Acoustic middle-ear-muscle-reflex thresholds in humans with normal audiograms: No relations to tinnitus, speech perception in noise, or noise exposure

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Abstract

The acoustic middle-ear-muscle reflex (MEMR) has been suggested as a sensitive non-invasive measure of cochlear synaptopathy, the loss of synapses between inner hair cells and auditory nerve fibers. In the present study, clinical MEMR thresholds were measured for 1-, 2-, and 4-kHz tonal elicitors, using a procedure shown to produce thresholds with excellent reliability. MEMR thresholds of 19 participants with tinnitus and normal audiograms were compared to those of 19 age- and sex-matched controls. MEMR thresholds did not differ significantly between the two groups at any frequency. These 38 participants were included in a larger sample of 70 participants with normal audiograms. For this larger group, MEMR thresholds were compared to a measure of spatial speech perception in noise (SPiN) and a detailed self-report estimate of lifetime noise exposure. MEMR thresholds were unrelated to either SPiN or noise exposure, despite a wide range in both measures. It is possible that thresholds measured using a clinical paradigm are less sensitive to synaptopathy than those obtained using more sophisticated measurement techniques; however, we had good sensitivity at the group level, and even trends in the hypothesized direction were not observed. To the extent that MEMR thresholds are sensitive to cochlear synaptopathy, the present results provide no evidence that tinnitus, SPiN, or noise exposure are related to synaptopathy in the population studied.

INTRODUCTION

Cochlear synaptopathy, the loss of synapses between inner hair cells and auditory nerve fibers, has been convincingly demonstrated in rodent and recently primate models as a consequence of noise exposure and aging (Fernandez et al., 2015; Furman et al., 2013; Kujawa & Liberman, 2009; Sergeyenko et al., 2013; Valero et al., 2017). In humans, there is post-mortem histological evidence for age-related synaptopathy (Viana et al., 2015; Wu et al., 2018). However, the evidence for noise-induced synaptopathy in humans is inconsistent, and reliant on indirect measures.

Broadly speaking, there are two approaches to the study of synaptopathy in humans. First, is to select a measure of an assumed underlying cause - for example, self-report noise exposure - as a predictor variable and ask if this variable is associated with an outcome measure assumed to reflect synaptopathy - for example, wave I of the auditory brainstem response (ABR), which reflects auditory nerve function and is related to histological measures of synaptopathy in the rodent models (Kujawa & Liberman, 2009; Sergeyenko et al., 2013). Some studies have reported a relation of self-report noise exposure to wave I amplitude (Bramhall et al., 2017; Stamper & Johnson, 2015), or to the ratio of wave I amplitude to wave V amplitude (wave I/V ratio; Grose et al., 2017), or to the ratio of summating potential amplitude to action potential amplitude (SP/AP; Liberman et al., 2016), with the latter two measures having been proposed as differential measures of synaptopathy. However, most recent studies have not reported any relation for listeners with normal audiograms (Fulbright et al., 2017; Grinn et al., 2017; Guest et al., 2017b; Prendergast et al., 2017a; Prendergast et al., 2018; Spankovich et al., 2017). A problem with this approach with respect to noise exposure is that self-report measures are inherently highly variable, although we

have argued previously that the measurement error is small compared to the range of exposures in the population (Prendergast et al., 2017a).

The second approach is to assume that synaptopathy has perceptual consequences, and to try to determine whether or not individuals with a perceptual deficit, despite normal audiometry, have synaptopathy. Two deficits have received most attention: speech-perception-in-noise (SPiN) difficulties, and tinnitus. SPiN might be affected due to a reduction in information in the auditory nerve (Lopez-Poveda & Barrios, 2013), particularly at high sound levels, since synaptopathy seems to preferentially affect synapses with high-threshold, low-spontaneous-rate (low-SR), fibers (Furman et al., 2013). Tinnitus might be a perceptual consequence of synaptopathy due to increased neural gain, which may amplify spontaneous neural activity as a side effect of homeostatic compensation for a reduction in auditory nerve input (Schaette & McAlpine, 2011).

Bharadwaj et al. (2015) found a relation between envelope-following response (EFR) amplitude and performance on a spatial selective attention task with spoken digit targets and a pink noise masker. Although the EFR is thought to be largely dependent on brainstem generators, the response may be affected by low-SR fiber loss, especially at high levels and shallow modulation depths (Bharadwaj et al., 2014; Bharadwaj et al., 2015). Bramhall et al. (2015) reported a relation between ABR wave I amplitude and SPiN, although the relation was largely driven by audiometric thresholds and was not significant when these thresholds were included in the regression model. Liberman et al. (2016) reported that SPiN at low levels was related to the SP/AP ratio. However, it is uncertain if this relation would survive statistical control for high frequency audiometric thresholds, which were weakly associated with SPiN. In addition, synaptopathy is generally assumed to reflect coding at high levels because of its association with low-SR fibers, and it is not clear why performance at low levels should be affected. In contrast, several recent studies have reported no relation between ABR wave I amplitude and SPiN (Bramhall et al., 2018; Fulbright et al., 2017; Guest et al., 2018c; Prendergast et al., 2017b), nor between EFR amplitude and SPiN (Guest et al., 2018b; Prendergast et al., 2017b).

