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Effects of unilateral eye closure on middle ear muscle contractions

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ABSTRACT

Middle ear muscle contractions (MEMCs) are most commonly considered a response to high-level acoustic stimuli. However, MEMCs have also been observed in the absence of sound, either as a response to somatosensory stimulation or in concert with other motor activity. The relationship between MEMCs and non-acoustic sources is unclear. This study examined associations between measures of voluntary unilateral eye closure and impedance-based measures indicative of middle ear muscle activity while controlling for demographic and clinical factors in a large group of participants (N=190) with present clinical acoustic reflexes and no evidence of auditory dysfunction. Participants were instructed to voluntarily close the eye ipsilateral to the ear canal containing a detection probe at three levels of effort. Orbicularis oculi muscle activity was measured using surface electromyography. Middle ear muscle activity was inferred from changes in total energy reflected in the ear canal using a filtered (0.2 to 8 kHz) click train. Results revealed that middle ear muscle activity was positively associated with eye muscle activity. MEMC occurrence rates for eye closure observed in this study were generally higher than previously published rates for high-level brief acoustic stimuli in the same participant pool suggesting that motor activity may be a more reliable elicitor of MEMCs than acoustic stimuli. These results suggest motor activity can serve as a confounding factor for auditory exposure studies as well as complicate the interpretation of any impulsive noise damage risk criteria that assume MEMCs serve as a consistent, uniform protective factor. The mechanism linking eye and middle ear muscle activity is not understood and is an avenue for future research.

1. Introduction

Middle ear muscle contractions (MEMCs), which involve the stapedius (ST) and/or tensor tympani (TT) muscles, can alter middle ear impedance. For more than half a century, MEMCs have been considered a protective mechanism in some impulsive noise damage risk criteria (DRCs) (Price, 2007a, 2007b; Price and Kalb, 1991; Ward, 1968). DRCs for impulsive noise return estimates of the level and probability of hearing damage associated with sound exposure in order to prevent injury. Including a protective role for MEMCs in DRCs implies that, within the exposed population, MEMCs are pervasive, of sufficient magnitude to provide substantial protection, and reliable across different situations and operational environments. Warfighters engage in a wide range of activities including operating firearms, maintaining situational awareness, and communicating with team members. For example, warfighters often tightly close their eyes when discharging a fixed/crew-served weapon (e.g., mortar), but are explicitly trained to maintain a relaxed body position with eyes open at the point of firing their service rifle (U.S. Department of the Army, 2011, 2012). Both scenarios involve high-level noise, but it is unclear how the influence of non-acoustic factors, such as eye closure, might impact the likelihood of MEMCs and, therefore, the warfighters' noise exposure.

MEMCs typically reduce low frequency energy reaching the cochlea (Pang and Peake, 1986) while higher-frequency sound transmission is either minimally affected or amplified (Feeney and Keefe, 1999; Jones et al., 2017). The evolutionary function of MEMCs is not clear. In addition to potentially protecting the ear from environmental noise (Borg et al., 1984), posited roles include enhancing ventilation of the middle ear space (Mukerji et al., 2010), and reducing the masking from endogenous vocalization (Borg and Zakrisson, 1975). Alternatively, given the substantial evolutionary remodeling of the middle ear and nearby structures,

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MEMCs may have selective advantages unrelated to hearing (e.g. mandibular stabilization), or may be an evolutionary vestigial (Manley, 2010).

The small size and deep location of middle ear muscles complicate recording activity directly. As a result, studies that performed direct recordings have relied on the use of animals (Carmel and Starr, 1963, 1964; Starr and Salomon, 1965) or humans who either have significant auditory pathologies such as a perforated tympanic membrane or are under anesthesia (Borg and Zakrisson, 1975; Salomon and Starr, 1963). The impedance-based approach used in most human-based studies is convenient and non-invasive, but has limitations. Measurement validity can be compromised by movement of the measurement probe or external sound sources contaminating the ear canal recordings. Additionally, disambiguating ST and TT activity is not possible based solely on impedance changes. These limitations notwithstanding, the impedance-based approach has been an essential research tool for characterizing MEMCs among healthy and hearing disordered populations.

MEMCs can be elicited by a range of stimuli (Djupesland, 1976). The acoustic reflex (AR), elicited in response to moderate-to-high intensity sound exposure, is routinely used in clinical audiology to assess auditory system integrity (Hunter and Shahnaz, 2013). Beyond the AR, various forms of somatosensory stimulation of the external ear and face can reliably elicit MEMCs (Djupesland, 1976; Djupesland and Tvete, 1979; Klockhoff and Anderson, 1959, 1960), indicating that multiple afferent pathways converge onto middle ear motor neuron pools (Mukerji et al., 2010). Prior studies have reported MEMCs concurrent with general body movement (Carmel and Starr, 1963, 1964; Starr and Salomon, 1965), swallowing (Klockhoff, 1961; Wersall, 1958), yawning (Klockhoff, 1961), vocalization (Borg and Zakrisson, 1975; Carmel and Starr, 1963, 1964; Salomon and Starr, 1963; Simmons, 1964a, 1964b; Starr and Salomon, 1965), eye-related activity (Gruters et al., 2018; Salomon and Starr, 1963) and during sleep (De Gennaro et al., 2000; Slegel et al., 1991).

Eye-related motor activity has been linked to MEMC activity (Gruters et al., 2018; Salomon and Starr, 1963). Voluntary eye closure has been shown to activate the TT (as measured with direct muscle recording) with high reliability, little habituation, and contraction levels that scale with degree of eye closure (Salomon and Starr, 1963). However, involuntary protective eye closure can elicit TT activity that is highly variable, lags periorbital muscle activity and is prone to rapid habituation (Salomon and Starr, 1963). ST activity is not consistently observed during eye closure. Recently, Gruters et al. (2018) found interesting relationships between tympanic membrane pressure changes and saccadic eye movement in humans and rhesus monkeys. First, ear canal pressure changes were observed approximately 10 ms prior to the onset of a saccadic eye movement. These pressure changes lasted throughout the eye movement and into the eye fixation period between successive saccades. Second, the amplitude of ear canal pressure change was proportional to the amplitude of the saccadic eye movement. Finally, the amplitude and phase of the eardrum pressure changes were associated with the direction and amplitude of the eye saccades. Although the authors stated that the source of the ear canal pressure changes were not known, middle ear muscle activity was a likely possibility.

In summary, the middle ear muscle system is a site of complex sensorimotor integration and there is a need to understand the relationship between MEMCs, discrete motor activity, and acoustic and non-acoustic sensory elicitors. Studies can help elucidate the range of neural circuits controlling middle ear muscles, including afferent pathways across different sensory modalities and sites, and efferent pathways to the middle ear muscles from central structures. Non-acoustically elicited MEMCs have received little attention in both clinical applications and by those who assume a protective role for MEMCs against noise exposure (Price, 2007a, 2007b; Price and Kalb, 1991).

The aim of the current study was to assess the association between orbicularis oculi muscle activity associated with voluntary unilateral eye closure and synchronous middle ear muscle activity, inferred from ear canal pressure changes in response to a broadband click probe train.

