M. P., Keefe, D. H. (2008). Wideband absorbance tympanometry using pressure sweeps: system development and results on adults with normal hearing. *J Acoust Soc Am*, 124, 3708-3719.
- Makary, C. A., Shin, J., Kujawa, S. G., Liberman, M. C., Merchant, S. N. (2011). Age-related primary cochlear neuronal degeneration in human temporal bones. *J Assoc Res Otolaryngol*, 12, 711-717.
- Mepani, A. M., Kirk, S. A., Hancock, K. E., Bennett, K., de Gruttola, V., Liberman, M. C., Maison, S. F. (2020). Middle Ear Muscle Reflex and Word Recognition in "Normal-Hearing" Adults: Evidence for Cochlear Synaptopathy? *Ear Hear*, 41, 25-38.
- Mitchell, C., Phillips, D. S., Trune, D. R. (1989). Variables affecting the auditory brainstem response: audiogram, age, gender and head size. *Hear Res*, 40, 75-85.
- Montgomery, J. M., Nyhan, B., Torres, M. (2018). How conditioning on posttreatment variables can ruin your experiment and what to do about it. *Am J Pol Sci*, 62, 760-775.

- Neely, S., Liu, Z. (1993). EMAV: Otoacoustic emission averager. In *Tech Memo No. 17*: Boys Town National Research Hospital Omaha.
- Osterhammel, D., Osterhammel, P. (1979). Age and sex variations for the normal stapedial reflex thresholds and tympanometric compliance values. *Scand Audiol*, 8, 153-158.
- Otte, J., Schunknecht, H. F., Kerr, A. G. (1978). Ganglion cell populations in normal and pathological human cochleae. Implications for cochlear implantation. *Laryngoscope*, 88, 1231-1246.
- Paul, B. T., Bruce, I. C., Roberts, L. E. (2017). Evidence that hidden hearing loss underlies amplitude modulation encoding deficits in individuals with and without tinnitus. *Hear Res*, 344, 170-182.
- Prasher, D., Cohen, M. (1993). Effectiveness of acoustic reflex threshold criteria in the diagnosis of retrocochlear pathology. *Scand Audiol*, 22, 11-18.
- Prendergast, G., Guest, H., Munro, K. J., Kluk, K., Leger, A., Hall, D. A., Heinz, M. G., Plack, C. J. (2017). Effects of noise exposure on young adults with normal audiograms I: Electrophysiology. *Hear Res*, 344, 68-81.
- Robles, L., Ruggero, M. A. (2001). Mechanics of the mammalian cochlea. *Physiol Rev*, 81, 1305-1352.
- Ruggero, M. A., Rich, N. C., Recio, A., Narayan, S. S., Robles, L. (1997). Basilar-membrane responses to tones at the base of the chinchilla cochlea. *J Acoust Soc Am*, 101, 2151-2163.
- Schaette, R., McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J Neurosci*, 31, 13452-13457.
- Schairer, K. S., Feeney, M. P., Sanford, C. A. (2013). Acoustic reflex measurement. *Ear Hear*, 34 Suppl 1, 43S-47S.
- Schmiedt, R. A., Mills, J. H., Boettcher, F. A. (1996). Age-related loss of activity of auditory-nerve fibers. *J Neurophysiol*, 76, 2799-2803.
- Sergeyenko, Y., Lall, K., Liberman, M. C., Kujawa, S. G. (2013). Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. *J Neurosci*, 33, 13686-13694.
- Shaheen, L. A., Valero, M. D., Liberman, M. C. (2015). Towards a Diagnosis of Cochlear Neuropathy with Envelope Following Responses. *J Assoc Res Otolaryngol*, 16, 727-745.
- Shehorn, J., Strelcyk, O., Zahorik, P. (2020). Associations between speech recognition at high levels, the middle ear muscle reflex and noise exposure in individuals with normal audiograms. *Hear Res*, 392, 107982.
- Singer, J. D., Willett, J. B. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. New York: Oxford University Press.
- Trune, D. R., Mitchell, C., Phillips, D. S. (1988). The relative importance of head size, gender and age on the auditory brainstem response. *Hear Res*, 32, 165-174.
- Valero, M. D., Burton, J. A., Hauser, S. N., Hackett, T. A., Ramachandran, R., Liberman, M. C. (2017). Noise-induced cochlear synaptopathy in rhesus monkeys (*Macaca mulatta*). *Hear Res*, 353, 213-223.
- Valero, M. D., Hancock, K. E., Liberman, M. C. (2016). The middle ear muscle reflex in the diagnosis of cochlear neuropathy. *Hear Res*, 332, 29-38.