With respect to tinnitus, the literature is also inconsistent. Several studies have reported a reduction in ABR wave I at high levels in tinnitus participants with normal hearing compared to non-tinnitus controls (Bramhall et al., 2018; Gu et al., 2012; Schaette and McAlpine, 2011). However, in the Gu et al. study the groups were not audiometrically matched; for the analysis in which a significant effect on wave I was observed, the tinnitus group exhibited substantially higher audiometric thresholds at frequencies of 8 kHz and above. In the Bramhall et al. study there were also audiometric differences between the groups, although the authors controlled for distortion-product otoacoustic emission amplitude in the analyses. In the Schaette and McAlpine study there was a small audiometric elevation (3.5 dB) in the tinnitus group at 12 kHz, and thresholds measured at 16 kHz were not reported. It is not clear the extent to which small high-frequency audiometric elevations might affect ABR wave I amplitude, although wave I is dependent on basal generators, including those above 8 kHz (Don & Eggermont, 1978). These positive findings were not replicated by Gilles et al. (2016), Guest et al. (2017a; 2017b), or Shim et al. (2017), who all found no relation between presence of tinnitus and wave I amplitude. Paul et al. (2017) reported a reduction in the EFR in their tinnitus participants compared to controls. However, there was flaw in the original statistical analysis and the finding was non-significant after this was corrected (Roberts et al., 2018). Guest et al. (2017b) also reported a non-significant reduction in EFR amplitude in their tinnitus participants, but this was not apparent in a subsequent analysis (Guest et al., 2017a) and most likely represents random variability.

Despite their widespread use, there are concerns that neither ABR wave I amplitude nor EFR amplitude are sensitive to synaptopathy. In rodent models, the compound action potential is unaffected by loss of the fibers with the lowest SRs (Bourien et al., 2014), and the EFR, while sensitive to synaptopathy at modulation rates around 1 kHz, is insensitive at the lower rates (typically 100 Hz) used in the human studies (Shaheen et al., 2015). An alternative non-invasive measure is the acoustic middle-ear-muscle reflex (MEMR), a sound-evoked reflex contraction of the stapedius muscle that results in a reduction in transmission through the middle ear. The MEMR, particularly the acoustic threshold level for activation of the MEMR (hereafter referred to as “MEMR threshold”), is highly sensitive to the presence of synaptopathy in the mouse model, more so than ABR wave I (Valero et al., 2016; Valero et al.,

2018). This sensitivity is consistent with the hypothesis, based on threshold and tuning curve considerations, that the MEMR is largely driven by low-SR fibers (Kobler et al., 1992; Liberman & Kiang, 1984). Recently, Wojtczak et al. (2017) reported a large reduction in the amplitude of the MEMR in tinnitus patients compared to controls.

Following these new insights, the present paper reports an analysis of previously unpublished MEMR threshold data collected as part of two published studies examining the relation between tinnitus, SPiN, and electrophysiological measures of synaptopathy in listeners with normal audiometric hearing (Guest et al., 2017b; Guest et al., 2018c). The data were analyzed to answer the following questions:

1. Is there a relation between MEMR threshold and presence of tinnitus?

2. Is there a relation between MEMR threshold and SPiN?

3. Is there a relation between MEMR threshold and self-report noise exposure?

MATERIALS AND METHODS

Participants

Over the course of 18 months, 83 young adults (aged 18-39) were recruited to two parallel studies investigating possible etiologies of tinnitus (Guest et al., 2017b) and impaired SPiN (Guest et al., 2018c). In these studies, recruitment specifically targeted individuals who experienced tinnitus and SPiN difficulties, respectively. Participants with tinnitus reported *prolonged spontaneous tinnitus* (i.e. occurring spontaneously and lasting for >5 minutes at a time; Davis, 1995); the tinnitus was required to be chronic and with a stable percept. In practice, the vast majority of participants reported tinnitus that was constant in quiet (see Guest et al., 2017b). Participants with SPiN impairment reported significant listening difficulties in noise *and* exhibited poor performance on a laboratory SPiN task. All 83 exhibited clinically normal audiometric thresholds (≤20 dB HL at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz) and reported no history of ear surgery, neurological disorder, head trauma, or ototoxic exposure. MEMR thresholds were not obtained from 13 of the 83 participants, due to time constraints, participant preference, or measurement difficulties (e.g. inadequate acoustic seal in the ear canal or unstable compliance); the remaining 70 participants (including 19 with tinnitus) comprise the present sample. Of these, 67 had normal tympanometric results (compliance 0.3-1.6 cm^3, pressure -50 to +50 daPa), including all of the participants with tinnitus. Two participants had compliance of 0.2 cm^3 in both ears; one had compliance of 2.4 cm^3 in one ear; all three had MEMR thresholds <95 dB HL at 2 kHz and were included in the analyses. Participant characteristics are outlined in Table 1.

Audiometric thresholds

Pure-tone air-conduction audiometric thresholds (at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz) were obtained from both ears as described in Guest et al. (2017b). Extended high-frequency (EHF) audiometric thresholds (to 1/3-octave narrowband noise centered at 10 and 14 kHz) were obtained from both ears as described in Guest et al. (2017b).

Middle-ear-muscle reflex thresholds

Middle-ear-muscle reflex thresholds were measured from both ears using a GSI Tympstar diagnostic middle-ear analyzer. The probe was encompassed in a Grason KR-Series clinical ear tip and delivered a probe tone at 226 Hz. Stimuli were ipsilateral pulsed pure tones at 1, 2, and 4 kHz with a duration of 1.5 seconds. Reflex thresholds were determined by observing changes in middle-ear compliance following presentation of the stimuli. A reflex response was defined as a reduction in compliance of 0.02 ml or greater with appropriate morphology and no evidence of significant measurement artifact. If significant measurement artifact was observed during the response period, the presentation was repeated. For each threshold measurement, stimulus level commenced at 70 dB HL and ascended in 5 dB steps until a response was observed. Stimulus level then decreased by 10 dB and ascended in 2 dB steps until

threshold was obtained. Threshold was defined as the lowest stimulus level at which three clear responses were observed over the course of three, four, or five artifact-free presentations. This procedure has previously been shown to yield thresholds with excellent reliability (Guest et al., 2018b). For two participants out of 70, time constraints led to the use of an abbreviated threshold-finding procedure, involving 5 dB rather than 2 dB steps.

