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Pervasiveness of Early Middle Ear Muscle Contractions

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14. ABSTRACT
Middle ear muscle contractions (MEMCs) are expected to reduce the transmission of energy through the middle ear. Some damage-risk criteria (DRCs) for impulsive noise include MEMCs as a form of protection. Early MEMCs (eMEMCs) have been assumed in some DRCs to reach maximum effect prior to the arrival of the hazardous impulse. Despite decades of conjecture leading to the inclusion of eMEMCs in DRCs, there is no conclusive evidence of protective eMEMCs in the scientific literature. The inclusion in MIL-STD-1474E of one model utilizing eMEMCs elevates greatly the need for certainty that eMEMCs are pervasive (i.e., present in 95% of the population with 95% confidence) in the military population. The current study was designed to determine whether eMEMCs are pervasive in the military population, either as conditioned responses or the result of prior experiences. Participants were adults with excellent or very good hearing sensitivity, and no evidence of dysfunction affecting the ear or relevant cranial nerves. Changes in the levels of a click-based probe signal developed in the ear canal were used as the indicator of MEMCs. None of the experimental tasks tested here produced proportions of eMEMCs approaching the requirement for pervasiveness. There was a very low likelihood of observing an eMEMC for any of the tasks relying on prior experience.

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14. Abstract (continued)
Fewer than 11% of participants showed a tendency toward eMEMCs when discharging live ammunition. This project found no evidence of pervasive eMEMCs and provides unambiguous evidence that eMEMCs are not a dependable form of protection against impulsive noise. Any DRC including eMEMCs will require modification and subsequent validation.

Summary

Background

Middle ear muscle contractions (MEMCs) are expected to reduce the transmission of energy through the middle ear. Some damage-risk criteria (DRCs) for impulsive noise include MEMCs as a form of protection. Early MEMCs (eMEMCs) have been assumed in some DRCs to reach maximum effect prior to the arrival of the hazardous impulse. Despite decades of conjecture leading to the inclusion of eMEMCs in DRCs, no conclusive evidence exists of protective eMEMCs in the scientific literature. The inclusion in MIL-STD-1474E of one model utilizing eMEMCs greatly elevates the need for conclusive evidence that eMEMCs are pervasive (i.e., present in 95% of the population with 95% confidence) in the military population.

Purpose

The current study was designed to determine if eMEMCs are pervasive in the military population, either as conditioned responses (CRs) or the result of prior experiences.

Methods

Participants were adults with excellent or very good hearing sensitivity, and no evidence of dysfunction affecting the ear or relevant cranial nerves. Changes in the levels of a click-based probe signal developed in the ear canal were used as the indicator of MEMCs. Participants were assigned to one of nine experimental tasks. Three tasks were designed to develop conditioned eMEMCs, with conditioning stimuli varying by sensory modality (visual or auditory) and the level of attention available to the conditioning stimulus. Six tasks, ranging from laboratory simulations through discharge of an M4 carbine on an outdoor target range, provided an opportunity to demonstrate eMEMCs among U.S. Army Service Members and civilians with recent firearm experience. Pervasiveness was evaluated in terms of a tendency for a participant to exhibit an eMEMC and in terms of the proportions of individual impulses eliciting eMEMCs.

Results

None of the nine experimental tasks produced proportions of eMEMC approaching the requirement for pervasiveness. The greatest likelihood of exhibiting eMEMCs was observed as a CR to an attended stimulus with a matching sensory modality (95% confidence of as low as 50% prevalence). However, the likelihood of a conditioned eMEMC declined precipitously when the sensory modality of the conditioning stimulus did not match the unconditioned stimulus and when attention to the conditioning stimulus was unavailable. Results showed a very low likelihood of observing an eMEMC for any of the tasks relying on prior experience. Fewer than 11% of participants showed a tendency toward eMEMCs when discharging live ammunition**.**

Conclusion

This project found no evidence of pervasive eMEMCs and provides unambiguous evidence that eMEMCs are not a dependable form of protection against impulsive noise. Any DRC including eMEMCs will require modification and subsequent validation.

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Background

Maintaining readiness and preserving force strength are priorities for the U.S. Army Medical Command. The noise produced by the discharge of military weapon systems can damage the hearing of the Service Member (Bunch, 1942; Guild, 1918; Murray & Reid, 1946; Ward, 1968). Hearing loss impacts the military and its personnel in multiple ways. Degraded auditory information compromises mission effectiveness (Casto & Casali, 2013; Garinther & Peters, 1990; Keller et al., 2017; Mentel et al., 2013; Sheffield et al., 2017). Hearing loss can necessitate military occupational specialty (MOS) changes or early separation from service (U.S. Army, 2019), and can compromise communication effectiveness and quality of life following separation from service (Mulrow et al., 1990).

Current medical standards for induction and retention require that Service Members have minimum auditory detection thresholds commensurate with their duties. Although not a medical standard, current and proposed future materiel are evaluated for noise production using MIL-STD-1474E (U.S. Department of Defense, 2015). Training procedures and doctrine are developed to optimize warfighter capacity, monitor health, identify policy deficiencies, and prevent unnecessary morbidities. Hearing loss associated with military service can be reduced or prevented by reducing noise exposures to levels that are unlikely to produce damage to the auditory system. In the U.S., recommendations for the protection from hearing loss due to gunfire date back to the 1940s (Ades et al., 1953; McIlwain et al., 2008).

In the U.S. Army, the prevalence of hearing impairment varies with the Soldier's MOS (Ahroon et al., 2011). For example, Ahroon et al. (2011) reported that many of the MOS categories with excess prevalence of hearing impairment involve the use of weapons or exposure to explosive/impulsive sounds, which suggests that the processes used to procure and use military weapon systems might rely on a deficient understanding of the risks to the users of the weapon systems. A damage-risk criterion (DRC) is an algorithm used to link a physical phenomenon like noise to the risk of damage to a body system like hearing. DRCs are used to evaluate the risk of harm posed by an exposure, and to inform procurement and policy accordingly. DRCs are used before acquisition to inform decision makers about the likely consequences of procurement. After acquisition, DRCs are used to develop training and usage doctrine and support health hazard evaluations and assessments.

The earliest regulation regarding noise exposure in the U.S. Department of Defense (DoD) was implemented by the U.S. Air Force (U.S. Air Force, 1956), but knowledge gaps regarding the hazards of impulsive noises delayed the development of DRCs for these brief, intense signals. At the time, DRCs were developed for chemical, radiation, and noise exposures, and the selection of variables representing the acoustic exposures was based on: whether the variable could be measured accurately; whether the variable correlated to an aspect of hearing deemed important; and parsimony regarding descriptors of the exposure, the outcome, and the relationship between the two (Rosenblith, 1958). The first DoD standard implementing a DRC for impulsive noise was promulgated in the early 1970s, based on research work conducted between the 1950s and 1970s (CHABA [Committee on Hearing - Bio-Acoustics - and Biomechanics], 1965; G. R. Garinther et al., 1975; Loeb & Fletcher, 1968). High-level impulse noise exposures that varied the level, exposure repetitions, hearing protection and A-duration of

the initial blast overpressure were investigated with Service Members during the Albuquerque Blast Overpressure Walk-up studies (Johnson, 1993).

Some DRCs for impulsive noises invoke early middle ear muscle contractions (eMEMCs) in anticipation of a high-level impulse noise or exposure as a form of endogenous hearing protection (Price & Kalb, 2018; Zagadou et al., 2016). This is because an eMEMC is expected to operate via a reduction in energy transfer through the middle ear when the muscles are contracted. In these DRCs, the eMEMC is expected to initiate and reach maximum force prior to the arrival of the acoustic impulse at the ear. Reflexive middle ear muscle contractions (rMEMC), reacting in response to a high-level impulse noise or exposure, have long been suspected of reducing the transmission of low-frequency energy into the cochlea. Early suggestions that contraction of the stapedius reduces intracochlear pressure have been attributed to Urbantschich, in the late 19th century (Dejerine, 1914). Even at that time, contraction of the middle ear muscles was expected to reduce hearing sensitivity (Dejerine, 1914; Levi, 1885). This insight was advanced for its time, given that hearing loss was only just beginning to be seen as different from a mental disorder (Mott, 1916; Wilson, 1917). Suggestions regarding the role and importance of MEMCs come with the frequent caveat that the process leading to these contractions is understood poorly (Rosenblith & Stevens, 1953). The limited information available regarding the conditions under which an MEMC will be elicited has facilitated conjectures that protective MEMC will always be present both as rMEMCs and as eMEMCs (Price, 2007).

Over 100 years have elapsed since the idea of a protective MEMC was considered initially, but the neural mechanisms by which MEMCs are elicited remain unclear (Mukerji et al., 2010). It has, however, become clearer that rMEMCs are not simple reflexes. They are described more accurately as complex responses, the presence of which is influenced by a variety of factors (Hodges & Ruth, 1987), are not restricted to the auditory domain (Djupesland, 1964; Tasko, Deiters, et al., 2020), are affected by auditory and demographic factors (Flamme et al., 2017), are not pervasive in any definable demographic group (Flamme et al., 2017; McGregor et al., 2018), and do not generalize from longer to shorter stimulus durations (Deiters et al., 2019; Rossi & Solero, 1983, 1984). The presence of rMEMCs cannot be expected for each presentation of a short-duration elicitor, even for an individual who demonstrates clinical acoustic reflexes and who demonstrates a trend toward exhibiting rMEMCs across multiple trials (Tasko, Flamme, et al., 2020). In short: The complexity of the response might make it unreasonable to depend on any stimulus eliciting rMEMCs across a broad range of people or settings.

MIL-STD 1474E (U.S. Department of Defense, 2015) has two metrics for analyzing impulse noise: $L_{IAeq100ms}$ and the Auditory Hazard Assessment Algorithm for Humans (AHAAH). The U.S. Air Force and U.S. Navy use the LIAeq100ms metric to evaluate noise exposures produced by materiel. The U.S. Army uses the AHAAH model which includes two classes of protective MEMCs, the unwarned and warned options, which differ only in the relative time of onset of the MEMC (Price, 2007). In the unwarned case, an rMEMC is used. The unwarned rMEMC is expected to follow the onset of the impulse by 9 milliseconds (ms) and then rise to a maximum within about 50 ms, with a morphology similar to a capacitive system that follows a time constant of 11.7 ms (Price, 2007). In the warned case, an eMEMC is assumed

to be elicited by the listener's conscious expectation of an impending impulse, or through an unconscious association between preparatory events and the impulse. In the warned eMEMC case, the contraction has reached maximum effect before the impulse arrives, by simply replicating the rMEMC time constants and rise time with onset occurring 50 ms prior to the impulse. In AHAAH, the effect of the eMEMC, relative to the rMEMC, is on the order of 10 decibels (dB) of additional protection at the level of the cochlea (Murphy et al., 2011). For small arms impulses, the numbers of permissible exposures increase by slightly less than an order of magnitude when warned exposures are assumed (Figure 1) (Flamme et al., 2019).

Figure 1. Allowable number of rounds (ANOR) returned by AHAAH as a function of impulsive peak level. Triangles represent ANOR under the unwarned assumption (i.e., no eMEMC). Circles represent ANOR under the warned assumption (i.e., eMEMC).