2. Methods

2.1. Participants

Study participants included 190 noninstitutionalized adults (71 % female) ranging from 18 to 55 years of age and drawn from the general population around Kalamazoo, Michigan between 2015 and 2017. Oversight of human research subject protection was provided via institutional review boards at Western Michigan University (Protocol #15-04-09) and the U.S. Army Medical Research and Materiel Command (HRPO # A-18436.2).

Participants exhibited no indication of auditory dysfunction, no history of Bell's Palsy, no current ear pain, no history of concussion, and no unexplained dizziness. Exclusion criteria included excessive cerumen, signs of middle ear abnormality, any pure tone air conduction threshold poorer than 10 dB HL from 125 Hz to 1 kHz or poorer than 20 dB HL from 2 to 8 kHz, threshold asymmetry greater than 20 dB, abnormal middle ear function via conventional and wideband acoustic immittance and wideband tympanometry, absent clinical acoustic reflexes, or evidence of abnormal trigeminal or facial nerve function.

2.2. Instrumentation

Pure tone audiometry was conducted using the Nelson Acoustics Audiometric Research Tool (ART, VIacoustics, Austin, TX) and automatic audiometry software utilizing National Instruments (NI) Hybrid PXI/PXIe-4461 modules (National Instruments, Austin, TX). Air conduction signals were delivered with Sennheiser HDA-200 circumaural earphones (Sennheiser, Wedemark, Germany) and bone conduction signals were delivered through a RadioEar B-71 bone oscillator (RadioEar, Middelfart, Denmark) placed on the forehead. Stimulus calibration and test procedures followed ANSI standards (ANSI S3.6, ANSI S3.1) and pure tone (octave intervals 0.125–8 kHz, inclusive, and 3 and 6 kHz) and bone conduction thresholds (octave intervals 0.5–4 kHz, inclusive) were established using a modified Hughson-Westlake procedure (Carhart and Jerger, 1959).

An Interacoustics Titan® middle ear analyzer (Interacoustics, Middelfart, Denmark) was used to perform conventional tympanometry, wideband tympanometry and immittance (using a band-limited click stimulus), acoustic reflex thresholds, and acoustic reflex decay. Tympanometry was conducted with a single pressure sweep from +200 to -300 daPa. Ipsilateral and contralateral acoustic reflex traces were obtained in each ear using a 226 Hz probe tone for pure-tone elicitors (700–800 ms) at 0.5, 1, 2 and 4 kHz to a maximum of 100 dB HL. Elicitors were presented in 5 dB increments from 80 dB HL to the level producing a repeatable immittance change of 0.05 mmho, or at 100 dB HL, whichever occurred first.

Surface EMG recordings were made using a Delsys Bagnoli® EMG system with active, double-differential electrode sensors (Delsys, Natick, MA). A gain multiplier of 1000 (i.e., 60 dB) and band-pass filtering (20–450 Hz) was applied to all EMG signals prior to analog-to-digital conversion. Five EMG sites were monitored during the main experiment. Sites were identified using standard procedures (Criswell and Cram, 2011) and skin was prepared by gentle scrubbing with alcohol, followed by "tacking" the area with biomedical tape. A common ground electrode was placed over the olecranon of the ulna at the elbow joint. Of primary importance for this study was the orbicularis oculi (OOC) recording site (ipsilateral to the OAE probe), which is active during eye closure. The OOC sensor was placed as close as possible to the inferior margin of the lower eyelid, while minimizing any interference by the sensor during tight eye closure. The integrity of the OOC EMG signal was confirmed through a series of maximal effort eye closures. Mandibular motion can influence ear canal geometry. Therefore, synchronous EMG recordings from the masseter muscle (mandibular elevator), and the suprahyoid muscle complex (mandibular depressor) were made throughout the experiment. EMG traces associated with individual eye closure trials were inspected visually and any evidence of concomitant masseter and/or suprahyoid muscle activity was flagged and included as a potential predictor variable in the subsequent regression analysis.

An Etymotic Research ER-10X® OAE probe (Etymotic Research, Elk Grove Village, IL) was used to present probe stimuli and acquire the signal in the ear canal. The ear in which the OAE probe was positioned and the OOC EMG recording site were both contralateral to the participant's preferred trigger finger. The probe stimulus was a train of clicks presented every 50 ms (20 Hz presentation rate). Each click was the finite impulse response of a digital filter with a passband from 0.2 – 8 kHz. Probe clicks were digitally sampled at 44.1 kHz and presented at 93 dB peak SPL as measured in an IEC-60318-4 occluded ear simulator. Probe fit was monitored throughout the experiment via visual inspection and assessment of the ear recordings of a swept sine wave (0.1−16 kHz) stimulus.

Instrumentation used during the main experiment was controlled with custom MATLAB (The MathWorks, Inc., Natick, MA) scripts using a Windows-based (Microsoft, Redmond, WA) PC workstation (Dell model 7910, Dell, Austin, TX) connected to a NI PXIe-1082 chassis. A NI PXI/PXIe (hybrid) 4461 dynamic signal analyzer and PXIe-4499 modules were used to produce the probe click and synchronously sample all input channels during recording. All input channels were sampled at 44.1 kHz (24 bit).

The calibration of all acoustic transducers was verified at the beginning and end of each test day.

2.3. Experimental procedure

The complete experiment required participants to make two laboratory visits ($<$ 2 h/visit). The first visit included obtaining informed consent to participate, questionnaire measures focused on ear and hearing health as well as current and past noise exposure, otoscopy, pure-tone audiometry, a brief screening of trigeminal and facial nerve function, and a clinical middle ear evaluation.

The main experimental visit occurred within two weeks of the candidacy visit. Upon arrival, participants were questioned about changes in ear pain, hearing, and dizziness since the initial visit. The visit began and ended with video otoscopy, pure tone air conduction thresholds, and clinical middle ear testing (without acoustic reflexes) to rule out changes in auditory function since the initial visit and/or following the main experiment. In addition to the voluntary eye closure task, which is the focus of this report, the main experiment consisted of nine conditions employing various acoustic stimuli, a single condition in which puffs of air were presented to the face and a single associative learning task. For a complete description of test conditions, see Deiters et al. (2019) and Tasko et al. (2020). The test condition in which voluntary eye closure was elicited occurred first, second, tenth or eleventh in the twelve-condition sequence.

Participants were seated in front of a large computer display with the OAE probe and the EMG electrodes in place. Additionally, an Etymotic Research ER-4PT® insert earphone (Etymotic Research, Elk Grove Village, IL) was placed in the ear contralateral to the OAE probe to deliver instructions and stimuli unrelated to the present report. The experimental task required participants to close one eye using light, tight, or maximum muscular effort level, as indicated using a visual analog display (Fig. 1A). Single eye closure was employed in the current study to allow for continuous viewing of the analog display and because it is likely that military personnel fire small arms weapons with either both eyes open or one eye closed. An empty display indicated an open eye (no closure effort). Participants were instructed to close the test eye with an effort level they believed matched the fill level of the display (Fig. 1B). The display could be 3/10 full, prompting a low level of muscular effort (light closure effort), 8/10 full prompting a greater level of muscular effort (tight closure effort), or completely full, prompting maximum muscular effort (maximum closure effort). Eye closure prompts were 3 s long and the interstimulus interval was 5 s. For each participant, a total of 14 trials were presented. The experimental run lasted 114 s. The first and last trials were always a filled display, prompting maximum closure effort. The twelve intermediate trials included six light closure effort trials and six tight closure effort trials, presented in random order.