Speech perception in noise

The SPiN measure combined high overall sound levels, competing talkers, and spatial cues, and is described fully in Guest et al. (2018c). Participants were required to discern Coordinate-Response-Measures phrases ("Ready Baron, go to {colour} {number} now"), delivered at 0° azimuth in the presence of two-talker interference (delivered at -60° and +60° azimuths, at a combined sound level of 80 dB SPL). The participant's task was to report the colour and number spoken by the target talker (a 16-alternative, forced-choice task). Target sound level varied according to a one-down, one-up decision rule, yielding a threshold signal-to-noise ratio (SNR) corresponding to the 50% point on the psychometric function.

Lifetime noise exposure

Cumulative exposure to sound levels >80 dBA was estimated via detailed structured interview (the Noise Exposure Structured Interview, NESI). The reporting procedures are described in Guest et al. (2017b) and presented as an instrument for use by other researchers in Guest et al. (2018a). In short, participants were directed to (i) identify occupational and/or recreational noisy activities in which they had engaged; (ii) for each activity, divide the lifespan into periods in which exposure habits were approximately stable; (iii) estimate exposure duration for each period, based on frequency of occurrence and duration of a typical exposure; (iv) estimate exposure level, based on vocal effort required to hold a conversation or, for personal listening devices, typical volume control setting; (v) report usage and type of hearing protective equipment. The resulting data from all activities and life periods were combined to yield NESI units of lifetime noise exposure, a measure linearly related to the total lifetime energy of exposure above 80 dBA.

Analysis

Statistical analyses were performed using *R* (R-Core-Team, 2015). For the tinnitus comparisons, the 19 tinnitus participants were individually matched with 19 controls on the basis of age and sex. (Matching aimed to minimize the difference in the mean ages of the two groups, with the constraint that each pair of participants should be of the same sex and differ in age by less than two years.) Audiometric thresholds were allowed to vary freely between groups, since high-frequency audiometric loss might be a biomarker for cochlear synaptopathy at lower frequencies (Liberman et al., 2016). However, in practice, thresholds were found to differ little between groups at frequencies up to 10 kHz (Fig. 1). When testing for correlations between MEMR thresholds and lifetime noise exposure, the full sample (n = 70) was used. When testing for correlations between MEMR thresholds and SPiN, only native speakers of English were included (n = 63). Tinnitus comparisons were conducted by way of *t*-test or Wilcoxon-Mann-Whitney test, as appropriate. Potential correlations with noise exposure and SPiN were sought by way of Spearman's rho or Pearson's r, as appropriate. All tests were two-tailed ($\alpha = 0.05$). Statistics reported in the text and figures are uncorrected for multiple comparisons.

RESULTS

For 68 out of 70 participants, MEMR thresholds were obtained from both ears, then averaged; for two participants (neither of whom reported tinnitus), thresholds were recorded from the right ear only, due to recording difficulties. Of the resulting 414 MEMR measurements, 93.7% yielded thresholds within the limits of the middle-ear analyzer; for the remaining 26 measurements (all recorded at 2 or 4 kHz), thresholds exceeded these limits, and were ascribed a value 2 dB greater than the maximum output level (106 dB HL at 2 kHz or 102 dB HL at 4 kHz).

Tinnitus

Fig. 2 presents MEMR thresholds for the tinnitus and control groups, which were compared via unequal-variance *t*-test at 1 kHz, Student's *t*-test at 2 kHz, and Wilcoxon-Mann-Whitney test at 4 kHz. These tests revealed no significant difference between groups at 1 kHz ($t(29.7) = -1.0335$, $p = 0.31$), 2 kHz ($t(36) = -1.6403$, $p = 0.11$), or 4 kHz ($W = 140$, $n_1 = n_2 = 19$, $p = 0.24$).

Speech perception in noise

Participants presented a wide range of thresholds on the CRM (SNRs of -21.9 to -5.5 dB), despite normal pure-tone audiometric thresholds. Fig. 3 illustrates the relations between MEMR thresholds (at 1, 2, and 4 kHz) and CRM thresholds; no significant correlations, nor any consistent trends, are evident.

Following reviewer recommendations, we also compared MEMR thresholds between a group with self-reported SPiN impairment and an age- and sex-matched control group ($n_1 = n_2 = 24$). This post hoc analysis revealed no significant differences between the groups at 1 kHz ($t(46) = -1.23$, $p = 0.23$), 2 kHz ($t(46) = -1.81$, $p = 0.08$), or 4 kHz ($U = 265.5$, $p = 0.65$).

Lifetime noise exposure

Lifetime noise exposure varied widely between participants, with a range greater than a factor of 1000 in terms of NESI units, which are linearly related to total energy of exposure. Fig. 4 illustrates the relations between NESI units of lifetime noise exposure and MEMR thresholds (at 1, 2, and 4 kHz); no significant correlations, nor any consistent trends, are evident.

DISCUSSION

With respect to our three research questions, our results reveal no relation between MEMR threshold and the presence of tinnitus, no relation between MEMR threshold and SPiN, and no relation between MEMR threshold and noise exposure. These findings are consistent with our previously reported findings on the same cohort using ABR wave I amplitude and EFR amplitude as measures of synaptopathy (Guest et al., 2017a; Guest et al., 2017b; Guest et al., 2018c). Overall, we have found no evidence that our cohort had noise induced-cochlear synaptopathy, or that synaptopathy is a cause of tinnitus or SPiN difficulties in our cohort.