One proponent of a protective eMEMC speculated that human ears are capable of producing an eMEMC in response to a number of circumstances (Price, 2007). With a multitude of unconditioned stimuli (UCS) occurring together, including acoustic impulses and a number of unconditioned facial stimuli, users of the AHAAH model are expected to assume that an eMEMC must occur during weapon discharge (Fedele et al., 2013). Citing early studies demonstrating the middle ear may be conditionable (Brasher et al., 1969; Marshall et al., 1975; Yonovitz & Harris, 1976), this conjecture led to the adoption of the warned metric for use in the AHAAH model and use of a maximum, protective eMEMC when calculating auditory risk using that model. For example, MIL-STD 1474E specifies use of the warned AHAAH metric when calculating auditory risk for Service Members firing their own weapon. The eMEMC is believed

to be elicited through the repeated exposure to a stimulus (stimuli), in this case, the Service Member firing their own weapon. The conditioned stimulus (CS) is embedded in act of firing the weapon (e.g., the CS may be the act of thinking about firing the weapon or the Service Member placing the finger on the trigger), the conditioned response (CR) is the eMEMC activation *prior* to the acoustic impulse, the unconditioned stimulus (UCS) is the impulsive signal created when the weapon is fired, and the unconditioned response (UCR) is the rMEMC that occurs following the impulsive signal.

Flawed reasoning exists in the warned AHAAH option, which assumes maximal eMEMC prior to the arrival of the acoustic impulse. While acknowledging the lack of definitive, scientific data in support of the conditionability of the MEMC, proponents of a protective eMEMC failed to acknowledge that eMEMC conditioning studies have not shown pervasive levels of conditioning, and often resulted in no evidence of conditioning (see Table 1) (Bates et al., 1970; Brainerd & Beasley, 1971; Brasher et al., 1969; Djupesland, 1964, 1965; Marshall et al., 1975; Yonovitz & Harris, 1976). Further, the circumstances under which these studies took place did not emulate military training or activities.

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Table 1. Studies Assessing Conditionability of the eMEMC

Reference	% conditioned	Conditioning stimulus	Unconditioned stimulus	Notes
Djupesland (1965)*	80%	Toy pistol pointed at non-operated ear, informed it would make a loud, unpleasant sound	None	"… general muscular contraction, including contraction of the tympanic muscles" correlated with the "sight of a toy pistol pointed at the non-operated ear"
Djupesland (1964)*	?	"You are going to hear a loud noise" + threatened with pistol"	None	General observations on diagnosis and localization of ossicular chain defect.
Brasher et al. (1969)*	0%	?	Pistol shot with blanks	"No significant correlations were found between any of the middle-ear muscle responses (reflexive or anticipatory contraction) and TTS…"
Marshall et al. (1975)*	86%	Countdown and visualization of noisy toy	Sound of noisy toy	Anticipatory middle-ear reflex activity from noisy toys
Yonovitz (1976)*	80%	1kHz pure tone, 750 msec, 30 dB HL	Electrocutaneous external ear canal stimulation	"Temporal conditioning was occasionally present," (p. 13).
Bates et al. (1970)	50%	Delay conditioning paradigm	95- or 110-dB HL	"…6 of the 12 Ss showed little or no evidence of conditioning"
Brainerd and Beasley (1971)	0%	1. Time interval of 45 sec. 2. light with fixed luminescence	1kHz tone, 10 dB above reflex threshold, 1 sec dur 1. fixed 45 second ISI 2. ISI varied (30, 45, 60 s)	"…no CRs were elicited from any S in response to the CS during the test trials"
Ward et al. (1961)	0%	Fixed and variable temporal conditioning, 20 dB of TTS at 1 frequency	Click stimulus	Comparison of TTS pre- and post-temporal conditioning, no significant difference between fixed and variable intervals.

* Cited by Price (2007) as evidence of MEMC conditionability.

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Some proponents of including eMEMC in DRCs also speculated that an eMEMC must be elicited due to the abundance of UCS (e.g., acoustic impulses, facial stimuli, air puffs, contact with the rifle stock, etc.) present during intense gunfire. The Albuquerque blast overpressure walk-up study (BOP study) did not evaluate the MEMC for conditioned or unconditioned responses. Following the completion of the exposure conditions, volunteer subjects who exhibited no threshold shifts were recruited to participate in the "no-countdown" exposure. All of the other exposure conditions were preceded by a countdown that warned the subjects of the pending blast and presumably activated the MEMC response through classical conditioning. However, the BOP study did not evaluate the acoustic reflex or monitor the MEMC just prior to the blast. Thus, no definitive statement regarding the status of the MEMC can be claimed. Classical conditioning, in the simplest form, requires repeated presentation of one CS during the time interval surrounding the UCS, eventually resulting in a conditioned response (CR, i.e., the eMEMC) through associative learning (Buser, 2006).

In anticipatory conditioning, the CS is often presented prior to the UCS. The warned AHAAH option has been specified in MIL-STD-1474E for use in cases where the gunner knows the weapon will discharge, with advanced countdown to firing, or in closely spaced firing events (e.g. firing a machine gun) (U.S. Department of Defense, 2015). The AHAAH model presumes the conditioned eMEMC (i.e., CR) begins at precisely the same time and without variation relative to a number of different CSs pairing to a single UCS (i.e., weapon discharge). However, conditioning is a complex phenomenon, with more than 12 categories (i.e., extinction, inhibition, temporal properties, stimulus competition) and at least 85 models in use to explain different aspects of those categories (Luzardo et al., 2017). Early classical conditioning theories aimed to explain associative learning, but failed to capture the effects of timing differences (Pearce & Bouton, 2001). Timing models have been designed to explain various timing complexities, but fail to interweave associative learning (Machado, 1997). These complexities cannot be explained easily using a single model.

More recently, attempts have been made to produce omnibus models encompassing a variety of models developed to explain special cases (Luzardo et al., 2017). Many models fail to explain the outcome when more than one CS is paired with a UCS or when the timing between the CS-UCS differ significantly. In such cases, the potency of each CS is reduced or can lead to changes or complete elimination of the CR (Holland & Schiffino, 2016; Luzardo et al., 2017). These complexities are problematic for DRCs, because the circumstances under which military weapons are used allow multiple CSs, inconsistent CS-UCS timing, and fail to account for individual differences in the magnitude, timing, or persistence of a CR. Further, these studies suggest that the abundance of stimuli present in field conditions could interfere with the development of eMEMCs rather than promote them.

Stimuli that can elicit an rMEMC are present in every weapon discharge (e.g., the acoustic impulse); however, MEMC activity occurring following weapon discharge cannot be relied upon to provide a source of expectation of MEMC activation occurring prior to weapon discharge. The magnitude and morphology of CRs are not necessarily identical to UCRs (Kaulich et al., 2010). The magnitude and morphology of eMEMCs have not been the subject of rigorous prospective study, so it is not known whether it is reasonable to expect that eMEMCs should be modeled as rMEMCs that mature prior to impulse arrival. Also, the CR is more labile

than the UCR. Introduction of differing CSs increases inter- and intra-subject variability (Marshall et al., 1975; Simmons, 1964; Simmons et al., 1959), and this variability is difficult to implement within a single mathematical model.

The operational details regarding the conditions under which the warned assumption should be applied are poorly specified. For example, are the benefits of the eMEMC expected to extend to adjacent shooters on a firing range during Initial Entry Training (IET)? Further, the expected timing and contraction rate of the eMEMC has never been specified, with the exception that the response is assumed to approximate the maximum rMEMC before the impulse arrives. At present, the decision regarding whether to apply warned (eMEMC) or unwarned analysis is subject to the analyst's notion of whether "…the person being exposed is aware that the impulse is coming…" (Price & Kalb, 2018). It is not clear when delays in an expected impulse become so great (e.g., if circumstances demand re-targeting) that an early contraction would be expected to return to rest, therefore reverting to an unwarned response. Nor is there clear evidence to ensure that model users will apply the *warned* or *unwarned* metric consistently in a given circumstance. These are substantial deficiencies in an acquisition standard such as MIL-STD-1474E or for an investigation of the health hazards for personnel in the vicinity of the impulsive source.

It is tempting to assume that a listener with robust rMEMCs will also have robust eMEMCs. The justification for assuming that exposed personnel would exhibit an eMEMC was not based on empirical evidence that eMEMC can be seen in a large proportion of exposed people (Price & Kalb, 2018), but was founded on the assumption that an auditory system that supports an rMEMC should also produce an eMEMC. However, the eMEMC would be expected to rely on a wider array of neural systems than a simple reflex. Furthermore, empirical analyses of rMEMCs indicate that listeners with a tendency to exhibit rMEMCs based on combined responses across multiple trials do not exhibit consistent rMEMCs for the individual trials (Tasko, Flamme, et al., 2020).

Early animal and human studies of conditioned eMEMCs did not provide evidence of a pervasive response (Bates et al., 1970; Brasher et al., 1969; Djupesland, 1965; Marshall et al., 1975; Simmons et al., 1959; Yonovitz, 1976), and often resulted in a complete failure to detect conditioned eMEMC activity (Brainerd & Beasley, 1971; Ison et al., 1979; Simmons, 1964; Ward et al., 1961). These studies presumably permitted attention to the signal or task, and a greater likelihood of conditioning is found in studies where participants are allowed to attend to, or are informed of, the CS-UCS relationship (Buser, 2006; Clark & Squire, 1998). However, it must be noted that contingency awareness implies conscious knowledge, attention, or declarative memory to any conditioning stimuli and cannot be expected to generalize to modern training of warfighters where focus is maintained on reaching an objective target. No DRC that includes an MEMC has specified a protocol for entraining the eMEMC response, which implies an assumption that the eMEMC is developed through a passive process.

Additional factors may be related to eMEMC responses. Non-auditory activity, such as a startle, flinch, or wince, is sometimes observed in parallel to an MEMC response (Deiters et al., 2020; Simmons et al., 1959). However, the startle response is variable among individuals, with emotional and attentional factors increasing variability considerably (Grillon & Baas, 2003). A history of post-traumatic stress disorder (PTSD) has led to startle response increases or

reductions (Grillon & Baas, 2003). There may be a link between startle responses and middle ear activity (Deiters et al., 2020), but not for all individuals or under all listener states (Greisen & Neergaard, 1975). Additionally, marksmanship training involves a number of protocols that actively train and promote an inhibitory response to the non-auditory actions that may occur reflexively during gunfire (e.g., flinch, eye closure, movement, abrupt trigger pull), which ultimately leads to increased marksmanship precision (U.S. Department of the Army, 2011, 2012). The startle cannot be relied upon in a military setting because a startle or flinch leads to decreased accuracy and is minimized during marksmanship training (MacCaslin & Levy, 1956; Moore, 1962; U.S. Department of the Army, 2011, 2012).

The act of discharging a weapon requires attention to multiple concerns, such as motor control to maintain the target, breathing patterns, and unintended obstacles and non-targets in the foreground and background, none of which include active attention to the loudness of the weapon. There are substantial time constraints imposed during training and combat. In urban combat, for example, a potential target is expected to be visible for only 2-4 seconds (Scribner, 2002). During this time, the warfighter is expected to sight the potential target, confirm the potential target's status as ally or adversary, decide whether to engage the target, and complete the engagement.