2.4. Data processing

Following data acquisition, the OOC EMG recording was notch filtered at 60 Hz to eliminate possible artifacts from power line noise, and a root-mean-square (RMS) trace of each EMG channel was generated using a moving average over a 50-ms rectangular window (Fig. 1B). The onset and offset of OOC EMG activity was detected using a two-step process that included an automated detection algorithm followed by visual confirmation and modification if necessary (Tasko et al., 2020). The automated algorithm extracted the OOC RMS trace for each trial for a time window beginning one second before, and ending three seconds following the eye close display. Closure onset was defined as the point where the RMS trace first exceeded the 95th percentile of the RMS distribution and closure offset was the point where the RMS trace dropped below the 95th percentile. EMG onset and offset times were used to segment the recording into a consecutive series of eye closure and eye open (i.e., baseline) intervals. The eye closure interval was defined as the period bound by EMG onset and offset times. The baseline interval was defined as the period between the offset of the preceding eye close interval (or in the case of the first trial, the beginning of the record) and the onset of the next eye close interval.

A change in middle ear status in response to eye closure was assessed using a method modified from Keefe et al. (2010). The difference was calculated between each 50-ms click waveform developed in the ear canal during eye closure (Fig. 1C, left plot) and the ensemble-average waveform of all 50-ms clicks located within the previous baseline interval (Fig. 1C, middle plot). The RMS magnitude of the deviation between the individual and average baseline click (Fig. 1C, right plot) served as the estimate of middle ear status change for that 50-ms click. This process was repeated for all recorded clicks for a given baseline interval-eye closure interval sequence. Fig. 1D is a stair plot that includes 0.5 s of a baseline interval followed by a complete eye closure interval. Each stair represents RMS deviation of a click (hereafter termed ear click deviation). The RMS magnitude of the OOC EMG activity was calculated for the same 50-ms window as the ear canal clicks allowing synchronized comparison of OOC and ear canal activity (Fig. 1E). Mean ear click deviation and mean OOC EMG activity was determined for the last three seconds of each baseline interval and for each eye close interval (i.e. trial-level means). Exploratory analysis revealed that distributions of the mean ear click deviation and the mean OOC EMG activity exhibited a strong positive skew. There-

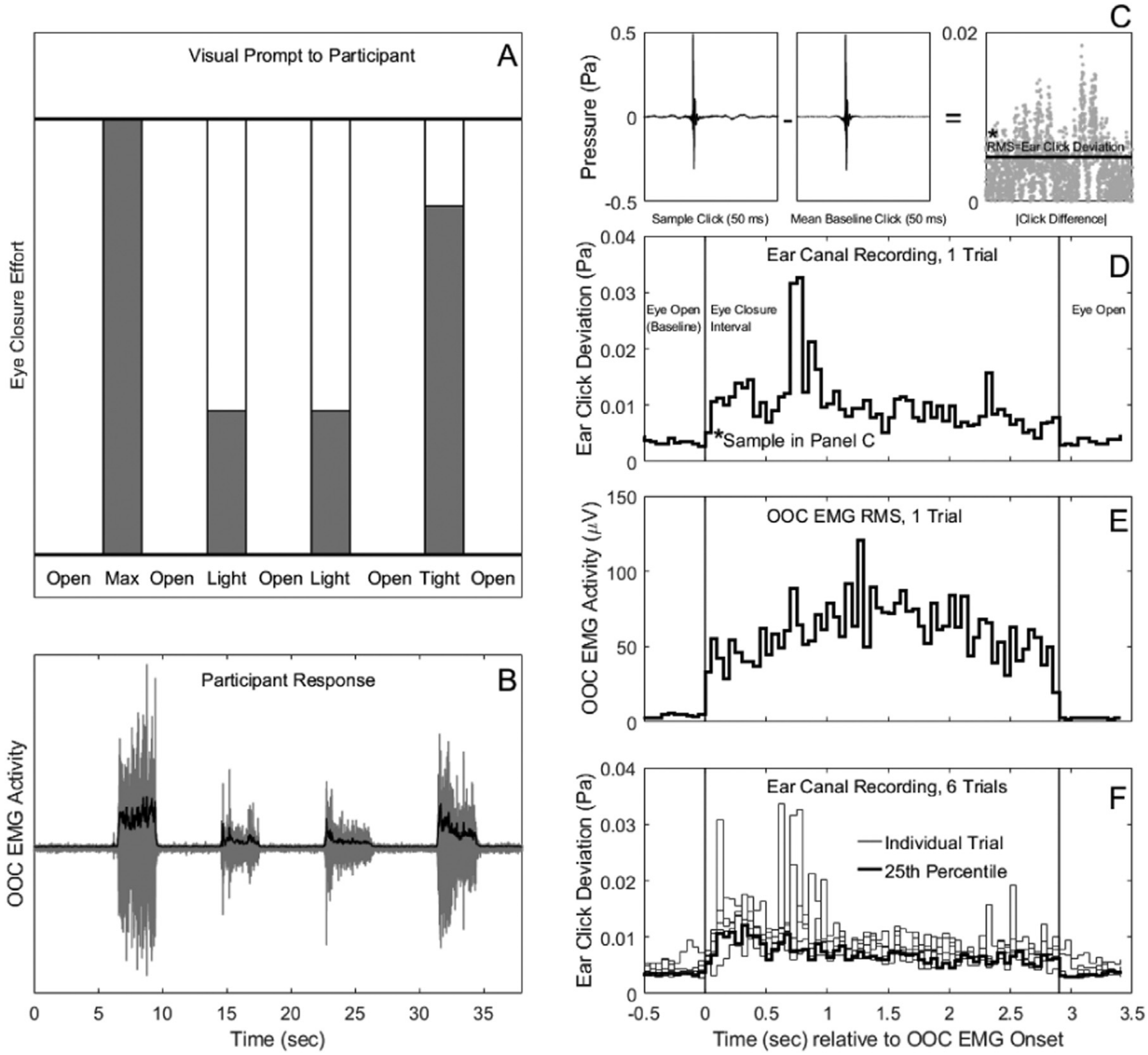

Fig. 1. Panel A: Representation of the visual display used to prompt participants to perform unilateral eye closures. The first 40 s of a 114 second record is shown. A thermometer-type display alternated between an empty display, prompting eye opening, and a partially or fully filled display, prompting different closure effort levels (light, tight, and maximum). Each closure prompt lasted 3 s and eye open intervals (baseline) were 5 s. Panel B: OOC EMG activity recorded from a participant responding to visual prompts from Panel A. The gray line is the raw EMG trace and the black line is the RMS of the raw signal (50-ms window). OOC EMG activity lags the visual prompts due to visuomotor processing. Panel C: Processing steps applied to click train recorded in participants' ear canals. The left plot represents a single 50-ms broadband click waveform obtained during an eye closure interval. The middle plot represents the mean "baseline" waveform, generated using all click waveforms occurring 3 s prior to eye closure onset when the eye was open. The right plot represents magnitude of the waveform deviations, generated by subtracting the mean baseline click from the individual click. These magnitudes were used to calculate overall ear click deviation. These processing steps were repeated for all clicks recorded during the eye-open baseline and eye-closure intervals. Panel D: Stair plot of the ear click deviation during an eye closure trial. Time is referenced to the onset of OOC EMG activity. Each step represent ear click deviation for successive 50-msec clicks. The ear click deviation increases at onset of the eye closure interval and decreases at the end of the eye closure interval. The asterisk marks the location of click waveform from Panel C. Panel E: Stair plot of the OOC EMG activity for the same 50-ms intervals in Panel D. Panel F: Stair plot of ear click deviation for an ensemble of 6 ear canal recordings (light gray lines) drawn from a single participant across all replicates of tight eye closure. The heavy black line represents the 25th percentile of the data distribution (i.e., trial-level data for this participant on this task) and was used by judges to assess for the presence or absence of MEMCs (see text).