Middle-ear-muscle reflex thresholds and tinnitus

Consistent with our results, some studies have not found any evidence for a relation between tinnitus and electrophysiological measures of synaptopathy (Gilles et al., 2016; Guest et al., 2017a; Guest et al., 2017b; Shim et al., 2017). However, others have reported such a relation using similar methodologies (Bramhall et al., 2018; Gu et al., 2012; Schaette & McAlpine, 2011). A problematic question is why our findings are so different from those of Wojtczak et al. (2017), who reported a huge reduction, by a factor of about four, in MEMR amplitude in their tinnitus participants compared to controls.

There were some methodological differences between the two studies: three major differences in MEMR methods, plus two notable differences in participant characteristics. First, we used clinical equipment incorporating a tonal (226 Hz) probe and coupler calibration of sound level, whereas Wojtczak et al. used a wideband probe and in-situ calibration. It is likely that these aspects added undesirable between-subject variability to our measurements, since between-subject differences are expected in ear-canal acoustics and in the pattern of MEMR effects across probe frequency (Feeney et al., 2017). However, the wideband-probe approach can increase measurement error (Feeney et al., 2017), and our clinical paradigm produces thresholds with excellent reliability (Guest et al., 2018b). In short, our measurements may reflect greater between-subject (though not within-subject) variability from factors other than synaptopathy, potentially reducing study power. The second and third differences in MEMR methods are that Wojtczak et al. used MEMR amplitude as their metric, whereas we used MEMR threshold, and used wideband noise bursts (0.5-10 kHz) to elicit the MEMR, whereas we used tonal elicitors. In both regards, our measure more closely resembles the measure shown to be most sensitive

to synaptopathy in the recent mouse study (Valero et al., 2018). In that study, threshold measures were more predictive of synaptopathy than suprathreshold amplitude, and a high-frequency narrowband elicitor, targeting the basal synaptopathic region, was more sensitive to synaptopathy than a wideband elictor. Hence, the mouse results suggest that our tonal elicitors should provide more sensitivity than the wideband elicitor used by Wojtczak et al. Of course, this reasoning does not hold if synaptopathy in humans is restricted to frequencies well above the highest frequency of our eliciting stimulus (4 kHz); however, in age-related synaptopathy, at least, the 2-5 kHz region appears most vulnerable (Wu et al., 2018). Relatedly, it is possible that our highest elicitor frequency was too low to excite the region associated with tinnitus, which would be expected to be most synaptopathic. However, a recent study by Wojtczak and Beim (2018) using filtered noise elicitors found that the average reflex strength of the control group was still significantly greater than that of the tinnitus group when frequencies above 4 kHz were removed. Hence, the fact that the elicitor extended to higher frequencies in the study of Wojtczak et al. is unlikely to account for the difference between their results and ours.

Statistical power should be considered. Sample sizes of the two studies were similar, with one additional participant per group in the present study. Between-subject variability of MEMR measurements in humans is high, as is evident in both studies. In Wojtczak et al., this variability was overcome by a very large effect size (Cohen's d = 3.34 at 88 dB SPL). Accurate power analysis for the present study is prevented by disparities between our MEMR methods and those of Wojtczak and colleagues. However, if we assume, conservatively, an effect size four times smaller than that of Wojtczak et al. (i.e. Cohen's d = 0.84), then the two-tailed power of our study to detect this effect ($\alpha = 0.05$) is 74%. Instead, we observed a consistent trend for *lower* median MEMR thresholds in tinnitus participants than in controls; at 2 kHz, this trend borders on statistical significance if analyzed via a one-tailed test ($t(36) = -1.64$, $p = 0.05$). Hence, we consider it unlikely that low statistical power explains the differing results.

Differences in participant characteristics may offer an explanation. Audiometric thresholds in the present study were well-matched between tinnitus and non-tinnitus participants up to 10 kHz. In the Wojtczak et al. study, the tinnitus participants had elevated thresholds compared to controls, with a difference of about 8 dB at 8 kHz, and thresholds were not measured at higher frequencies. Although the effect of group was still highly significant after controlling for audiometric threshold, the measurements were limited to a minimum of 0 dB HL, which may have biased thresholds for the controls upwards. It is unclear, however, if this could account for the large group differences in MEMR amplitude they observed. Finally, participants were considerably older in the Wojtczak et al. study, with a mean age of 46 years for the tinnitus participants compared to mean age of 27 here. We have suggested previously that synaptopathy may be a significant etiology of tinnitus only in older participants, but not in the young (Guest et al., 2017b).

Middle-ear-muscle reflex thresholds and speech perception in noise

Our results are consistent with the majority of recent findings, showing no association between SPiN difficulties and electrophysiological measures of synaptopathy for listeners with normal audiograms (Bramhall et al., 2018; Fulbright et al., 2017; Guest et al., 2018c; Prendergast et al., 2017b). As described in the Introduction, it could be argued from rodent findings that the electrophysiological measures used in humans are relatively insensitive to synaptopathy. However, there is compelling evidence from the mouse model that the MEMR is highly sensitive to synaptopathy (Valero et al., 2018). It is possible of course that synaptopathy is present in our cohort, but that it has relatively insignificant effects on SPiN. Oxenham (2016) has argued, from a signal detection theory perspective, that the perceptual effects of even 50% deafferentation may be insignificant.