The rate of fire for commonly used small arms weapon systems ranges from a maximum of 900 rounds per minute in the automatic setting to a maximum of 90 rounds per minute in the 3-round burst setting down to a sustained rate of 12 rounds per minute (67 ms to 5000 ms interstimulus interval for repeated shots) (Jenkins & Lowrey, 2004). The term "closely spaced events" (MIL-STD-1474E, U.S. Department of Defense (2015), p. 38) has no accepted definition, and whether the firing rates above could be considered closely-spaced appears to be an arbitrary decision left to the analyst. If an rMEMC is elicited, the contraction could persist across successive impulses in a maximum rate of fire, but in many cases, especially involving sustained rates of fire, offset of the rMEMC (i.e., muscle relaxation) would occur prior to the subsequent impulse (Jones et al., 2018). There is little evidence to inform expectations about the fate of rMEMCs across extended repeated elicitation intervals, but there is evidence that the likelihood of observing an rMEMC declines with increasing elicitor presentations (Deiters et al., 2019).

Despite limited supporting empirical evidence, noise exposure metrics invoking eMEMCs as protective factors have been included in MIL-STD-1474E (U.S. Department of Defense, 2015), a standard used by the U.S. automotive industry (SAE, 2011), and in other impulsive noise injury models (Zagadou et al., 2016). The military standard guides the acquisition of military materiel, including weapon systems, and as it is currently written, the AHAAH model must be used for procurement of materiel for the U.S. Army if that materiel produces peak sound pressure levels exceeding 140 dB sound pressure level (SPL) (MIL-STD-1474E, section B.5.1, p. 41). The SAE standard has been used for evaluating the risk of harm to the auditory system from the noise produced during airbag deployment (SAE, 2011). The integrated cochlear energy (ICE) model, proposed as an improvement to AHAAH, includes a warned eMEMC option; however, the developers also acknowledge the lack of scientific evidence for a pervasive MEMC response (Zagadou et al., 2016, 2017). Hence, they have included the ability to either delay or remove the MEMC from the model altogether.

No line of research is likely to return complete certainty about the pervasiveness of the conditioned eMEMC response, but the categories of certainty and uncertainty defined by Rosenblith et al. (1954) may be useful for the present study. In the 1954 report on the relations between hearing loss and noise exposure, the authors utilized certainty categories as "this we know," "this is reasonably certain," "of this we have considerable doubt," and "on this we still cannot make an intelligent guess." At the outset of the present study, the conjecture of a pervasive protective eMEMC falls squarely in the category of "on this we still cannot make an intelligent guess," and this status makes it unfortunate that a DRC relying on this phenomenon is embedded within MIL-STD-1474E.

The purpose of this report is to describe a series of studies designed to evaluate whether eMEMC are pervasive in test conditions ranging from (a) the development of conditioned eMEMCs in controlled laboratory environments wherein participants attended to the CS, to (b) the detection of eMEMC for active-duty military Service Members discharging M4 carbines on an outdoor firing range. To increase the likelihood of developing a CR, tasks varying across the dimensions of sensory modality and the attention provided to the CSs were included. An additional set of six tasks were administered to regular firearm users or active-duty military Service Members to increase the probability of detecting pervasive eMEMCs on at least one task. These tasks varied in their fidelity to weapon use in the military, ranging from conducting a tracking/triggering task with a toy rifle mounted in a yoke in a university laboratory setting, to M4 carbine targeting and discharge of live ammunition on an outdoor military firing range. The results of this project will provide a greater understanding of the relations between impulsive noise exposure and middle ear muscle activity, which will increase the certainty about whether eMEMCs should be considered a potential protective factor in DRCs.

Methods

The methods were divided into two studies, each examining a different class of eMEMCs. In both studies, the primary outcome of interest was the change in the level of a band-limited click developed in the ear canal due to changes in impedance presumably resulting from an MEMC. In Study 1, a classical conditioning process was completed during three tasks to examine the conditionability of eMEMCs across sensory modalities and level of attention to the CS. The conditioning process involved repeated presentation of a CS just prior to presentation of the UCS in order to examine the likelihood that the CS prompted the development of an eMEMC. The task termed attended visual (AV) involved participants attending to a visual CS paired to an auditory UCS. The attended auditory (AA) task involved participants attending to an auditory CS paired to an auditory UCS, and the unattended auditory (UA) conditioning task involved participants performing a distractor activity during an auditory CS-UCS pairing. Study 1 was conducted in a university lab setting (Kalamazoo, MI) with civilian participants who were not regular firearm users. Laboratory classical conditioning tasks were completed to provide a framework for the understanding of the conditionability of eMEMCs in previously unconditioned participants with very good hearing and normal middle ear function.

Study 2 included participants who were regular firearm users, with typical firearm experience in military, occupational, and/or recreational settings. Study 2 did not include an

eMEMC conditioning paradigm, but assumed the firearm user was previously conditioned and an unspecified CS, embedded in the act of firing a weapon, elicits an eMEMC. The generalizability of Study 2 varies across six tasks, from laboratory use of a toy cap gun to use of an M4 with ammunition by active duty Soldiers on a firing range, in order to assess the presence of eMEMCs and whether the progression to a more generalizable setting impacts the likelihood of eliciting a previously conditioned eMEMC. Tasks contributing to Study 2 were completed at the university lab and at a military lab setting (Ft. Rucker, AL).

The tasks labeled simulated trigger (ST) and dry fire (DF) were conducted in the university lab setting, and involved participants firing a toy gun or a modified gun at a target on a monitor. Subsequently, the tasks termed simulated shooter/simulated spotter (SH/SP) were conducted using a simulated M4 carbine inside a laboratory. The live fire active/live fire waiting (LA/LW) tasks were conducted using an M4 carbine on a military firing range. The SH/SP and LA/LW tasks required participants to alternate between firing their weapon (SH and LA) and attending downrange while another participant fired a weapon (SP and LW). Figure 2. provides a visual flowchart of participant counts.

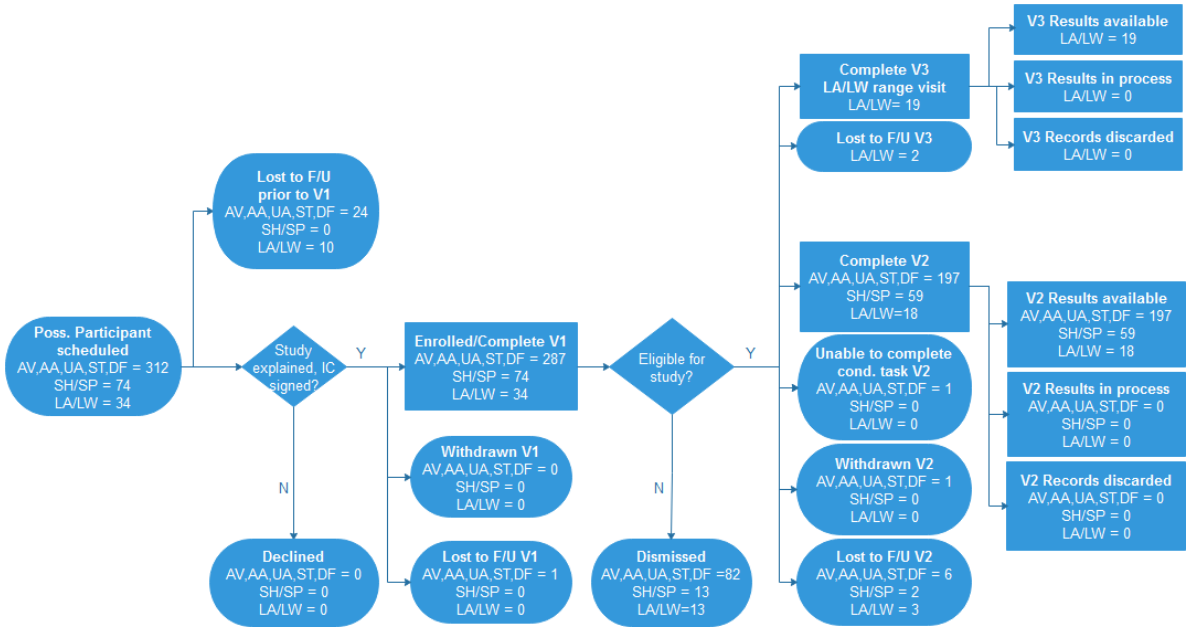

Figure 2. The eMEMC project participant flowchart includes participant counts for each site and study. Attended vision (AV), attended auditory (AA), unattended auditory (UA), dry fire (DF), and simulated trigger (ST) tasks were completed at the university lab. Simulated shooter/simulated spotter (SH/SP) and active and waiting live fire tasks (LA/LW) were completed at the military lab. V1=enrollment visit, V2= laboratory/military experiment visit, V3=live fire range experiment visit.

The instrumentation and software used during the eMEMC project was often shared or duplicated between Study 1 and Study 2. A complete list of instrumentation and software is included in Table 2, with additional details included in Study 1 and 2 Instrumentation sections.

Table 2. Instrumentation and Software Used During the eMEMC Project

Instrumentation /Software	**Manufacturer/Developer**	**Location**
Arduino Uno microprocessor	Adafruit Industries	New York, NY
Brüel & Kjær Nexus microphone power supply	Brüel & Kjær	Nærum, Denmark
Brüel & Kjær Type 4321 calibrator	Brüel & Kjær	Nærum, Denmark
Brüel & Kjær Type 4930 artificial mastoid	Brüel & Kjær	Nærum, Denmark
Dell PC workstation	Dell	Austin, TX
Dell Precision Tower 7910	Dell	Austin, TX
Delsys Bagnoli® 8-channel electromyography system	Delsys	Natick, MA
Engagement Skills Trainer training simulator (EST2000)	Cubic	San Diego, CA
ER-10X® otoacoustic emissions probe	Etymotic Research	Elk Grove Village, IL
ER-4PT® high-output commercial insert earphone	Etymotic Research	Elk Grove Village, IL
force sensitive resistor (FSR400)	Interlink Electronics	Camarillo, CA
G.R.A.S. Type 12AA power supply	G.R.A.S. Sound & Vibration	Holte, Denmark
G.R.A.S. Type 26AC preamplifier	G.R.A.S. Sound & Vibration	Holte, Denmark
G.R.A.S. Type 42AP Intelligent Pistonphone	G.R.A.S. Sound & Vibration	Holte, Denmark
G.R.A.S. type 43AA ear simulator (IEC 60318-1)	G.R.A.S. Sound & Vibration	Holte, Denmark
G.R.A.S. type RA0045 occluded ear simulator (IEC 60318-4)	G.R.A.S. Sound & Vibration	Holte, Denmark
MATLAB software	The MathWorks	Natick, MA
National Instruments (NI) NI PXIe-4499 module	National Instruments	Austin, TX
NI Hybrid PXI/PXIe-4461 modules	National Instruments	Austin, TX
NI PXI-4498 module	National Instruments	Austin, TX
NI PXI-6620 M Series Multifunction DAQ	National Instruments	Austin, TX
NI PXI-6621 M Series Multifunction DAQ	National Instruments	Austin, TX
NI PXIe-1062Q chassis	National Instruments	Austin, TX
NI PXIe-8360 MXI-Express	National Instruments	Austin, TX
Nelson Acoustics Audiometric Research Tool (ART)	Viacoustics	Austin, TX
Quest QC-20 acoustic calibrator, Class 1	3M Detection Solutions	Oconomowoc, WI
RadioEar B-71 bone oscillator	RadioEar	Middelfart, Denmark
Sennheiser HDA-200 circumaural earphones	Sennheiser	Wedemark, Germany
Stata software	StataCorp	College Station, TX
TDT RP2.1 Enhanced Real-Time Processor	Tucker-Davis Technologies	Alachua, FL
Titan® middle ear analyzer	Interacoustics	Middelfart, Denmark
Uninterruptible Power Supply (UPS)	Cyber Power Systems	Shakopee, MN
Welch-Allyn Digital Macroview® video otoscope	Welch-Allyn	Skaneateles, NY
Whisperwatt Diesel powered AC Generator	MQ Power Corp.	Carson, CA
Windows 7 Professional software	Microsoft	Redmond, WA

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Study 1: Conditioned eMEMCs

Participants.