fore, mean ear click deviation and mean OOC EMG activity were log-transformed prior to statistical analysis.

2.5. Estimating occurrence rates of eye closure-related changes in ear click deviation

One aim of this study was to estimate the occurrence rate of MEMCs during unilateral eye closure. The presence/absence of an observable stimulus-linked change in the ear click deviation was determined using binary judgements by three independent raters (authors GF, ST, and KD) who were blinded to the participant identity and eye closure effort level. Judgements were based on examination of a trace representing the 25th percentile of the time-aligned ensemble of ear click deviation traces for a given participant and eye closure effort level (Fig. 1F). This procedure has been used in a previous study focused on acoustically elicited MEMCs (Deiters et al., 2019). Two criteria were used to determine the occurrence of MEMCs. A "loose" criterion required 2/3 agreement among the judges and a "strict" criterion required unanimity.

2.6. Statistical analysis

2.6.1. Regression analysis

Stata® software (StataCorp LLC, College Station, TX) was used to perform descriptive and inferential statistical analysis. The primary independent measure, eye closure effort, was defined nominally (baseline/open eye, light, tight and maximum closure effort) and as OOC EMG activity (RMS voltage for 50 ms window synchronous to ear canal recordings). The primary dependent measures were the ear click deviation recorded in the ear canal and the binary judgement of the 25th percentile of the ear click deviation trace ensemble. Additionally, a large number of measures related to pure-tone audiometry, middle ear testing results, participant characteristics, and experimental design features were considered potential predictor variables.

Two separate regression modeling procedures were used for the continuous variable ear click deviation and the binary MEMC occurrence. A mixed-effects linear regression model was used to predict ear click deviation based on eye closure-related OOC EMG activity, while controlling for participant, audiologic and experimental variables. This analysis was performed at the trial-level, nested within participants. A logistic regression model was used to predict occurrence of MEMC based on measures of eye closure effort while controlling for participant, audiometric and experimental variables. As MEMC judgements were based on an ensemble of trials for a given eye closure effort level and participant, this analysis was performed at the participant level. For both analyses, participant identity was treated as a random factor and all other measures were treated as fixed factors. Development of the final model was similar for the two different models, and involved a multi-step process (Hosmer et al., 2013). First, univariable analyses were conducted with all potential predictor variables. Any univariable model with a p-value ≤ 0.20 was identified as a potentially important predictor variable and was retained for inclusion in the multivariable model. A p-value threshold greater than 0.05 was used to reduce the risk of prematurely eliminating a variable that may offer some predictive value in the multivariable model. A final multivariable model was developed by initial forced-entry of all near-significant variables. Variables failing to retain significance in the multivariable context were sequentially discarded until the model contained only significant ($p \leq 0.05$) variables using robust standard errors (Huber, 1967).

2.6.2. Reliability of Judges' ratings

Cohen's kappa (Cohen, 1960) was used for interrater reliability of the three judges' ratings based on visual inspection of the 25th percentile trace of the ensemble of responses for a given effort level within a given participant (Fig. 1, Panel F, heavy black line). Twenty-five participant-effort level sets could not be reviewed because it was not possible to confidently identify clear EMG boundaries. Each judge evaluated a total of 545 (light effort: 185; tight effort: 185; maximum effort: 175) traces across the 190 participants. Absolute agreement was 90.3, 90.9 and 96.2% for each combination of raters. Expected agreement based on chance alone for the same three judge pairings was determined to be 80.5, 80.9 and 87.5%, respectively. Absolute agreement was high, due to the high expected agreement based on chance, and Cohen's kappa values for the three judge pairings were 0.50, 0.52 and 0.70, which placed agreement levels in the moderate to substantial range (Cohen, 1960).

3. Results

3.1. OOC EMG activity across nominal levels of eye closure

Across the participant group, OOC EMG activity systematically varied as a function of eye closure effort level (Fig. 2A). During the baseline interval when the eye was open, OOC EMG activity approached zero and levels systematically increased with each nominal step in eye closure effort. A similar pattern was observed when OOC EMG activity was expressed as a percentage of maximum voluntary contraction (not shown). A mixed-effects linear regression model was performed in which nominal effort level was treated as a fixed factor and participant identity was a random factor. The overall model was significant (Wald $X^2 = 34062.73$, $p < 0.000005$) and post-hoc Bonferroni-adjusted ($p < 0.05$) pair-wise testing indicated that OOC EMG activity was significantly different for all eye closure effort levels. In summary, the visual analog scaling procedure was effective in eliciting a systematic grading of OOC EMG activity across a wide range of levels from the participant group.

3.2. Ear click deviation across nominal levels of eye closure

Ear click deviation across nominal levels of eye closure are summarized in Fig. 2B. Similar to OOC EMG activity, ear click deviation increased with each nominal step in eye closure effort. A mixed-effects linear regression model was significant (Wald $X^2 = 4947.15$, $p < 0.000005$) and post-hoc Bonferroni-adjusted ($p < 0.05$) pairwise testing indicated that ear click deviation was significantly different for all eye closure effort levels. In summary, open and lightly closed eyes were associated with the lowest ear click deviation and tight and maximal eye closure effort were associated with highest ear click deviation.

3.3. Multivariable mixed-effects linear regression: predicting ear click deviation

The aim of this analysis was to develop a model that characterized the relationship between OOC EMG activity and ear click deviation while controlling for a range of experimental, participant, and audiologic variables. The first step was to use univariable models to identify potential variables for the multivariable mixed-effects linear regression model. Univariable results alone have limited interpretive value as the models do not control for confounds and effect modifications by other variables. Univariable models were generated for the 46 potential predictor variables: OOC EMG, nominal eye closure effort, three experiment-specific variables, age, self-reported impulse exposure, self-reported tinnitus, 14 pure-tone audiometric thresholds and 24 clinical acoustic reflex variables (Table 1). Univariable models having a Wald X^2 test statistic p-value ≤ 0.20 were included in the multivariable model. In addition to nominal effort and OOC EMG activity, 16 other variables related to experimental design and audiologic results met the criterion for inclusion in the multivariable analysis.