Given the many auditory and non-auditory factors that are known to contribute to SPiN (Yeend et al., 2017), it is perhaps unsurprising that the contribution of synaptopathy might be obscured. It should be remembered, however, that our recruitment specifically targeted participants who experienced listening difficulties, and the variation in SPiN performance in our cohort was therefore greater than that expected of a random sample of listeners in the same age range. Moreover, results were confirmed in a supplementary analysis based on self-reported listening difficulties. Our results suggest that the

problems experienced by these listeners are not primarily a consequence of reduced auditory nerve function, at least as measured by MEMR thresholds.

Middle-ear-muscle reflex thresholds and noise exposure

The majority of recent studies have found no relation between common recreational noise exposure and electrophysiological measures of synaptopathy in humans with normal audiometric hearing (Fulbright et al., 2017; Grinn et al., 2017; Guest et al., 2017b; Prendergast et al., 2017a; Prendergast et al., 2018; Spankovich et al., 2017). It is possible that more extreme exposures, such as those associated with firearm use, may be more synaptopathic (Bramhall et al., 2017). It was hoped that an MEMR threshold measure, being potentially more sensitive than the electrophysiological measures used previously, would reveal an effect, but this was not the case in the present study.

Although humans may be less susceptible to synaptopathy than rodents (Dobie & Humes, 2017), noise-induced synaptopathy has been observed in the macaque model, in the absence of significant threshold shifts. Hence, it seems plausible that noise-induced synaptopathy might also occur in humans with normal audiometry. However, in the macaque study, the degree of synaptopathy associated with temporary threshold shift was 12-27% (Valero et al., 2017), which may be at the limit of the sensitivity of the measures used in humans. For example, ABR wave I amplitude has a coefficient of variation of about 0.3 in humans (Guest et al., 2017b; Prendergast et al., 2018b). Combined with the fact that noise exposure in humans in necessarily much more variable than that in experimental animal studies, it is possible that large numbers of participants would be required to detect an effect. Most of the published studies may have been underpowered.

Self-report measures of noise exposure are likely imprecise. However, the range of estimated exposures in the present study (greater than a factor of 1000 in terms of total energy), are probably large compared to the imprecision of the tool. For example, one would certainly expect participant recollection to be sufficient to differentiate between more-than-weekly attendance at nightclubs and concerts over several years and total abstinence from loud music events. These are examples of NESI reports from the extremes of our distribution. Furthermore, in a previous study with the same cohort (Guest et al., 2017b), the noise-exposure tool was sufficiently sensitive to show a significant elevation in exposure in tinnitus participants compared to non-tinnitus participants, with only 20 participants in each group.

CONCLUSIONS

Despite the promise of the MEMR metric, and a wide range in the comparison variables (tinnitus, SPiN, and noise exposure) that are assumed to be associated with cochlear synaptopathy, the present results provide no evidence for synaptopathy in the population studied. Manifestations of cochlear synaptopathy in young humans with normal audiograms remain elusive. It is of course possible, likely even, that substantial noise-induced synaptopathy in humans co-occurs with a noise-induced audiometric loss. In the macaque study, the monkeys who were sufficiently exposed to incur a permanent threshold shift had a dramatic loss of synapses, up to about 75%, on the surviving inner hair cells (Valero et al., 2017). The challenge then is to separate the effects of hair cell loss from synaptopathy diagnostically. Given that the MEMR may be particularly dependent on low-SR function (Kobler et al., 1992; Liberman & Kiang, 1984), it could provide a useful measure in this regard.

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REFERENCES

Bharadwaj HM, Verhulst S, Shaheen L, Liberman MC, Shinn-Cunningham BG (2014) Cochlear neuropathy and the coding of supra-threshold sound. Front Syst Neurosci 8: 26.

Bharadwaj HM, Masud S, Mehraei G, Verhulst S, Shinn-Cunningham BG (2015) Individual differences reveal correlates of hidden hearing deficits. J Neurosci 35: 2161-72.

Bourien J, Tang Y, Batrel C, Huet A, Lenoir M, Ladrech S, Desmadryl G, Nouvian R, Puel JL, Wang J (2014) Contribution of auditory nerve fibers to compound action potential of the auditory nerve. J Neurophysiol 112: 1025-39.

Bramhall N, Ong B, Ko J, Parker M (2015) Speech perception ability in noise is correlated with auditory brainstem response wave I amplitude. J Am Acad Audiol 26: 509-17.

Bramhall NF, Konrad-Martin D, McMillan GP (2018) Tinnitus and auditory perception after a history of noise exposure: Relationship to auditory brainstem response measures. Ear Hear.

Bramhall NF, Konrad-Martin D, McMillan GP, Griest SE (2017) Auditory brainstem response altered in humans with noise exposure despite normal outer hair cell function. Ear Hear 38: e1-e12.

Davis, AC (1995) Hearing in adults. London, UK: Whurr.

Dobie RA, Humes LE (2017) Commentary on the regulatory implications of noise-induced cochlear neuropathy. Int J Audiol 56: 74-78.

Don M, Eggermont JJ (1978) Analysis of the click-evoked brainstem potentials in man using high-pass noise masking. J Acoust Soc Am 63: 1084-92.

Feeney MP, Keefe DH, Hunter LL, Fitzpatrick DF, Garinis AC, Putterman DB, McMillan GP (2017) Normative wideband reflectance, equivalent admittance at the tympanic membrane, and acoustic stapedius reflex threshold in adults. Ear Hear 38: e142-e160.

Fernandez KA, Jeffers PW, Lall K, Liberman MC, Kujawa SG (2015) Aging after noise exposure: acceleration of cochlear synaptopathy in "recovered" ears. J Neurosci 35: 7509-20.

Fulbright ANC, Le Prell CG, Griffiths SK, Lobarinas E (2017) Effects of recreational noise on threshold and suprathreshold measures of auditory function. Semin Hear 38: 298-318.