The median age of participants in this study was 21 years old (interquartile range [IQR]: 20 to 23 years, range: 18 to 55 years) and included 287 (70% females) participants. Fifteen participants from a laboratory portion of Study 2 are included in Study 1 participant descriptives due to the small sample size and similarities to Study 1 participant characteristics. Both the age of participants and gender imbalance likely resulted from the study location, as the fields of study at the university setting were primarily dominated by young adult females. Younger adults and female gender groups are the most likely to exhibit conventional ARs (Flamme et al., 2017), which suggests the present study results can be expected to overestimate responses likely to be observed among men or people with poorer hearing. Human research subject protection oversight and protocol approval was conducted by the institutional review boards at Western Michigan University (HSIRB Project Number 15-04-09) and MRDC-IRB (HRPO Approval Number A-18436.2) sites. Table 3 lists the inclusion criteria provided to interested participants prior to obtaining informed consent to participate, and included details likely to be known prior to participating in the study. Participant status regarding exclusion criteria could not be known with certainty prior to completing the enrollment visit of the study, and the result of the enrollment visit was to establish candidacy for the study and provide study baseline information. Participants were reimbursed for their participation.

Table 3. University Lab Experiment Visit Inclusion and Exclusion Criteria

Inclusion Criteria	
	Ages 18+ (19+ if non-active duty military at military lab)
	Willingness to participant in study 2-4 hours over 2 visits (3-8 hours over 3 visits for LA/LW)
	No current ear pain or history of Bell's Palsy, concussion, TBI*, unexplained dizziness
	Those involved in mock/live firing will be regular occupational/recreational shooters
Exclusion Criteria	
	Features/physical disabilities that interfere with eMEMC probe fit (e.g., birth defect, surgery, earrings)
	Ear canal size/shape inappropriate for use with ear canal probe tips
	Excessive cerumen, irritation, infection of ear (dismissal or postponement)
	Pure tone air conduction hearing thresholds poorer than 10 dB at octave-band center frequencies from 125 Hz - 1 kHz, poorer than 20 dB HL from 2 - 8 kHz
	Thresholds differing by more than 20 dB across ears at any frequency
	Abnormal middle ear function based on WBR, WBT
	Absence of conventional contralateral acoustic reflex (re: 0.02 mmho immittance change and growth) for the monitored ear at all elicitor frequencies
	Abnormal CN V (Trigeminal) or CN VII (Facial) nerve function

*TBI= traumatic brain injury

Pure tone air conduction thresholds.

Pure tone air conduction threshold results obtained during the enrollment visit were analyzed for participants who were eligible and completed the experiment visit (Table 4) and for participants who did not qualify to take part in the study based on their results from the enrollment visit (Table 5). In general, participants who were eligible for and completed the experiment visit exhibited excellent hearing sensitivity, with the 50th percentile thresholds at 0 dB HL in both ears and at all frequencies with the exception of 250 Hz on the right ear (2.5 dB HL) and 8 kHz on the right and left (5 dB HL). Pure tone air conduction thresholds for participants who were dismissed from the study (Table 5) were generally good, but showed considerably more variability as a number of these individuals (n = 52) were dismissed from the study because one or more pure tone thresholds met the exclusion criteria. Participants who did not meet the inclusion and exclusion criteria were also dismissed for a variety of reasons not relating to pure tone thresholds, including history of doctor-diagnosed concussion or TBI (n = 17), lack of conventional acoustic reflexes (n = 25), and a history of Bell's Palsy (n = 1). Some participants (n = 14) were dismissed for more than one reason.

Mean results from eight individuals who were eligible but did not complete the experimental visit were within one standard deviation of the mean results from the participants who were eligible and completed the experimental visit (Table 4) for all frequencies in both ears.

Table 4. Pure Tone Air Conduction Thresholds in dB HL, Obtained at the Enrollment Visit, for Eligible Participants who Completed the Experiment Visit (N = 190)

kHz	0.125	0.25	0.5	1	2	3	4	6	8
Left ear									
Min	-10	-10	-10	-10	-10	-15	-15	-15	-10
p10	-5	-5	-5	-5	-5	-10	-10	-5	-5
p25	0	0	-5	-5	-5	-5	-5	-5	0
p50	0	0	0	0	0	0	0	0	5
p75	5	5	5	5	5	5	0	5	10
p90	5	5	5	5	10	5	5	10	15
Max	10	10	10	10	20	15	20	15	20
mean	2	0	0	0	1	-1	-1	1	4
Sd	4	4	4	5	5	6	6	6	7
Right ear									
Min	-5	-10	-10	-10	-10	-15	-15	-15	-10
p10	0	-5	-5	-5	-5	-5	-10	-5	-5
p25	0	0	-5	-5	0	-5	-5	-5	0
p50	2.5	0	0	0	0	0	0	0	5
p75	5	5	5	5	5	5	0	5	10
p90	5	5	10	5	10	10	5	10	15
Max	10	10	10	10	20	20	15	20	20
mean	3	1	1	0	2	1	-1	1	5
Sd	4	4	5	5	5	6	5	6	7

Table 5. Pure Tone Air Conduction Thresholds in dB HL, Obtained at the Enrollment Visit, for Participants Who Did Not Qualify to Complete the Study (*N* = 81)

kHz	0.125	0.25	0.5	1	2	3	4	6	8
Left ear									
Min	-10	-5	-5	-5	-10	-10	-10	-10	-10
p10	-5	-5	-5	-5	-5	-5	-5	-5	0
p25	0	0	0	0	0	-5	-5	0	5
p50	5	5	5	5	5	5	2.5	5	15
p75	10	10	10	10	15	10	10	15	25
p90	15	15	20	20	25	25	35	35	45
Max	50	45	40	40	35	70	70	65	70
mean	6	6	6	5	8	7	8	11	17
Sd	9	9	10	10	11	15	16	16	16
Right ear									
Min	-5	-5	-5	-10	-5	-10	-10	-10	-10
p10	0	-5	-5	-5	-5	-5	-5	-5	0
p25	0	0	0	0	0	0	0	0	5
p50	5	5	5	5	5	5	0	5	12.5
p75	10	10	10	10	10	10	5	15	25
p90	15	15	15	15	15	20	25	30	40
Max	45	40	25	25	30	45	50	45	60
mean	6	5	5	5	6	7	5	10	16
Sd	8	8	7	8	8	11	12	13	15

Indicators of conductive impairment.

Results of bone conduction threshold testing shown in Table 6 represent the differences between the better ear pure tone air conduction thresholds and bone conduction thresholds at each frequency. Results for participants who completed the study were analyzed separately from results for participants who did not complete the study. Mean differences of 0 dB would be expected for a large dataset, but mean differences were greater than 0 dB at all frequencies examined, indicating that participant's bone conduction thresholds were typically slightly better than their best air conduction thresholds at the same frequency. These results did not correlate with abnormal conventional tympanometric results (Table 7) or the likelihood of rMEMCs. The small difference in central tendency could be associated with small errors in the Reference Equivalent Threshold Force Levels (RETFLs) for the bone oscillator, small errors in Reference Equivalent Threshold Sound Pressure Levels (RETSPLs) for HDA200 circumaural earphones, or incorrect coupling forces for the bone oscillator despite confirmation that the headband coupling force met ANSI S3.6-2010 standards. The interquartile range was similar between the two participant groups, with the participants who did not complete the study exhibiting higher maximum air-bone gap values.

Table 6. Air-bone Gap (dB) by Frequency for Participants Completing the Study and Those Who Did Not Complete the Study. Differences were Calculated as the Best Air Conduction Threshold Minus the Bone Conduction Threshold (Forehead Placement) at Each Frequency

	Participants completing the study				**Participants <u>not</u> completing the study**			
kHz	0.5	1	2	4	0.5	1	2	4
min	-10	-10	-20	-10	-10	-15	-15	-5
p10	0	-5	-5	-5	0	-5	-5	0
p25	5	0	-5	0	5	0	-2.5	5
p50	10	5	0	5	5	0	0	5
p75	10	5	5	10	10	5	5	10
p90	15	10	10	15	20	10	15	15
max	30	15	25	20	25	30	35	35
mean	7	3	2	6	7	3	2	7
sd	6	6	8	6	7	7	8	7

Tympanometry.

Results of conventional and wideband middle ear assessments indicated normal effective volume, static admittance, tympanic peak pressure, and tympanometric width for participants completing the experiment visit (Table 7). Mean results among participants who were ineligible to complete the experiment visit were also consistent with normal middle ear function. There were rare cases indicating abnormal peak pressure, but these cases did not appear to affect substantially the measure of tympanometric width, which is a sensitive indicator of middle ear dysfunction (Roup et al., 1998).

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Table 7. Tympanometric Results, Including Ear Canal Volume, Compliance, Peak Pressure, and Tympanometric Width, for Participants Who were Eligible to Take Part in the Study and Completed the Experimental Visit, and for Those Who Were Not Eligible to Complete the Study

		Mean	**SD**	**90% range**	***n***
Participants completing the study, Left ear					
	Volume (cm^3)	1.24	0.30	(0.83-1.81)	190
	Admittance (mmho)	0.76	0.68	(0.29-1.44)	189
	Peak Pressure (daPa)	-4.17	22.23	(-21.00-10.00)	189
	Tympanic Width (daPa)	79.38	32.56	(30.00-133.00)	189
Participants completing the study, Right ear					
	Volume (cm^3)	1.29	0.31	(0.85-1.86)	190
	Admittance (mmho)	0.70	0.49	(0.27-1.50)	190
	Peak Pressure (daPa)	-5.05	25.74	(-26.00-14.00)	190
	Tympanic Width (daPa)	80.81	34.10	(35.00-126.00)	190
Participants not eligible for the study, Left ear					
	Volume (cm^3)	1.36	0.66	(0.77-2.05)	79
	Admittance (mmho)	0.92	0.72	(0.28-2.77)	77
	Peak Pressure (daPa)	-14.66	38.22	(-88.00-9.00)	77
	Tympanic Width (daPa)	78.57	44.36	(15.00-142.00)	77
Participants not eligible for the study, Right ear					
	Volume (cm^3)	1.49	0.69	(0.80-2.81)	79
	Admittance (mmho)	1.00	0.84	(0.29-3.03)	77
	Peak Pressure (daPa)	-17.58	53.02	(-183.00-20.00)	77
	Tympanic Width (daPa)	75.55	45.16	(18.00-166.00)	77

The resonance frequencies for wideband absorbance at ambient pressure and wideband tympanometry testing, used to characterize the middle ear system (Keefe et al., 2015; Rabinowitz, 1977), were used to sub-divide the frequency spectrum during examination of rMEMCs. The resonance frequency for the ambient-pressure wideband absorbance measure was determined as the lowest frequency where the phase of the wideband absorbance function crosses zero. The mean resonance frequency for the wideband absorbance function was approximately 3500 Hz for eligible and ineligible participants alike (Table 8), with ineligible participant outliers showing atypically low wideband absorbance resonance frequencies, suggesting either increased effective mass or decreased stiffness in the middle ear system. The resonance frequency for the wideband tympanometry test is a quantity returned by the measurement system based on changes in absorbance as a function of static ear canal pressure. The mean wideband tympanogram resonance frequency was around 700 to 760 Hz, with a trend toward lower mean resonance frequencies among ineligible participants (Table 9).