Exploratory analyses revealed a curvilinear relationship between ear click deviation and OOC EMG activity. Therefore, the full multivariable mixed-effects regression model included first and second power of OOC EMG activity along with the variables identified using univariable analysis. Wald X^2 tests were used to verify which variables significantly improved the model fit. Fourteen variables related to audiologic results, participant characteristics or experimental design were dropped from the final model because they failed to significantly improve the model fit. The final, best-fit model (Wald $X^2 = 3329.38$; $p < 0.00005$) included the first and second power of OOC EMG activity, tympanometric volume and tympanometric gradient (Table 2). Although tympanometric volume and gradient significantly improved the model fit, the first and second power of the OOC EMG activity were the strongest predictors of ear click deviation. Fig. 3 is a scatterplot of the ear click deviation plotted as a function of OOC EMG activity at the individual trial level. The heavy black line represents the line of best fit based on the first and second power of OOC EMG activity. The gain in ear click deviation grows nonlinearly with OOC EMG activity.

3.4. MEMC occurrence rates

MEMC occurrence rates were calculated using loose (2/3 raters) and strict (unanimous) agreement criteria (Fig. 4, Panel A, left side). MEMC occurrence was lowest for light eye closure (55 to 75%), higher for tight eye closure (89 to 93%), and highest for the maximum closure effort (96 to 98%). Rate differences between the

Table 1
Univariable regression results for age, self-report, experimental conditions, hearing thresholds and clinical acoustic reflex variables. Linear regression results (dependent variable: ear click deviation) are summarized in middle column. Logistic regression results (dependent variable: MEMC occurrence) are summarized in right columns. Cells with Wald X^2 statistic and/or odds ratios denote predictor variables with a p-value of 0.20 or less and those with a dash denote those with a p-value greater than 0.20. For hearing threshold and clinical acoustic reflex variables, the odds ratios represent the likelihood of observing MEMCs for each unit change in the parentheses for that variable category.

Predictor Variables	Linear Regression (Ear canal deviation) Wald X^2 statistic	Odds Ratio	Logistic Regression (MEMC presence) Lower 95% CI	Upper 95% CI
Age, self-report and experimental variables				
OOC EMG Activity	2833.93 (↑)			
Eye Closure Effort Level	4947.15 (↑)			
Baseline (eye open)	All pairwise			
Light Effort	comparisons	1.00		
Tight Effort	were	6.70	3.83	11.74
Maximum Effort	significant	21.90	9.20	52.11
Order of Experimental Condition	8.85			
1st in overall sequence		1.00		
2nd in overall sequence	(↓)	0.45	0.24	0.84
10th in overall sequence	(↓)	-	-	-
11th in overall sequence	(↓)	0.63	0.34	1.16
MEMC during pneumotactile stimulus	-	-	-	-
Concomitant mandibular EMG activity	85.42 (↑)			
Age (decade)	-	-	-	-
Self-reported firearm/nail gun use	-	-	-	-
Self-reported tinnitus	-	-	-	-
Hearing thresholds (5 dB)				
Air conduction: Contralateral to OAE probe				
0.5 kHz	1.81 (↑)	1.21	0.96	1.52
4 kHz	3.26 (↑)	-	-	-
8 kHz	-	0.88	0.76	1.03
1, 2 kHz	-	-	-	-
Air conduction: Ipsilateral to OAE probe				
0.5 kHz	2.75 (↑)	-	-	-
1, 2, 4, 8 kHz	-	-	-	-
Bone conduction				
2 kHz	-	1.17	1.02	1.33
0.5, 1, 4 kHz	-	-	-	-
Contralateral clinical acoustic reflex variables				
Peak acoustic reflex magnitude (1 mmho)				
0.5, 1, 2, 4 kHz	-	-	-	-
Area under response curve (1 mmho·ms)				
1 kHz	1.96 (↑)	-	-	-
0.5, 2, 4 kHz	-	-	-	-
Acoustic reflex onset latency (100 ms)				
1 kHz	4.22 (↓)	0.84	0.73	0.98
2 kHz	2.28 (↓)	0.87	0.77	0.98
0.5, 4 kHz	-	-	-	-
Acoustic reflex duration (100 ms)				
0.5 kHz	2.46 (↑)	-	-	-
1 kHz	5.11 (↑)			
2 kHz	1.75 (↑)	1.07	0.97	1.19
4 kHz	-	-	-	-
Maximum presentation level (5 dB)				
0.5 kHz	2.72 (↓)	-	-	-
1, 2, 4 kHz	-	-	-	-
Tympanometric volume (1 ml)	3.70 (↑)	-	-	-
Static admittance (1 mmho)	5.43 (↓)	0.84	0.64	1.10
Tympanometric pressure (1 decaPa)	2.91 (↓)	-	-	-
Tympanometric gradient (1 decaPa)	6.64 (↑)	-	-	-

Table 2
Summary of the final multivariable linear regression model in which the predicted variable was ear click deviation. $X^2 = 3329.38$; $p < 0.00005$, N=2413.

	Regression Coefficient	Standard Error	z	p-value	95% CI Lower	Upper
(OOC EMG activity)^2	0.24	0.01	17.15	0.0005	0.21	0.27
OOC EMG activity	-0.35	0.046	-7.54	0.0005	-0.44	-0.26
Tympanometric volume	0.83	0.39	2.13	0.032	0.0069	0.16
Tympanometric gradient	0.001	0.0003	3.40	0.001	0.0005	0.002
Intercept	-2.53	0.068	-37.07	0.0005	-2.66	-2.39