Furman AC, Kujawa SG, Liberman MC (2013) Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. J Neurophysiol 110: 577-86.

Gilles A, Schlee W, Rabau S, Wouters K, Fransen E, Van de Heyning P (2016) Decreased speech-in-noise understanding in young adults with tinnitus. Frontiers Neurosci 10: 288.

Grinn SK, Wiseman KB, Baker JA, Le Prell CG (2017) Hidden hearing loss? No effect of common recreational noise exposure on cochlear nerve response amplitude in humans. Frontiers Neurosci 11: 465.

Grose JH, Buss E, Hall JW, (2017) Loud music exposure and cochlear synaptopathy in young adults: Isolated auditory brainstem response effects but no perceptual consequences. Trends Hear 21.

Gu JW, Herrmann BS, Levine RA, Melcher JR (2012) Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. J Assoc Res Otolaryngol 13: 819-33.

Guest H, Munro KJ, Plack CJ (2017a) Tinnitus with a normal audiogram: Role of high-frequency sensitivity and reanalysis of brainstem-response measures to avoid audiometric over-matching. Hear Res 356: 116-117.

Guest H, Munro KJ, Prendergast G, Howe S, Plack CJ (2017b) Tinnitus with a normal audiogram: Relation to noise exposure but no evidence for cochlear synaptopathy. Hear Res 344: 265-274.

Guest H, Dewey RS, Plack CJ, Couth S, Prendergast G, Bakay W, Hall DA (2018a) The Noise Exposure Structured Interview (NESI): An instrument for the comprehensive estimation of lifetime noise exposure. Trends Hear.

Guest H, Munro KJ, Plack CJ (2018b) Reliability and interrelations of proxy measures of cochlear synaptopathy. Presented at the British Society of Audiology Basic Auditory Science meeting, Newcastle, UK.

Guest H, Munro KJ, Prendergast G, Millman RE, Plack CJ (2018c) Impaired speech perception in noise with a normal audiogram: No evidence for cochlear synaptopathy and no relation to lifetime noise exposure. Hear Res 364: 142-151.

Kobler JB, Guinan JJ, Jr., Vacher SR, Norris BE (1992) Acoustic reflex frequency selectivity in single stapedius motoneurons of the cat. J Neurophysiol 68: 807-17.

Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci 29: 14077-85.

Liberman MC, Kiang NY (1984) Single-neuron labeling and chronic cochlear pathology. IV. Stereocilia damage and alterations in rate- and phase-level functions. Hear Res 16: 75-90.

Liberman MC, Epstein MJ, Cleveland SS, Wang H, Maison SF (2016) Toward a differential diagnosis of hidden hearing loss in humans. PLoS One 11: e0162726.

Lopez-Poveda EA, Barrios P (2013) Perception of stochastically undersampled sound waveforms: a model of auditory deafferentation. Frontiers Neurosci 7: 124.

Oxenham AJ (2016) Predicting the Perceptual Consequences of Hidden Hearing Loss. Trends Hear 20: 2331216516686768.

Paul BT, Bruce IC, Roberts LE (2017) Evidence that hidden hearing loss underlies amplitude modulation encoding deficits in individuals with and without tinnitus. Hear Res 344: 170-182.

Prendergast G, Guest H, Munro KJ, Kluk K, Leger A, Hall DA, Heinz MG, Plack CJ (2017a) Effects of noise exposure on young adults with normal audiograms I: Electrophysiology. Hear Res 344: 68-81.

Prendergast G, Tu W, Guest H, Millman RE, Kluk K, Couth S, Munro KJ, Plack CJ (2018) Supra-threshold auditory brainstem response amplitudes in humans: Test-retest reliability, electrode montage and noise exposure. Hear Res.

Prendergast G, Millman RE, Guest H, Munro KJ, Kluk K, Dewey RS, Hall DA, Heinz MG, Plack CJ (2017b) Effects of noise exposure on young adults with normal audiograms II: Behavioral measures. Hear Res 356: 74-86.

R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roberts LE, Paul BT, Bruce IC (2018) Erratum and comment: Envelope following responses in normal hearing and in tinnitus. Hear Res 361: 157-158.

Schaette R, McAlpine D (2011) Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. J Neurosci 31: 13452-7.

Sergeyenko Y, Lall K, Liberman MC, Kujawa SG (2013) Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. J Neurosci 33: 13686-94.

Shaheen LA, Valero MD, Liberman MC (2015) Towards a diagnosis of cochlear neuropathy with envelope following responses. J Assoc Res Otolaryngol 16: 727-45.

Shim HJ, An YH, Kim DH, Yoon JE, Yoon JH (2017) Comparisons of auditory brainstem response and sound level tolerance in tinnitus ears and non-tinnitus ears in unilateral tinnitus patients with normal audiograms. PLoS One 12: e0189157.

Spankovich C, Le Prell CG, Lobarinas E, Hood LJ (2017) Noise history and auditory function in young adults with and without Type 1 diabetes mellitus. Ear Hear 38: 724-735.

Stamper GC, Johnson TA (2015) Letter to the Editor: Examination of potential sex influences in . auditory function in normal-hearing, noise-exposed human ears, Ear Hear, 36, 172-184. Ear Hear 36: 738-40.

Valero MD, Hancock KE, Liberman MC (2016) The middle ear muscle reflex in the diagnosis of cochlear neuropathy. Hear Res 332: 29-38.

Valero MD, Hancock KE, Maison SF, Liberman MC (2018) Effects of cochlear synaptopathy on middle-ear muscle reflexes in unanesthetized mice. Hear Res 363: 109-118.