- Valero, M. D., Hancock, K. E., Maison, S. F., Liberman, M. C. (2018). Effects of cochlear synaptopathy on middle-ear muscle reflexes in unanesthetized mice. *Hear Res*, 363, 109-118.
- VBA, V. B. A. (2010). The Duty MOS Noise Exposure Listing. In.
- Verhulst, S., Altoe, A., Vasilkov, V. (2018). Computational modeling of the human auditory periphery: Auditory-nerve responses, evoked potentials and hearing loss. *Hear Res*, 360, 55-75.
- Viana, L. M., O'Malley, J. T., Burgess, B. J., Jones, D. D., Oliveira, C. A., Santos, F., Merchant, S. N., Liberman, L. D., Liberman, M. C. (2015). Cochlear neuropathy in human presbycusis: Confocal analysis of hidden hearing loss in post-mortem tissue. *Hear Res*, 327, 78-88.
- Wojtczak, M., Beim, J. A., Oxenham, A. J. (2017). Weak Middle-Ear-Muscle Reflex in Humans with Noise-Induced Tinnitus and Normal Hearing May Reflect Cochlear Synaptopathy. *eNeuro*, 4.
- Wu, P. Z., Liberman, L. D., Bennett, K., de Gruttola, V., O'Malley, J. T., Liberman, M. C. (2019). Primary Neural Degeneration in the Human Cochlea: Evidence for Hidden Hearing Loss in the Aging Ear. *Neuroscience*, 407, 8-20.

Figure 1. Schematic indicating the relationships between noise exposure, OHC function, cochlear synaptic integrity, and physiological measures. Noise exposure can damage both OHCs and cochlear synapses. Both types of damage can potentially impact ABR, EFR, and MEMR measurements, while only OHC damage impacts DPOAE measurements.

Figure 2. Audiometric thresholds are similar across noise exposure groups. Thin lines show audiometric thresholds from 0.25-16 kHz for individual participants, while thick lines show mean thresholds for each noise exposure group. Color-coding indicates the noise exposure group.

Figure 3. DPOAE levels are similar across noise exposure groups. Thin lines show DPOAE levels from 1-8 kHz (measured with a DP-gram) for individual participants. Thick lines show mean DPOAE levels for each noise exposure group. Color-coding indicates the noise exposure group.

Figure 4. Mean MEMR threshold and magnitude is reduced for the Veteran High Noise group compared with the Non-Veteran Control group. A) Boxplots illustrate the distribution of MEMR thresholds for each noise exposure group. The bottom, middle and top lines for each box represent the 1st quartile, median, and 3rd quartile, respectively, while the whiskers show the points furthest from the box that still fall within 1.5 interquartile ranges. Green triangles indicate the group mean and the diamond shows an outlier. B) Mean MEMR magnitude growth functions are plotted for each noise exposure group with thick color-coded lines. Thin lines show MEMR magnitudes for individual participants. C) Mean change in absorbed power is plotted by frequency for each noise exposure group.

Figure 5. Modeled mean MEMR magnitude is reduced for the Veteran High Noise group compared with the Non-Veteran Control group at high elicitor levels.

Modeled mean MEMR magnitude contrasts with the Non-Veteran Control group are plotted for the Veteran Medium Noise (circles) and Veteran High Noise (triangles) groups for each elicitor level. Values less than zero indicate a reduction in mean MEMR magnitude compared to the Non-Veteran Control group. Confidence intervals are shown with dashed lines.

Figure 6. Modeled relationship between average DPOAE level and MEMR magnitude suggests impact of OHC dysfunction on MEMR is minimal. The modeled relationship between average DPOAE level from 3-8 kHz (AvgDP) and MEMR magnitude is plotted for each noise exposure group (indicated by the line color) and elicitor level (shown in separate panels).

Figure 7. EFR and ABR measurements are more highly correlated with each other than with MEMR measurements. Scatterplots show relationships between pairs of physiological measurements. Pearson correlation coefficients, p-values, and regression lines are shown for each plot. EFR measurements are for an 80 dB SPL 4 kHz carrier tone 40% modulated at 110 Hz, ABR wave I amplitudes were obtained for a 90 dB peSPL 4 kHz toneburst, and contralateral MEMR magnitudes were collected using a 100 dB SPL broadband noise elicitor.

Table 1. Participant Characteristics by Noise Exposure Group

	Non-Veteran Control	Veteran Medium Noise	Veteran High Noise
Age (years)	26.8 (4.9)	30.1 (3.4)	30.0 (3.1)
Number of males	14 (40%)	9 (41%)	27 (77%)
LENS-Q score	4.1 (0.8)	8.4 (0.9)	9.7 (0.8)
Number with high MOS rating	N/A	N/A	26 (74%)
Report tinnitus in the test ear	N/A	5 (23%)	15 (43%)
PTA (0.5, 1, & 2 kHz; dB HL)	6.0 (4.8)	7.1 (5.3)	7.2 (4.3)
High freq. PTA (3, 4, & 6 kHz; dB HL)	3.6 (4.8)	4.7 (4.4)	6.8 (4.5)
EHF PTA (9-16 kHz; dB HL)	5.9 (8.5)	7.2 (8.9)	10.8 (13.6)
Average DPOAE (3-8 kHz; dB SPL)	4.3 (4.7)	3.0 (3.8)	2.5 (4.2)
Total Participants	35	22	35

For mean values, standard deviations are shown in (). For counts, percentages are shown in (). PTA – pure tone average, EHF – extended high frequency, LENS-Q – Lifetime Exposure to Noise and Solvents – Questionnaire, MOS – Military Occupational Specialty. High MOS rating refers to a high probability of hazardous noise exposure associated with that MOS.