Table 8. Wideband Absorbance Resonance Frequency (Hz) Results at Ambient Pressure for Participants who Completed the Study and for Those Who Were Not Eligible to Complete the Study

	Mean	**SD**	**90% range**	***n***
Participants completing the study, left ear	3504	816	(2594-4757)	190
Participants completing the study, right ear	3499	720	(2448-4757)	190
Participants not eligible for the study, left ear	3504	906	(1297-4757)	78
Participants not eligible for the study, right	3511	954	(1411-4757)	78

Table 9. Wideband Tympanometry Resonance Frequency (Hz) Results for Participants who Completed the Study and for Those Who Were Not Eligible to Complete the Study

	Mean	**SD**	**90% range**	***n***
Participants completing the study, left ear	743	127	(537-879)	189
Participants completing the study, right ear	760	123	(548-882)	189
Participants not eligible for the study, left ear	711	155	(398-872)	78
Participants not eligible for the study, right	718	149	(416-1006)	77

Maximum acoustic magnitudes.

Acoustic reflex results are described in terms of maximum change in admittance (mmhos) at the maximum level tested and are organized by ear, frequency and laterality in Table 10. Results only include participants who were eligible to take part in the study and completed the experimental visit of the study. Results are comprised of acoustic reflex levels only at the maximum level tested between presentation levels 80, 85, 90, 95, and 100 dB HL. The maximum level tested was often, but not always, the point where an admittance change of 0.05 mmhos or greater was observed repeatedly. In cases where the change in admittance was less than 0.05 mmhos, the value at the presentation level of 100 dB HL was used.

Table 10. Maximum Acoustic Reflex Magnitude (Change in Admittance, in mmhos), Ipsilateral and Contralateral for Left and Right Ears of Participants Who Completed the Study

Ear	**Stimulus Frequency**	**Mean**	**SD**	**90% range**	***n***
Ipsilateral					
Left	0.5	0.09	0.04	(0.04-0.16)	189
	1	0.09	0.04	(0.04-0.18)	189
	2	0.09	0.05	(0.01-0.19)	189
	4	0.08	0.04	(0.02-0.16)	189
Right	0.5	0.08	0.03	(0.03-0.15)	189
	1	0.09	0.04	(0.04-0.16)	189
	2	0.09	0.05	(0.01-0.18)	189
	4	0.08	0.05	(0.02-0.17)	189
Contralateral					
Left	0.5	0.06	0.04	(0.01-0.12)	189

	1	0.06	0.04	(0.01-0.11)	189
	2	0.07	0.04	(0.00-0.14)	189
	4	0.06	0.04	(0.01-0.13)	189
Right	0.5	0.06	0.03	(0.01-0.12)	189
	1	0.06	0.04	(0.01-0.13)	189
	2	0.06	0.05	(0.00-0.14)	189
	4	0.06	0.04	(0.00-0.14)	189

Video otoscopy.

Otoscopic video recording results were reviewed in .AVI format. Each video was examined for a variety of possible results including occlusive cerumen, exostoses, abnormal landmarks (scarring, irritation, opaque or perforated tympanic membrane) and the ability to visualize the tympanic membrane and manubrium. Results were unremarkable for participants completing the experiment visit.

Stimuli.

Enrollment visit stimuli.

Pure tone air conduction thresholds from 0.125 to 8 kHz, including inter-octave frequencies of 3 and 6 kHz, were obtained. Bone conduction thresholds, obtained using forehead transducer placement, were elicited at 0.5, 1, 2, and 4 kHz. All stimuli used for threshold testing met the requirements of ANSI S3.6-2010. Conventional ipsilateral and contralateral acoustic reflex (AR) traces were obtained in each ear using a conventional 0.226 kHz probe tone. Acoustic reflexes were assessed for pure tone elicitors at 0.5, 1, 2, and 4 kHz, with levels ranging from 80 to 100 dB HL. Acoustic reflex decay was assessed using a 1 kHz, 10 second tone presented contralaterally at 10 dB above the level at which a reliable 0.05 mmho response was obtained, or at 115 dB HL if a response of that level was not obtained at the highest acoustic reflex stimuli level (100 dB HL). Middle ear status was examined via conventional and wideband tympanometry (pressure sweep from +200 to -300 daPa), as well as wideband absorbance at ambient pressure.

eMEMC probe stimulus.

The stimulus used to detect MEMCs during the experiment visit involved measuring changes in the magnitude of the sound energy developed for a probe stimulus click train, which was presented at 20 Hz repetition rate (50 ms interval between clicks) (Keefe et al., 2010). The click stimuli were the impulse response of a finite impulse response (FIR) filter with a passband from 0.2 to 8 kHz, presented at 93 dB peak SPL in an IEC-60318-4 occluded ear simulator. The ER-10X probe function was verified using a one-second probe sweep (0.1 to 16 kHz, fs = 44.1 kHz), presented to the test ear before and after each portion of testing and when probe fit was uncertain.

Unconditioned stimulus.

The UCS, a 100 ms, 100 dBA field equivalent sound exposure level (SEL_A) white noise, gated using a 30 ms Hanning window, was presented 400 ms after the absence of expected tone or visual change (i.e., the CS), and was at a level capable of eliciting an rMEMC. The SEL_A metric is an integrated level normalized to a duration of 1 second. Probe click stimuli were presented in the test ear (contralateral to the participant's trigger finger), while the UCS was presented to the ear contralateral to the test ear. While the probe stimulus and UCS remained unchanged across tasks, the CS in each task differed by sensory mode and level of attention, as described below.

Attended visual CS.

The attended visual CS task (attended vision, AV) involved a change in an image on a video monitor viewed by the participant. The monitor displayed one image (e.g., a yellow rectangle) as a baseline visual stimulus. The CS was the change from the yellow rectangle to a blue oval (Figure 3). The rectangle and oval sizes had the same area in pixels, and the colors (yellow [255 255 051]; blue [051 255 255]) had equal luminance. The white noise UCS described above was presented to the earphone at a fixed interval after the visual stimulus changed from the yellow rectangle to the blue oval.

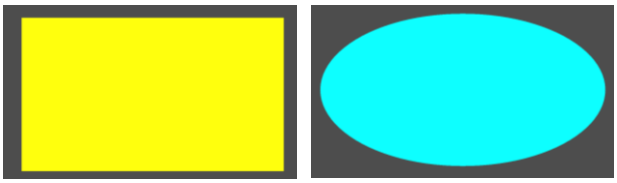

Figure 3. Participants were instructed to press a response button upon detecting a change in visual input from the yellow rectangle to the blue oval. This visual change was the conditioning stimulus, and was presented shortly before presentation of the white noise UCS.

Attended auditory CS.

The attended auditory (AA) conditioning task involved the use of a silent gap in a series of tones as the CS. The tones were a series of 100 ms, 1 kHz signals gated using a 30 ms Hanning window. The amplitude of each tone was 55 dB SPL as measured in an occluded ear simulator, which was easily audible but not sufficiently high to produce an rMEMC. A semi-random number of 100 ms tonebursts at a rate of 500 ms intervals were presented before a silent gap followed by the 100 dB SEL_A white noise UCS. The CS was an absence of tone at the expected 500 ms interstimulus interval. In order to ensure that participants attended to the stimulus, they were instructed to press a response button immediately upon detecting the silent gap (a missing 100 ms tone burst). Figure 4 shows a series of tones surrounding one silent gap and the corresponding white noise UCS. The silent gap was 900 ms total, ending with UCS presentation.

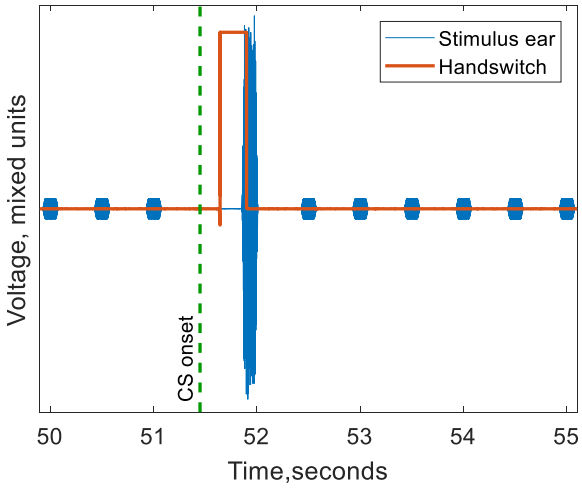

Figure 4. The CS onset was the silent gap after presentation of a series of 1 kHz tones presented in 500 ms intervals. The white noise UCS (large blue signal, presented near 52 second period) was presented 400 ms after the silent gap at the expected tonal interval (or 900 ms after the previously presented tone). The participant was instructed to press the response button as soon as the silent gap was detected (red line).

Unattended auditory CS.

The unattended auditory (UA) conditioning task was identical to the AA task with the exception of the level of attention given to the CS. A distractor activity was used. The distractor activity target, cursor icons, and basic behavior were modified from an open-source MATLAB video game (https://www.mathworks.com/matlabcentral/fileexchange/31330-dave-s-matlab-shooter, last accessed on 13 September 2019; Figure 5). The target (i.e., in red, see Figure 5) moved randomly around a video monitor, and the difficulty of the task was adaptive. If the participant was able to follow the target closely, the rate of target movement increased.

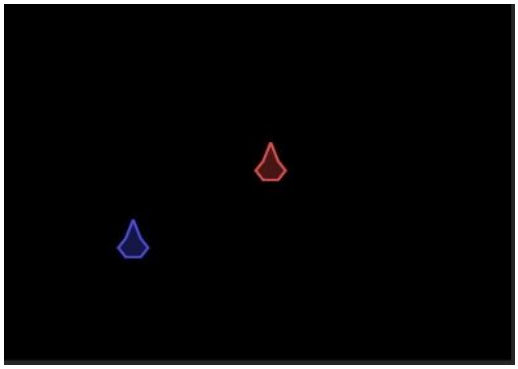

Figure 5. Example of target (red) and cursor (blue) used in the UA conditioning task.

Instrumentation.

The instrumentation used in the study is listed in Table 2. A Welch-Allyn Digital Macroview® video otoscope was used at both visits to examine the external ear and tympanic membrane, and to provide a record of the visual inspection. Additionally, middle ear assessments were obtained using an Interacoustics Titan® middle ear analyzer. Audiometric stimuli were delivered using the Nelson Acoustics Audiometric Research Tool (ART) automatic audiometry software utilizing National Instruments (NI) Hybrid PXI/PXIe-4461 modules. This system-controlled signal output to Sennheiser HDA-200 circumaural earphones (air conduction) and a RadioEar B-71 bone oscillator (bone conduction) and participant responses were tracked via a custom response switch box. Pure-tone testing was conducted in a double-walled sound booth with ambient noise levels permitting testing below -10 dB HL at all stimulus frequencies.