Valero MD, Burton JA, Hauser SN, Hackett TA, Ramachandran R, Liberman MC (2017) Noise-induced cochlear synaptopathy in rhesus monkeys (Macaca mulatta). Hear Res 353: 213-223.

Viana LM, O'Malley JT, Burgess BJ, Jones DD, Oliveira CA, Santos F, Merchant SN, Liberman LD, Liberman MC (2015) Cochlear neuropathy in human presbycusis: Confocal analysis of hidden hearing loss in post-mortem tissue. Hear Res 327: 78-88.

Wojtczak M, Beim JA (2018) The effect of elicitor bandwidth on a wideband measure of the middle-ear-muscle reflex in young normal-hearing individuals. Assocation for Research in Otolaryngology 41st Annual Midwinter Meeting, San Diego.

Wojtczak M, Beim JA, Oxenham AJ (2017) Weak middle-ear-muscle reflex in humans with noise-induced tinnitus and normal hearing may reflect cochlear synaptopathy. eNeuro 4.

Wu PZ, Liberman LD, Bennett K, deGruttola V, O'Malley JT, Liberman MC (2018) Primary neural degeneration in the human cochlea: Evidence for hidden hearing loss in the aging ear. Neurosci (in press).

Yeend I, Beach EF, Sharma M, Dillon H (2017) The effects of noise exposure and musical training on suprathreshold auditory processing and speech perception in noise. Hear Res 353: 224-236.

FIGURE CAPTIONS

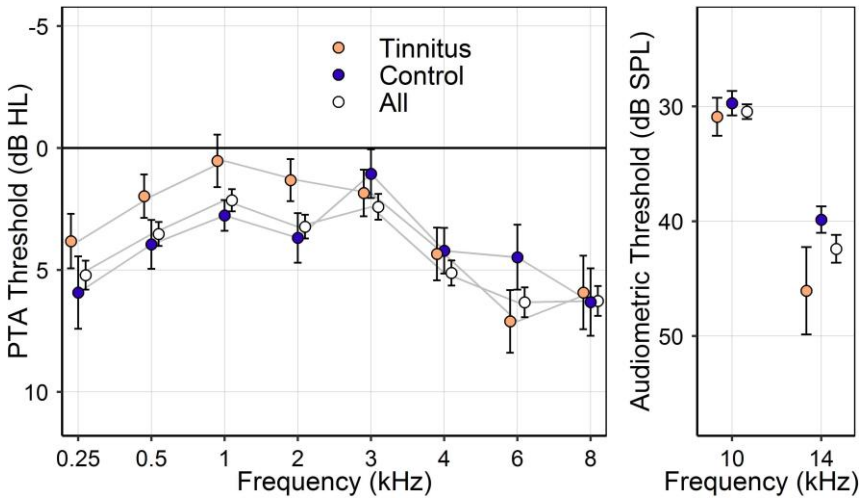
Table 1. Participant characteristics.

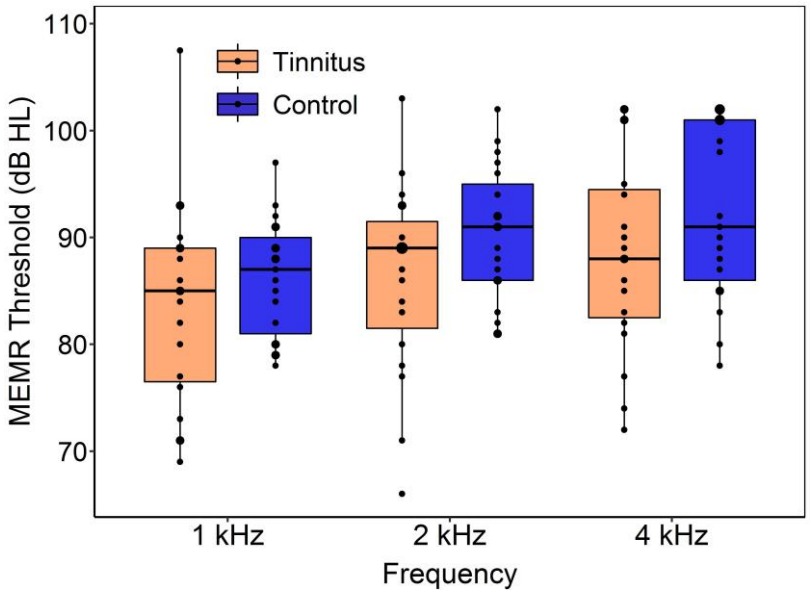
Fig. 1. Mean audiometric thresholds for the tinnitus and control groups, and for all participants in the study. Error bars represent the standard error of the mean (SEM). Mean thresholds differ between tinnitus and control groups by <3 dB across the standard audiometric range; mean EHF audiometric thresholds differ by 1.2 dB at 10 kHz and 6.2 dB at 14 kHz.

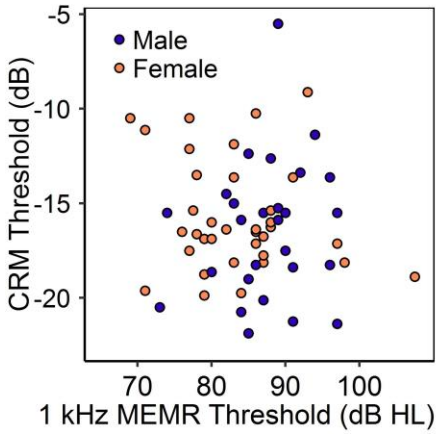
Fig. 2. Distributions of MEMR thresholds for tinnitus and control participants at 1, 2, and 4 kHz. Upper and lower hinges correspond to the first and third quartiles, upper whiskers to the highest value within 1.5 * IQR of the upper hinge (where IQR is the interquartile range), and lower whiskers to the lowest value within 1.5 * IQR of the lower hinge. Point size indicates number of observations at a given threshold. No significant differences in threshold between groups are evident.