Table 2. Modeled contrasts in mean MEMR magnitude between noise exposure groups.

Noise Exposure Group Contrast	Elicitor Level (dB SPL)	Modeled Contrast (dB)	95% Confidence Interval (lower bound)	95% Confidence Interval (upper bound)
Veteran High Noise - Control	55	-0.050	-0.450	0.349
Veteran Medium Noise - Control	55	-0.011	-0.465	0.444
Veteran High Noise - Control	60	-0.077	-0.454	0.301
Veteran Medium Noise - Control	60	-0.006	-0.436	0.424
Veteran High Noise - Control	65	-0.103	-0.465	0.259
Veteran Medium Noise - Control	65	-0.002	-0.414	0.410
Veteran High Noise - Control	70	-0.129	-0.483	0.224
Veteran Medium Noise - Control	70	0.003	-0.399	0.405
Veteran High Noise - Control	75	-0.156	-0.508	0.197
Veteran Medium Noise - Control	75	0.008	-0.394	0.409
Veteran High Noise - Control	80	-0.182	-0.541	0.177
Veteran Medium Noise - Control	80	0.012	-0.397	0.421
Veteran High Noise - Control	85	-0.208	-0.582	0.165
Veteran Medium Noise - Control	85	0.017	-0.408	0.442
Veteran High Noise - Control	90	-0.235	-0.628	0.159
Veteran Medium Noise - Control	90	0.022	-0.426	0.470
Veteran High Noise - Control	95	-0.261	-0.680	0.159
Veteran Medium Noise - Control	95	0.026	-0.451	0.504
Veteran High Noise - Control	100	-0.287	-0.737	0.163
Veteran Medium Noise - Control	100	0.031	-0.481	0.543

*Note that MEMR magnitude refers to the natural logarithm of the change in absorbed power across frequency as described in section 2.2.3.

Table 3. Modeled estimates of the relationship between MEMR magnitude and AvgDP for each noise exposure group and elicitor level.

Noise Exposure Group	Elicitor Level (dB SPL)	Slope Estimate	95% Confidence Interval (lower bound)	95% Confidence Interval (upper bound)
Non-Veteran Control	55	-0.008	-0.065	0.050
Veteran Medium Noise	55	0.045	-0.042	0.131
Veteran High Noise	55	0.032	-0.032	0.095
Non-Veteran Control	60	-0.004	-0.060	0.051
Veteran Medium Noise	60	0.049	-0.037	0.134
Veteran High Noise	60	0.035	-0.027	0.097
Non-Veteran Control	65	-0.001	-0.055	0.054
Veteran Medium Noise	65	0.052	-0.032	0.137
Veteran High Noise	65	0.039	-0.022	0.100
Non-Veteran Control	70	0.003	-0.051	0.057
Veteran Medium Noise	70	0.056	-0.028	0.140
Veteran High Noise	70	0.042	-0.018	0.102
Non-Veteran Control	75	0.006	-0.047	0.060
Veteran Medium Noise	75	0.059	-0.025	0.143
Veteran High Noise	75	0.046	-0.014	0.106
Non-Veteran Control	80	0.010	-0.044	0.064
Veteran Medium Noise	80	0.063	-0.021	0.147
Veteran High Noise	80	0.049	-0.011	0.110
Non-Veteran Control	85	0.013	-0.041	0.068
Veteran Medium Noise	85	0.066	-0.018	0.151
Veteran High Noise	85	0.053	-0.008	0.114
Non-Veteran Control	90	0.017	-0.039	0.073
Veteran Medium Noise	90	0.070	-0.016	0.155
Veteran High Noise	90	0.056	-0.006	0.119
Non-Veteran Control	95	0.020	-0.037	0.078
Veteran Medium Noise	95	0.073	-0.013	0.160
Veteran High Noise	95	0.060	-0.004	0.124
Non-Veteran Control	100	0.024	-0.036	0.084
Veteran Medium Noise	100	0.077	-0.011	0.165
Veteran High Noise	100	0.063	-0.002	0.129

Because MEMR magnitude has been log transformed, the percent growth in change in absorbed power across frequency for every 1 dB increase in AvgDP can be calculated as $100 * (e^{Slope\ Estimate} - 1)$. For example, the percent growth in change in absorbed power across frequency per 1 dB increase in AvgDP for the Non-Veteran Control group at an elicitor level of 75 dB SPL is $100 * (e^{0.006} - 1) = 0.6\%$.