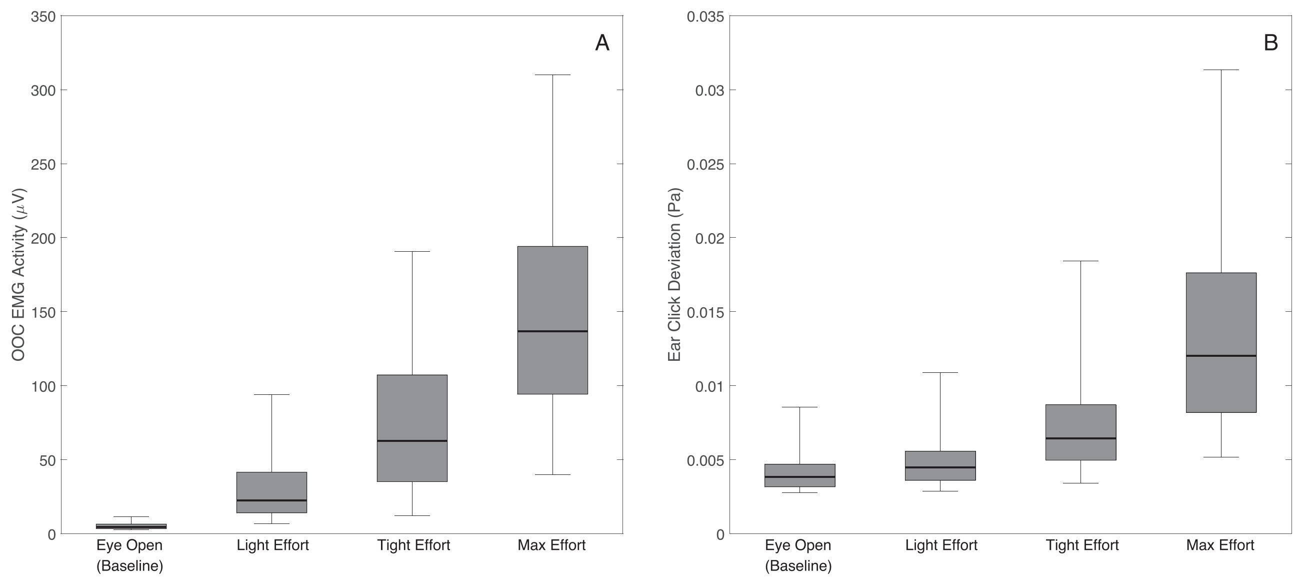

Fig. 2. Panel A: OOC EMG activity for eye open (baseline) and light, tight and maximum eye close effort levels. Panel B: Ear click deviation for eye open (baseline) and light, tight and maximum eye close effort levels. Observations are based on mean of individual trials within each participant at each effort level. For each plot, the horizontal line within each gray box represents the median value, the gray box bounds the interquartile range and the whiskers represent the 5th and 95th percentiles of the data.

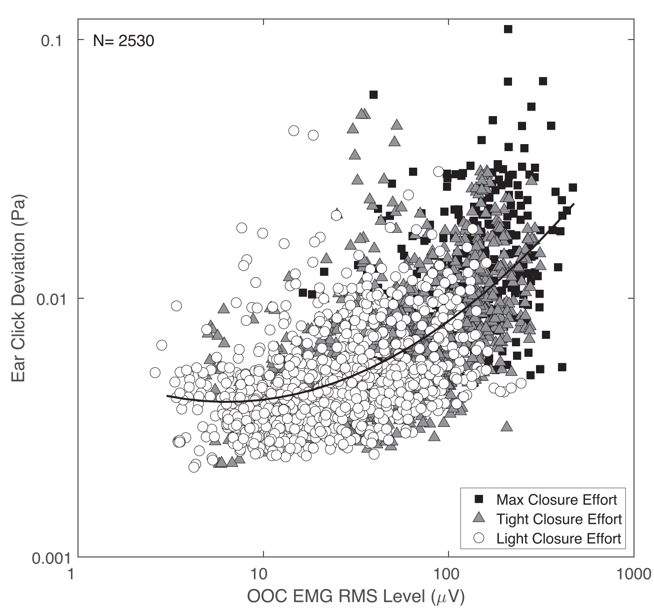

Fig. 3. Ear click deviation plotted as a function of OOC EMG activity for 2560 trials across 190 participants at three levels of eye closure effort. The solid black line represents the line of best fit based on a 2nd order mixed-effects linear regression model.

loose vs. strict criteria were largest for light eye closure, suggesting that light eye closure was difficult to discriminate from baseline EMG activity. MEMC occurrence rates systematically vary as a function of nominal eye closure effort level. However, even for the maximum eye closure effort, the lower bound of the 90% confidence intervals is less than the 95% level required to be considered a pervasive phenomenon. The MEMC occurrence rates associated with eye closure were generally higher than previously reported rates (Deiters et al., 2019) for a range of brief acoustic stimuli (100 ms pure tone, and white noise signals as well as recorded gunshots), presented at 100 dB SEL_A, measured in the same participant pool (Fig. 4, Panel A, right side).

MEMC occurrence was based on judgements of the presence/absence of an observable stimulus-linked change in the 25th percentile trace of ear click deviation. Fig. 4B, is a boxplot summary of the 25th percentile of the mean ear click deviation for the ensemble of trials within a participant condition organized by whether an MEMC was judged present. The 25th percentile of the ear click deviation is much larger for those conditions judged to have an MEMC present compared to those that do not. Mixed-effects linear regression analysis was performed with MEMC presence as a nominal predictor variable of ear click deviation. The overall model was significant (Wald $X^2 = 95.75$, $p < 0.000005$).

3.5. Multivariable logistic regression: Predicting the presence of MEMCs

Univariable logistic regression was performed across a range of variables with the aim of identifying those potentially predictive of MEMCs. Odds ratios and 95% confidence intervals are presented on the right side of Table 1 and variables with p-value $\leq$0.20 are marked with an asterisk. An odds ratio greater than one indicates the variable increases the likelihood of an MEMC and an odds ratio less than one reduces the change of an MEMC. In addition to nominal eye closure effort, 8 other variables related to experimental design and audiologic results had p-values $\leq$0.20.

All variables with a p-value $\leq$0.20 from the univariable analyses were included in the multivariable logistic regression model, with one exception. OOC EMG activity exhibited a continuous frequency distribution spanning a broad range of voltages. Therefore, to allow a greater level of resolution than allowed by the three nominal effort levels, the OOC EMG data was transformed into quintiles based on the distribution of OOC EMG activity (left side of Table 3). Wald tests were used to verify which variables significantly improved the model fit. The final, best-fit model only included OOC EMG activity as quintiles (Wald $X^2 = 35.93$; $p < 0.00005$). None of the variables related to hearing threshold, clinical middle ear testing, participant characteristics or experimental design features significantly improved the model fit. OOC EMG activity was linked strongly with MEMCs (right side of Table 3). The odds ratios associating OOC EMG activity with eye close related MEMCs were multiple orders of magnitude larger than for any other predictor variable, ranging from approximately 20 (comparing 2nd to 1st quintile) to over 7000 (comparing 4th and 5th quintiles to 1st quintile).