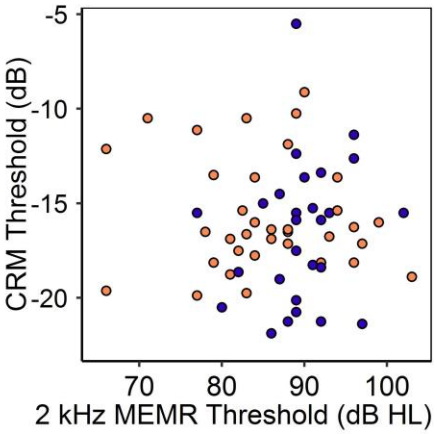
Fig. 3. CRM threshold as a function of MEMR thresholds at 1, 2, and 4 kHz. The lower right panel lists the correlation coefficient for each cohort (Pearson's r or Spearman's r_s, as appropriate). No significant correlations, nor any consistent trends, are evident.

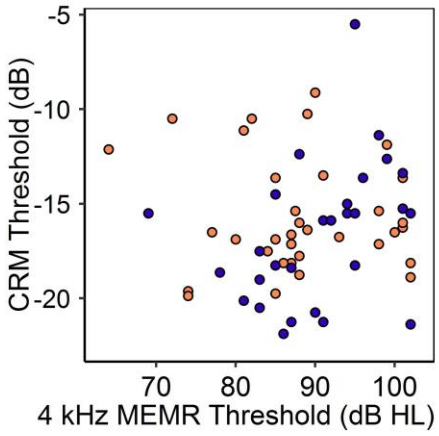
Fig. 4. MEMR thresholds at 1, 2, and 4 kHz as a function of lifetime noise exposure. Arrows indicate observations beyond the plotted range, from an 18-year-old participant with very little lifetime noise exposure (0.006 NESI units). The lower right panel lists the correlation coefficient for each cohort (Pearson's r or Spearman's r_s, as appropriate). No significant correlations, nor any consistent trends, are evident.











1 kHz

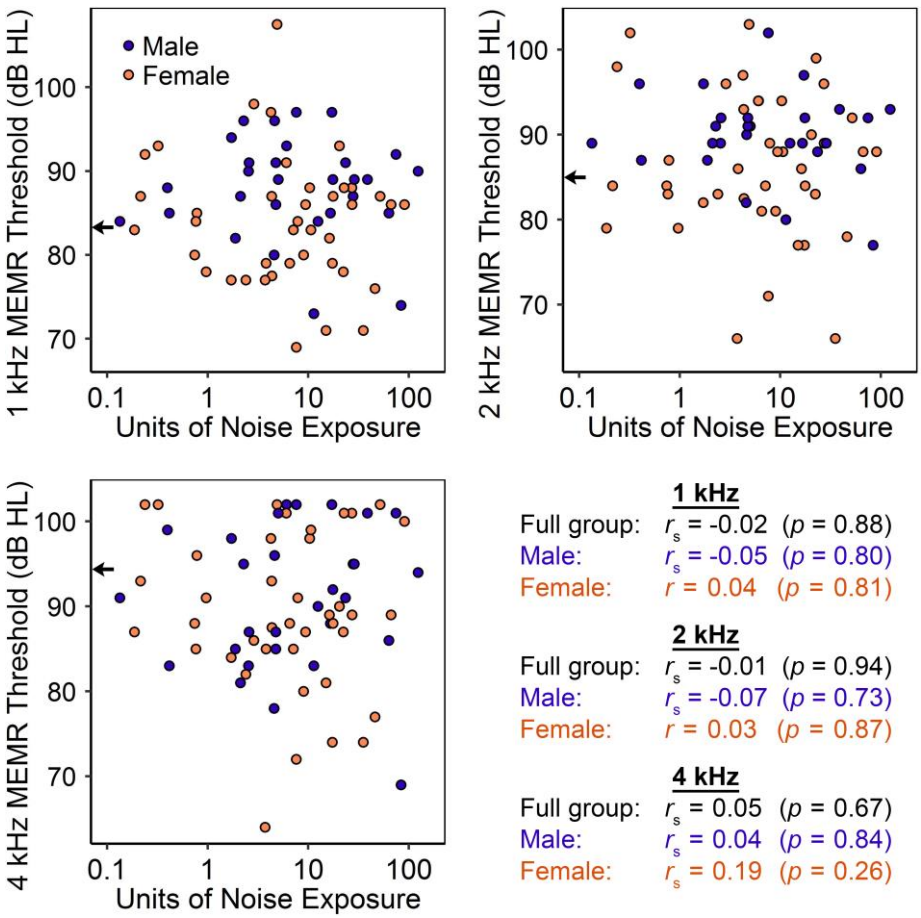
Full group: r = -0.1 (p = 0.45)
Male: r = 0.12 (p = 0.56)
Female: r_s = 0.15 (p = 0.4)

2 kHz

Full group: r = -0.05 (p = 0.71)
Male: r = 0.17 (p = 0.39)
Female: r_s = 0.01 (p = 0.95)

4 kHz

Full group: r_s = 0.19 (p = 0.14)
Male: r = 0.34 (p = 0.07)
Female: r_s = 0.06 (p = 0.72)



	Entire sample	Tinnitus	Controls for tinnitus
Number	70	19	19
Age (years)	Mean = 27.0 (SD = 5.6)	Mean = 26.6 (SD = 5.5)	Mean = 26.6 (SD = 5.5)
Female	40 (57%)	11 (58%)	11 (58%)