The experiment visit instrumentation was controlled using a Windows-based PC workstation (Dell model 7910) connected to a NI PXIe-1082 chassis. NI PXI/PXIe (hybrid) 4461 dynamic signal analyzer and PXIe-4499 modules were used to produce probe click and acoustic conditioning stimuli and simultaneously sample all input channels during recording. An Etymotic Research ER-10X® otoacoustic emissions probe was used to present probe click stimuli in the monitored ear and transduce the signal in the ear canal. An Etymotic Research ER-4PT® high-output commercial insert earphone was used to deliver acoustic conditioning and unconditioned stimuli. A Delsys Bagnoli® 8-channel electromyography system equipped with dry double-differential surface electrodes was used to monitor the activity of selected muscle groups.

A toy gun mounted on a stand was used to change the location of the blue cursor on the video monitor in the distractor activity (Figure 5) during the unattended auditory (UA) conditioning task. This toy gun was equipped with an inertial measurement unit (IMU), the output of which was routed to an Arduino Uno microprocessor, then integrated with the data stream from the NI modules. A hand switch with a push button was used during attended conditioning tasks (AA and AV). Custom MATLAB functions and scripts and Stata software (v.15) were used during data collection and in multiple levels of review and analysis.

Calibration procedure.

Acoustic stimuli were calibrated at the beginning and end of each test day. Calibration procedures were executed using MATLAB software. Instrumentation used for daily calibration procedures included the G.R.A.S. type 43AA ear simulator (IEC 60318-1), and a G.R.A.S. type RA0045 occluded ear simulator (IEC 60318-4). Bone conduction transducers were calibrated using a Bruel & Kjaer Type 4930 artificial mastoid. An ANSI Class 1 Quest QC-20 acoustic calibrator was used for daily calibration, and the accuracy of this calibrator was regularly validated using a G.R.A.S. Type 42AP Intelligent Pistonphone. Microphone signals were preamplified (G.R.A.S. Type 26AC) and routed through a power supply (G.R.A.S. Type 12AA) prior to digitization using an NI hybrid PXI/PXIe-4461 dynamic signal analyzer module mounted within an NI PXIe-1082 chassis.

Microphones were assessed in terms of sensitivity (mV/Pa) and harmonic distortion; receivers were evaluated in terms of magnitude and phase response as well as calibration offsets required for the nominal stimulus levels in the IEC 60318-4 occluded ear simulator. During daily calibration, deviations from expected calibration values necessitated inspection of the transducer and calibration hardware for errors and re-measurement. Across the 520 sensitivity measurements made with the occluded ear simulator, field microphone, and the ER-10X microphone, the interquartile range of measured sensitivities was: -0.09 to 0.03 dB for the ear simulator; -0.04 to 0.04 dB for the field microphone, and -0.30 to 0.70 dB for the ER-10X probe microphone. The interquartile range for the calibration offsets for the tone series in the AA and UA tasks was -0.41 to 0.30 dB, and the white noise interquartile range was -0.54 to 0.24 dB. Calibration results from the bone conduction transducer showed a good match to targets except for the 0.5 kHz stimulus at levels greater than 35 dB HL. This result was not surprising because the RadioEar B-71 bone oscillator model is known for having restricted maximum outputs for low-frequency signals. It is also noteworthy that performance below 30 dB HL was most important in the current study due to the audiometric inclusion criteria wherein only people with air conduction hearing thresholds better than 15 dB HL at 0.5 kHz were eligible to participate.

The calibration procedure for audiometric transducers included signal presentations at each stimulus frequency and level relevant to the study. The sound pressure levels developed in the ear simulators were compared with the levels specified in ANSI/ASA S3.6-2010 to determine calibration errors. Figure 6 represents sample results from a single calibration run. Daily calibration results for audiometric air conduction earphones (shown in Figure 6) indicated an excellent match to output targets down to approximately 0 dB HL. Below this point, the levels of the acoustic stimulus approach the internal electrical noise of the measurement apparatus and are no longer measurable acoustically. Separate measurements of the electrical output of the signal generator demonstrated that the range of linear output extended well below -10 dB HL, which is well within the allowable error tolerances of ANSI S3.6-2010.

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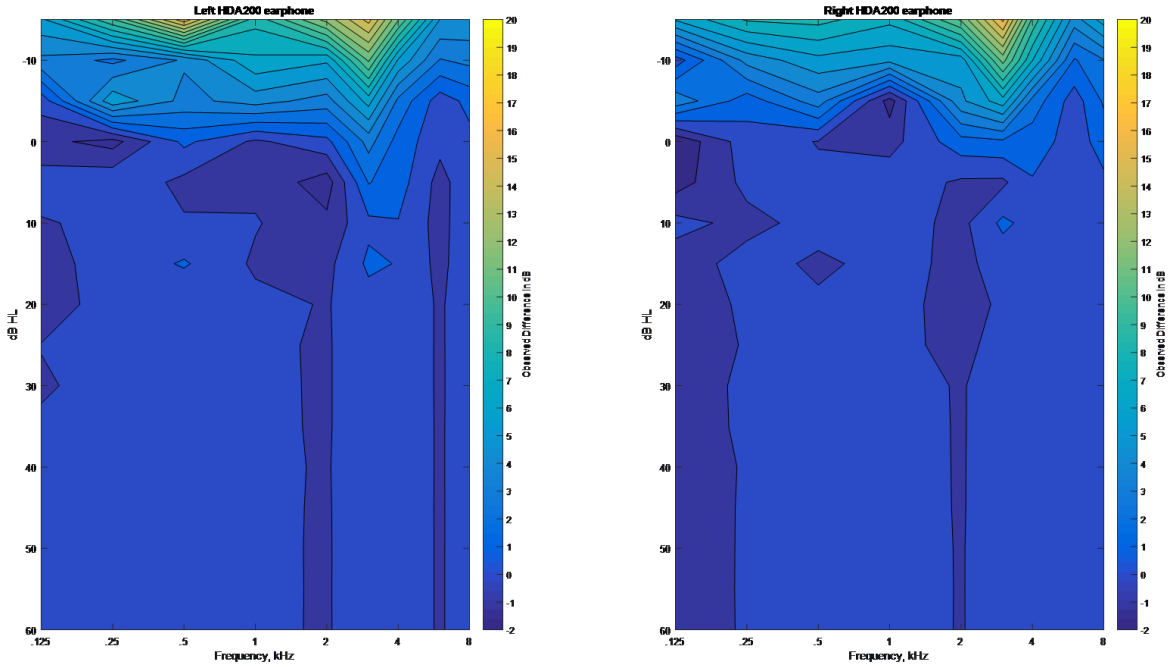

Figure 6. Contour plot of deviations from target for the left and right earphones used during audiometric assessments. Results indicate deviations within +/- 1 dB across the range of tested levels, except at levels below approximately 0 dB HL, where the signal levels approach the internal noise of the artificial ear system.

Data collection procedure.

Data collection at Western Michigan University site, conducted during years 2015 to 2017, involved completion of an enrollment visit and, if eligible based on the required inclusion/exclusion criteria (Table 3), an experiment visit. The conditioning task was completed during the experiment visit.

Participants volunteering for the laboratory components of this study were asked to complete two visits to the university research laboratory (Figure 7), with each visit taking up to two hours. Determination of eligibility for participation in the experimental visit was the primary objective of the first visit. During the enrollment visit, participants completed questionnaires, otoscopic examination, pure tone air and bone conduction audiometry, clinical assessment of the integrity of cranial nerves V and VII (trigeminal and facial nerve, respectively), and conventional and wideband middle ear assessment to rule out cases of middle ear disorders and to obtain clinically-accepted evidence of acoustic reflexes. Automated unmasked bone conduction testing, using forehead placement of the bone oscillator, was completed during the enrollment visit only. Air and bone conduction thresholds were determined via the modified Hughson-Westlake procedure (Carhart & Jerger, 1959), and were defined as the lowest presentation level producing greater than a 50% likelihood of response on at least three ascending trials.

The procedures in the experiment visit focused on confirmation of stable hearing status between the first and second visit, acquisition of experiment conditioning eMEMC data, and repeat confirmation of stable hearing status following the experiment data acquisition. For participants assigned to the AV conditioning task, approximately 40 CS-UCS trials and four trials only including the CS were presented. Although the interval between visual stimulus change and unconditioned auditory stimulus was fixed during a participant session, there was slight variation between participants. Participants assigned to the AA task attended to approximately 25 CS-UCS trials and four trials where only the CS was presented. Participant attention was monitored and achieved by pressing a response button upon detection of the change in image (in AV, Figure 3) or acoustic pattern (in AA, Figure 4). Participants assigned to the UA conditioning task were presented with 45 CS-UCS trials and four trials including only the CS. The number of CS-UCS trials presented, although variable across conditioning tasks, is typical of the number presented in studies of associative learning (Bates et al., 1970; Flaten & Hugdahl, 1990; Harris & Andrew, 2017).

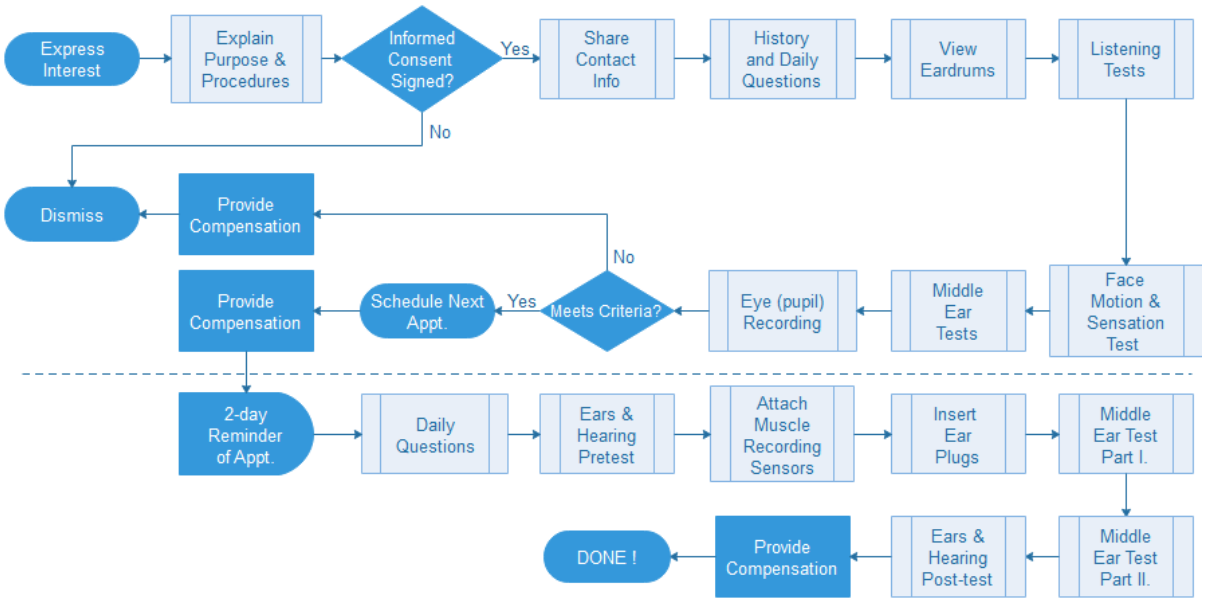

Figure 7. University lab participant visit flowchart. The dashed horizontal line separates the enrollment visit and the experiment visit details.