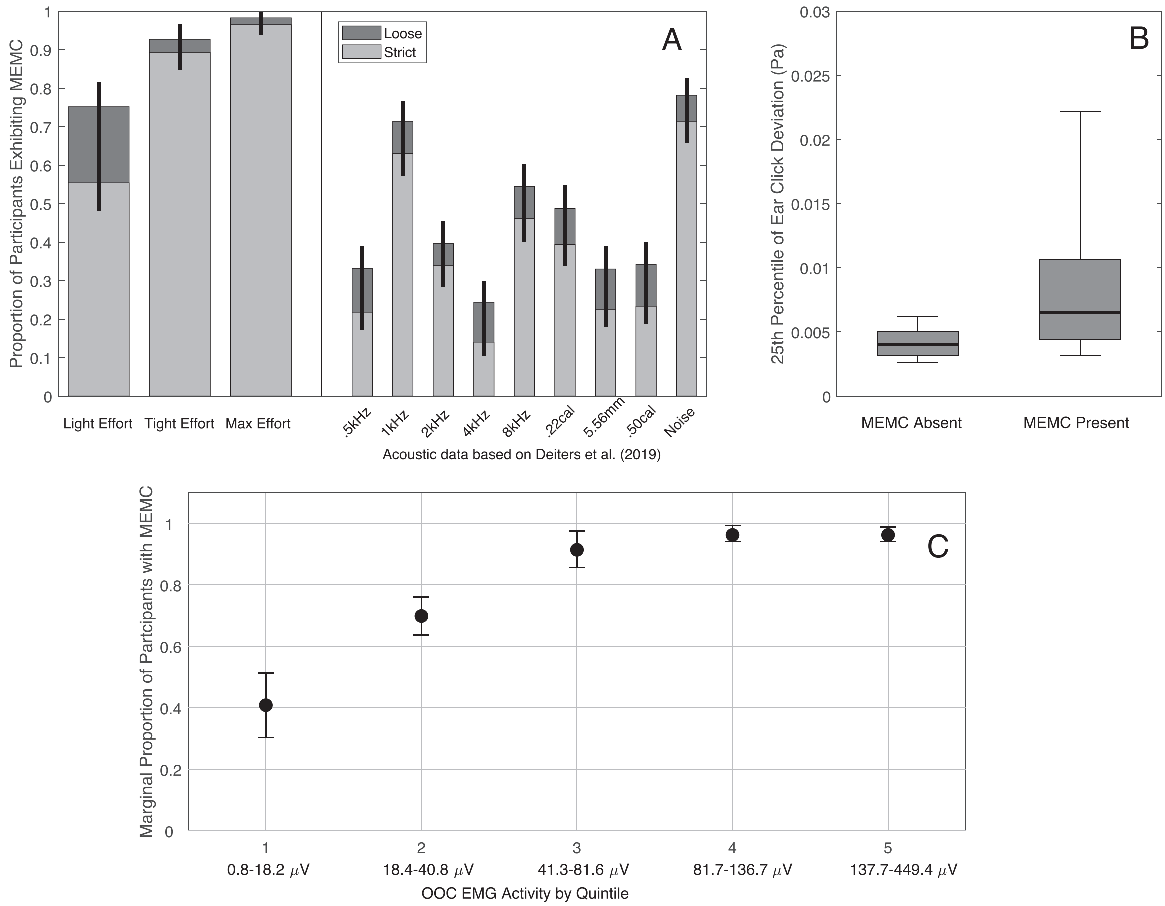

Fig. 4. Panel A: Proportion of participants judged to exhibit MEMCs coincident with eye closure (left side) and in response to brief acoustic stimuli (right side). The acoustic results are based on Deiters et al. (2019) and include the same participant pool as the current study. For each condition, the dark bar represents proportions of participants based on a strict agreement across judges (3/3 judges must agree MEMCs are present) and the light bar represents proportions based on a less strict or "loose" criterion (2/3 judges agree MEMCs are present). The thick, black vertical lines represent the lower and upper limits of the 95% confidence intervals for the strict and loose criteria respectively. Panel B: 25th percentile of ear click deviation grouped by presence or absence of MEMC (strict agreement). The horizontal line within each gray box represents the median value, the gray box bounds the interquartile range and the whiskers represent the 5th and 95th percentiles of the data. Panel C: Marginal proportion of participants judged to exhibit MEMCs (strict criteria) for each quintile based on OOC EMG activity. The solid circles represent the mean marginal proportion for each quintile and the error bars represent the 95% confidence interval.

Table 3
The left side of the table is a summary of the OOC EMG levels after division into quintiles of the overall distribution. The quintiles were included in the final multivariable logistic regression model along with other variables with a *p*-value at or below the near-significant level of 0.20. The only variables that significantly contributed to the final model fit were the OOC EMG quintiles. The right side of the table is a summary of the odds ratios (relative to 1st quintile) and the lower and upper 95% confidence intervals (CI).

Multivariable analysis OOC EMG Quintile	Minimum (µV)	Maximum (µV)	N	Odds Ratio	Odds Ratio 95% CI Lower	Upper
1 (1st–20th percentile)	0.8	18.2	109	1.00		
2 (21st–40th percentile)	18.4	40.8	109	20.08	3.98	101.28
3 (41st–60th percentile)	41.3	81.6	109	942.63	72.00	12341.31
4 (61st–80th percentile)	81.7	136.7	109	8724.86	405.12	187903.50
5 (81st–100th percentile)	137.7	449.4	109	7897.06	337.53	184765.20

Marginal proportions and pairwise comparisons using Bonferroni adjustment ($p < 0.05$) were used in post-hoc analyses. These comparisons revealed that the 1st and 2nd quintiles were significantly different from each other and the 3rd through 5th quintiles, and the 3rd, 4th and 5th quintiles were not significantly different from each other (Fig. 4C). Marginal proportions of participants exhibiting MEMCs ranged from approximately 0.4 (1st quintile) to greater than 0.95 (4th and 5th quintile). The relationship between marginal quintiles of OOC EMG activity was similar to a quadratic function, showing a linear increase in the marginal proportions between the 1st and 3rd quintiles, and approaching asymptote between the 3rd and 5th quintile. Therefore, OOC EMG activity greater than the lower limit of the 3rd quintile was very likely to elicit MEMCs.

4. Discussion

4.1. Voluntary unilateral eye closure predicts variations in click recordings within the ear canal

The aim of this study was to assess the relationship between unilateral voluntary eye closure and middle ear muscle activity in a large group of participants with no auditory dysfunction. Middle ear muscle activity was inferred from changes in ear canal recordings to a broad-band click probe. OOC EMG activity was a strong predictor of ear click deviations (Fig. 3). MEMC occurrence rates increased with nominal eye closure effort level and OOC muscle activity (Fig. 4). Further, multivariable linear and logistic regression analysis revealed that this relationship between eye closure and MEMCs was minimally affected by participant hearing thresholds, clinical middle ear testing results, and experimental design variables (Tables 2 and 3). These results are consistent with and expand upon previous studies that have examined relations between the eye-related motor activity and MEMCs. Salomon and Starr (1963), who directly recorded MEMCs in two adult humans, observed eye closure related TT responses that increased in magnitude with an increase in eye closure force, typically lasted the duration of the eye closure gesture, and exhibited little habituation across repeated closures. Although MEMC magnitude was not measured in the current study, results are consistent with the view that at greater eye closure effort level, MEMCs with larger magnitudes would be easier to identify, increasing occurrence rates. Furthermore, when observing individual trials, the impedance changes used to identify MEMCs often paralleled OOC muscle activity levels (Fig. 1).

4.2. MEMCs are more commonly elicited by eye closure than brief acoustic elicitors

Results from this study indicated that OOC muscle activity associated with eye closure was a reliable elicitor of MEMCs. Recently, Deiters et al. (2019) assessed rate of MEMCs in response to brief acoustic elicitors using the same participant group, data processing, and MEMC identification procedure as the current study. Acoustic elicitors included brief tones, white noise and recorded gunshots. Results of the Deiters et al. study are displayed alongside the current results for voluntary eye closure in Fig. 4A. Overall, rates of MEMCs in response to brief acoustic elicitors were lower than rates for voluntary eye closure. Specifically, rates ranged from as low as 20–30% to as high as 70–80% depending upon the characteristics of the acoustic elicitor. Moderate levels of OOC activity elicited higher rates than any of the acoustic elicitors. Additionally, unlike the current study, Deiters et al. (2019) found that rates of MEMCs for brief acoustic stimuli were significantly influenced by clinical acoustic reflex testing results (i.e. clinical acoustic reflex magnitude and latency). The comparison of the current results with those of Deiters et al. (2019) indicates that voluntary eye closure is a more robust elicitor of MEMCs than brief acoustic elicitors in participants with excellent hearing sensitivity, normal middle ear function, and present clinical acoustic reflexes. What is less clear are the reasons for these rate differences. The acoustic reflex is elicited by sound mediated over the auditory pathways. The originating source of voluntary eye closure-mediated MEMCs is not so well-defined. It is possible that a copy of a cortically derived motor command used to generate eye closure is also sent to the middle ear muscles. Alternatively, the response may be mediated via proprioceptive responses arising from the periorbital movement. This issue would be best addressed by comparing relative timing of OOC muscle activity and middle ear impedance changes. Unfortunately, that requires a level of temporal precision not possible using the current study's methodology for detecting MEMCs.