Data management and analyses.

Multiple levels of data review and analysis were completed on data collected during Study 1 using custom MATLAB functions and Stata statistical software. Otoscopic recordings were reviewed manually for obstructions or abnormalities (e.g., cerumen obstruction, tympanic membrane perforation).

Evaluation of eMEMCs.

Evaluation of the presence of an eMEMC was completed using a method modified from Keefe et al. (2010) and previously described in Deiters et al. (2019). Waveforms obtained from probe click recordings occurring during baseline periods (Figure 8) were compared to the

waveforms from probe click recordings occurring during CS and UCS presentation, producing difference waveforms (Figure 9). The RMS differences in each trial were time-synchronized relative to the onset of the UCS across trials (Figure 10), permitting the estimation of percentiles of the time-synchronized RMS differences as a function of time. Trials including presentation of only the CS were not included in the current evaluation of eMEMCs. Activity consistent with eMEMCs was seen as a 25th percentile RMS difference in the interval shortly before the onset of the UCS. A comparison of CS-UCS and CS only trials was not conducted in the current study because eMEMC activity occurs prior to the UCS onset. The presence of a UCS (i.e., gunfire) is expected in AHAAH, and the presence of an eMEMC during periods that do not include gunfire are not of interest in the current evaluation. RMS differences occurring in response to UCS onset are considered rMEMCs.

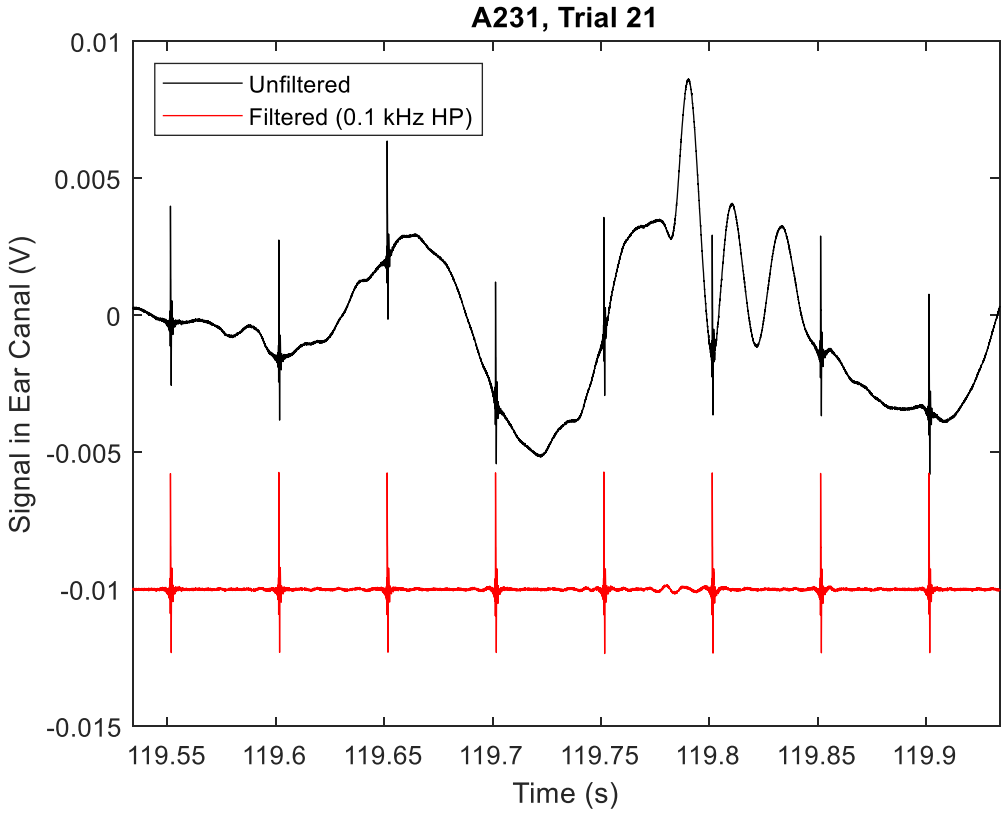

Figure 8. Click-based probe signals, before and after high-pass filtering. Plot represents pressure (Volts) at the probe microphone as a function of time (seconds). Click-based probe signals were presented with a 50 ms inter-stimulus interval. Unfiltered recordings (black curve) revealed that the probe signals were combined with physiological noise in the ear canal, which was minimized using a high-pass filter at 0.1 kHz (red curve, which was displaced by -0.01 V for clarity).

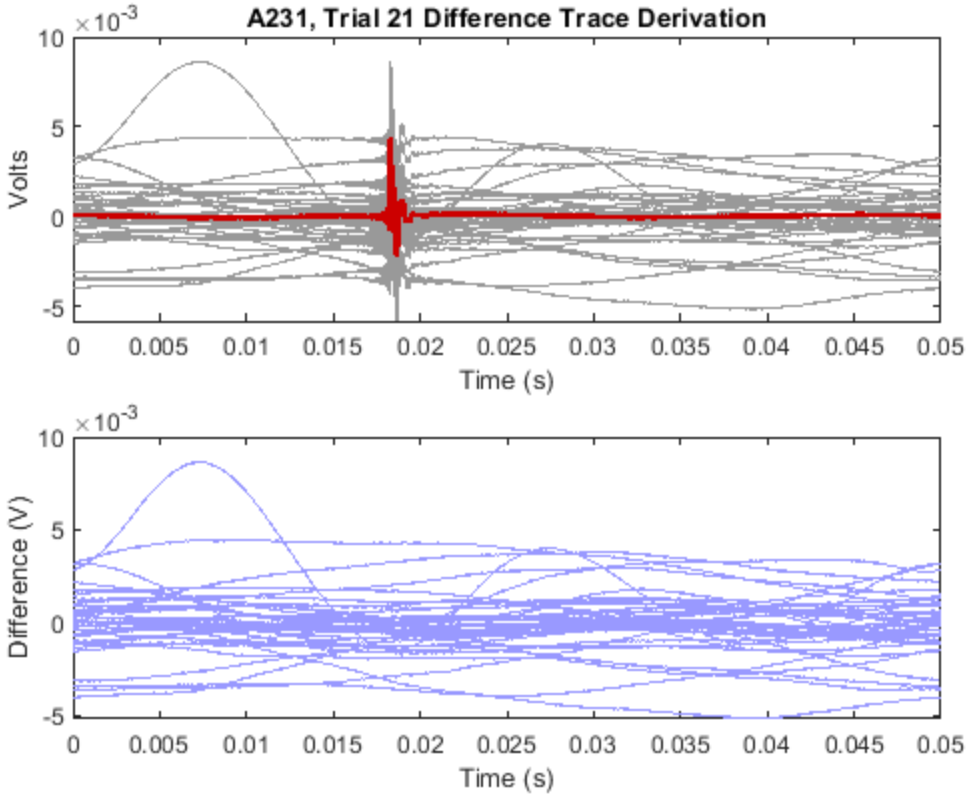

Figure 9. Derivation of difference waveforms for click-based probe signals. Upper plot represents pressure (Volts) as a function of time. Gray curves represent the 0.05 second intervals associated with individual click signals. The red curve represents the time-aligned mean of all clicks outside the elicitor interval (i.e., baseline). Lower plot represents the difference (individual click minus baseline) waveforms for each click within the elicitor interval for a single trial.

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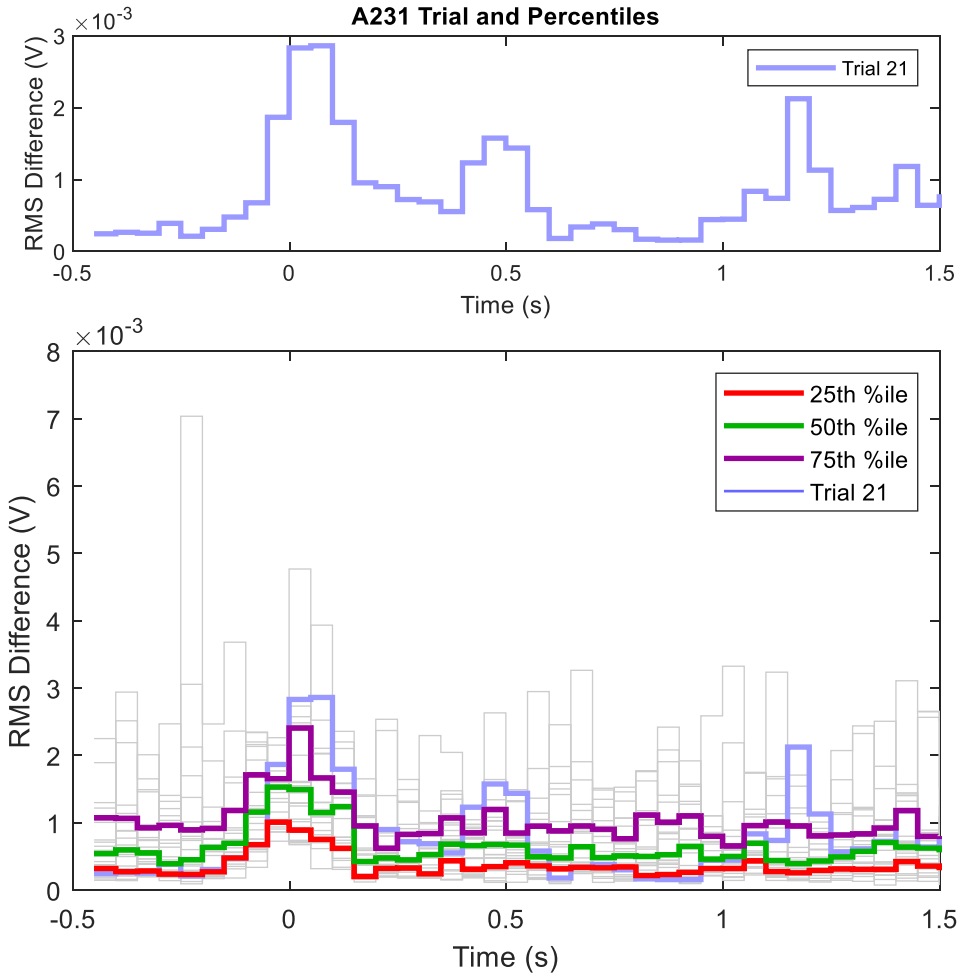

Figure 10. RMS difference plots for a single trial (upper) and for all trials within a task. The upper plot represents the RMS amplitude of the difference wave associated with each 50 ms probe click interval (re: UCS onset) within one trial. Gray stairstep curves in the lower plots represent the RMS difference values across all trials. Selected percentiles (25th, 50th, 75th) of all trials within the task are represented in red, green, and purple curves. The light blue plot represents the single trial RMS difference curve shown in the upper panel. In this example, the RMS difference prior to time=0 indicates the participant demonstrated a tendency to produce an eMEMC.