The pioneering study of Salomon and Starr (1963) and more recent work by Gruters et al. (2018), who examined eye saccade and eardrum pressure changes, suggest that eye and ear motor activity may arise from a common motor command. It is also possible that the effector muscles are different for the acoustic and non-acoustic responses. ST is generally agreed to be the primary effector mediating the acoustic reflex (Borg et al., 1984). Salomon and Starr (1963) reported that during eye closure, TT was active while ST was typically silent. The method used in this study, or any non-invasive study of middle ear muscle function, cannot discern relative contribution of TT and ST. The key point is the acoustic reflex represents a single example of a wide range of complex sensorimotor responses that involve the middle ear muscles. This has wide-ranging implications for studies that employ acoustic reflex measures and assume a protective MEMC function during noise exposure.

4.3. Implications for studies of MEMCs

The powerful influence of voluntary motor activity, such as eye closure, on middle ear muscle activity serves as a potential experimental confound for MEMC studies. In such experiments, little, if any attention has focused on explicitly monitoring concomitant motor activity other than to instruct participants to sit quietly and keep movement to a minimum. Depending on the timing of concomitant motor activity such as eye closure, there is a potential to obscure the presence of acoustically elicited MEMCs or misinterpret as an acoustic reflex when one did not occur. This is a timely issue given a resurgence of interest in middle ear muscle function. Recent studies suggest that narrow-band acoustic elicitors of MEMCs may be a highly sensitive indicator for synaptopathy in mice (Valero et al., 2016; Valero et al., 2018). Assessing the viability of measures of MEMCs as a marker of synaptopathy in humans is currently underway though, to date, a clear relationship between MEMCs and synaptopathy in humans has not been observed (Mepani et al., 2019; Wojtczak et al., 2017). Moving forward, sensitive and specific procedures for testing MEMCs will require careful control for influential variables. Motor activity, such as eye-related motor activity, must be monitored in human and animal studies.

4.4. Implications for damage risk criteria for impulsive noise

This study also has implications for the evaluation of DRC for impulsive noise, because some DRC assume a protective role for MEMCs. The most notable example is the Auditory Hazard Assessment Algorithm for Humans (AHAAH), which is an electro-acoustic model of sound transmission through the ear (Price and Kalb, 2018). Though AHAAH distinguishes between warned and unwarned exposures, both types assume a role for MEMCs. For an unwarned exposure, AHAAH assumes the initial impulse will elicit a reflexive MEMC which, due to the response latency, will be too slow to provide protection for the initial impulse, but will provide sustained, maximal protection for subsequent impulses. For a warned exposure, AHAAH assumes MEMCs will reach maximum effect before the initial impulse and will be maximally contracted throughout an exposure series. Finally, regardless of whether the exposure is warned or unwarned, the model assumes MEMCs are present and identical for all exposed persons and do not vary across individuals or situational context (Price and Kalb, 2018). The results of the current study call into question this last assumption. As a DRC, AHAAH is designed to be applied across a wide range of operational environments. In such settings, the warfighter will be responding to a continuously varying set of environmental demands that in one moment may imply tight eye closure in anticipation of a blast, while in the next moment demand a visual scan of the field, followed by aiming and firing a weapon with both eyes open. The current results indicate that eye closure effort significantly impacts the likelihood of MEMCs. Therefore, it is unlikely that MEMCs are present and identical across all warfighter operational contexts.

Finally, the current results also have implications for studies designed to compare different DRC for impulsive noise. Such studies are designed to incrementally increase, or "walk-up" the number of impulsive noise exposures to participants while carefully monitoring hearing status (Johnson, 1997). The tight control of exposure level is critical to properly assess candidate DRC. Such studies must monitor and control for non-acoustic factors, such as eye-related motor activity, that can influence MEMCs and resultant exposure at the cochlea.

4.5. Study limitations

This study inferred MEMCs based on RMS changes in the amplitude of a 50-ms broad-band click (0.2–8 kHz) probe stimulus relative to levels drawn from a baseline period. Because RMS calculations do not account for the direction of impedance change, there may be cases where impedance decreased during the period of elicitor presentation, thus providing increased sound transmission into the cochlea. Additionally, one cannot rule out the potential contribution of the medial olivocochlear system to ear canal changes reported in this study. Finally, although the ear canal recording was high-pass filtered at 0.1 kHz to remove noise unrelated to the probe stimulus, one cannot rule out the possibility that eye closure activity may have introduced exogenous noise within the bandwidth of the probe signal, which could contribute to ear click deviation (Bell and Jedrzejczak, 2021). Frequency-based analyses of ear click deviations could help resolve this issue.

MEMC occurrence rate was based on the binary judgement of 25th percentile traces of effort level-specific responses from three separate raters. While this procedure has the advantage of face validity, the procedure relies on a subjective rating. Therefore, rater error and/or bias is a possibility. There are two reasons to suggest rater error and/or bias minimally influenced the results. First, a high degree of absolute agreement in these data (90–96%) was observed. Second, very similar results were reported for analysis that used ear click deviation rather than subjective judgements of MEMC (Fig. 3, Table 2).

The current results are based on a large group of participants with excellent hearing sensitivity and normal middle ear function. Additionally, all participants exhibited acoustic reflexes in response to conventional acoustic reflex stimuli. It is unclear how these results generalize to individuals with absent clinical acoustic reflexes, poorer hearing sensitivity, and/or middle ear anomalies. It is also unclear how these results generalize to involuntary eye closure, shorter eye closure durations, contralateral unilateral eye closure, effects of closing both eyes simultaneously, or when individuals are not focused on the eye closure efforts.

5. Conclusions

In this laboratory study, middle ear activity, inferred from ear canal pressure changes in response to a broad-band click probe train, was measured in response to three levels of voluntary eye closure in a large group of participants with excellent hearing sensitivity and normal middle ear function. Orbicularis oculi (OOC) EMG recordings were used to monitor and quantify eye closure effort level. Evidence of MEMCs were commonly observed during EMG-verified eye closure gestures. The presence and magnitude of MEMCs were greater for higher levels of eye closure effort. Multivariable analysis revealed that OOC EMG activity was a strong predictor of the presence and magnitude of MEMCs. Occurrence rates of MEMCs at moderate levels of eye closure muscle activity was generally greater than previously published rates for brief acoustic stimuli. MEMCs were nearly certain to occur at high levels of eye muscle activity. These results suggest that eye-related motor activity is a powerful elicitor of MEMCs that can serve as a confounding factor for acoustic reflex studies. These results also complicate the interpretation of impulsive noise DRC that assume MEMCs serve as a consistent, uniform protective factor.

Author Statement

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript is in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations.

Informed consent was obtained from participants in this study and privacy rights were observed.

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