Energy changes in the ear canal were examined in 50 ms intervals because the click interval was 50 ms, leading to the use of a stairstep plot (Figure 10). Thus, if the energy in the ear canal increased relative to baseline at any point during the 50 ms interval, the stairstep for that entire interval increased. For an individual, the RMS differences for all conditioned task trials were averaged and the 25th percentile of results was plotted for further examination. The 25th percentile was used as it provided the greatest likelihood of exhibiting a consistent RMS difference without interference from excessively noisy RMS differences coincident with individual trials. The traces in Figure 11 represent the 25th percentile of the responses to trials that paired the CS with a UCS. Since the CS precedes the white noise UCS, an RMS difference

change occurring prior to the elicitor would suggest some consistent eMEMC activity has occurred. The dashed vertical line in each of the panels represents the interval just prior to the onset of the UCS. Given the uncertainty of timing, eMEMCs would need to be evident prior to this interval in order to be counted as an eMEMC response. The upper left plot is an example of such a shift. Note there is a rise from the baseline beginning 150 ms before the elicitor onset (0 sec).

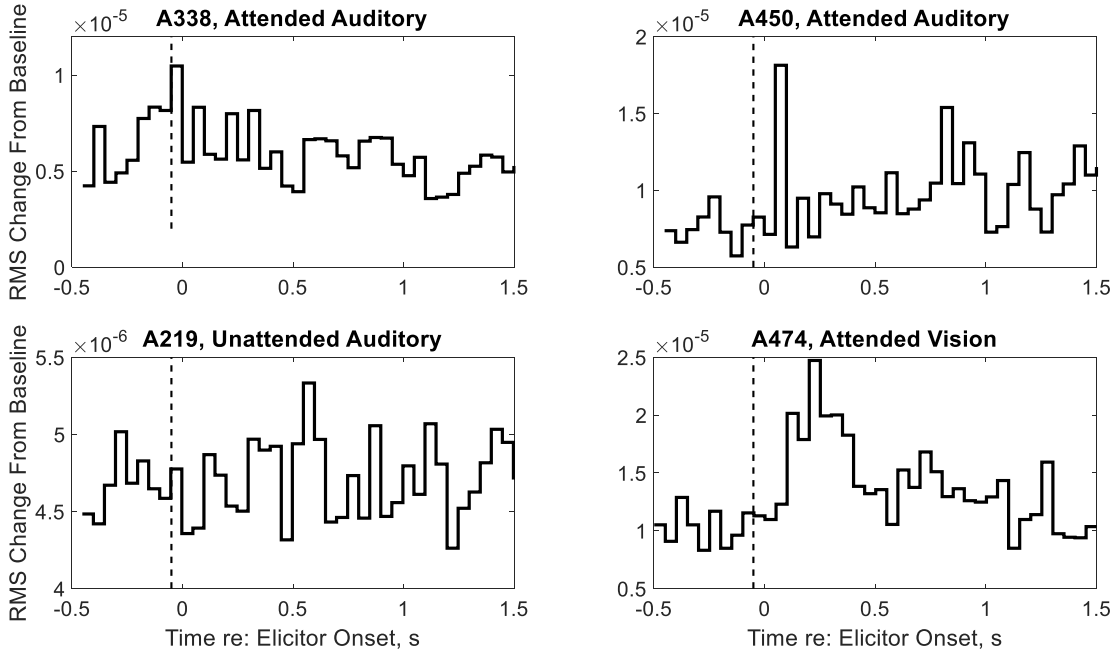

Figure 11. Example results from the Attended Auditory, Unattended Auditory, and Attended Vision conditioned tasks. The horizontal axis represents time, with time=0 representing the onset of the UCS (i.e., a white noise). The vertical axis represents the difference in RMS over the modulus of an FFT band (200-8000 Hz). Each trace represents the 25th percentile of the responses associated with trials that paired the CS and UCS. Evidence of conditioning would be seen as a sustained rise in amplitude occurring in the 50 ms intervals prior to the UCS onset (to the left of the vertical dashed line). A conditioned eMEMC seems likely for the Attended Auditory task for participant A338 (upper left panel), as illustrated by the increase in amplitude prior to time=0 and left of the vertical dashed line. However, a conditioned eMEMC is unlikely in any of the other examples. The two right panels show possible rMEMCs to the white noise UCS (increased RMS change after elicitor onset), but there is no clear rMEMC for the Unattended Auditory example in the lower left corner.

Analyses of lab-based conditioned eMEMCs were based on qualitative judgments of the 25th percentile plots (Figure 11) from three independent raters. All raters provided indications of evidence of the presence of an eMEMC at the task-level occurring after the onset of the CS and prior to the onset of the UCS. Consistent RMS differences occurring -50 ms relative to the UCS onset and later are likely a result of the UCS occurring within the 50 ms click interval immediately preceding the nominal UCS onset (i.e., artifactual) and would be considered an

rMEMC. The presence of rMEMCs in response to conventional stimuli and brief tones are discussed elsewhere (Deiters et al., 2019; Flamme et al., 2017; McGregor et al., 2018). Rater judgments were based on the presence of a stimulus-linked eMEMC response, and no attempt was made to judge whether any CR might have provided any potential protection at the moment the UCS arrived (i.e., relaxed definition). Thus, an early response that degraded to baseline prior to UCS arrival was considered a response even though it would not have affected exposure. The inclusion of a response that degraded to baseline prior to UCS arrival had an upward biasing effect on the proportion of people demonstrating eMEMCs. Proportions reported here should be distinguished from those that might be protective, which are fewer in number.

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Study 2: eMEMC Associated with Prior Experience

The presumed conditioning in this series of tasks is associated with the participant's prior experience as a regular firearm user. The developers of the AHAAH model assume those with awareness of an impending impulse (i.e. Simulated Trigger, Dry Fire, Simulated Shooter, Live Fire – Active) will exhibit an eMEMC, whereas those presented with an "unannounced" intense stimulus will not exhibit an eMEMC (Price & Kalb, 2018). The current study provided an examination into both mechanisms of awareness to impending impulses.

Participants (Laboratory experiments).

Simulated trigger / dry fire (ST/DF) – University lab.

The Simulated Trigger (ST) and Dry Fire (DF) tasks were conducted at the university lab in conjunction with the AA, UA, and AV conditioned tasks detailed in Study 1. Participants who were considered regular firearm users (i.e., those who had fired at least 10 rounds in the past 30 days) were tasked to complete either the ST or DF task, in a previously generated random order. A total of 9 participants (33% female) completed the DF task and 6 participants (33% female) completed the ST task. The median age of participants completing ST and DF tasks was 22 years (IQR: 21 to 27 years, range: 18 to 48 years). Additional participant details were described in Study 1. Enrollment of participants considered regular firearm users was limited, possibly due to the strict inclusion and exclusion parameters, despite exhaustive efforts to recruit law enforcement, individuals with a military background, recreational hunters, or at firearm stores and firing ranges.

Simulated shooter/simulated spotter (SH/SP) – Military lab.

The SH/SP tasks included 59 participants recruited at the military lab (USAMRDC IRB Protocol Number M-10588). Seventy-four participants provided informed consent and completed the military lab SH/SP enrollment visit. Data were available for 59 participants who subsequently completed the SH/SP experiment visit. Although all participants completing the experiment visit reported prior regular firearm use, only 32 reported firing at least 10 rounds within the past 30 days (the study definition of regular firearm user). Of the 59 participants completing the experiment visit, 18 failed to meet audiometric criteria, typically exhibiting one threshold outside of the acceptable range. There were two participants completing the experiment visit who failed to exhibit conventional acoustic reflex thresholds at any frequency or laterality meeting a clinically accepted level of 0.02 mmhos; one of those participants also did not meet the audiometric criteria. Figure 12 provides flowchart details for participants completing SH/SP tasks. Of those participants completing the experiment visit, 17 met audiometric, conventional acoustic reflex, health related and firearm use inclusion and exclusion criteria used for the other tasks described in this report (Table 3).

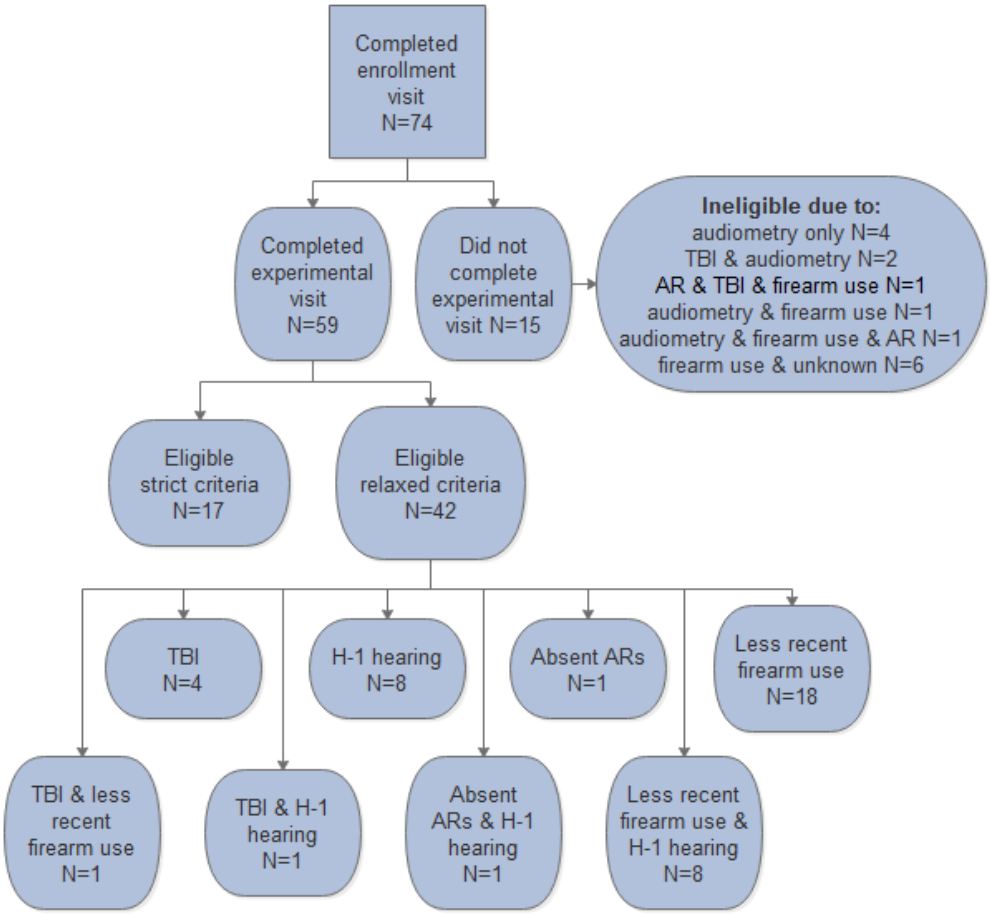

Figure 12. Military lab SH/SP study participant flowchart. Of the 59 participants completing the experiment visit, 17 were eligible based on strict inclusion and exclusion criteria. Forty-two participants were allowed to complete the experimental visit based on relaxed criteria. TBI=traumatic brain injury, ARs=acoustic reflexes, H-1 hearing level defined as average threshold at 0.5, 1, and 2 kHz not more than 25 dB hearing level (HL), with no individual level greater than 30 dB HL at these frequencies, and threshold at 4 kHz not more than 45 dB HL (AR 40-501 Standards of Medical Fitness, 2008).

Narrowband noise air conduction threshold.

The upper tail of the distribution of air conduction thresholds for participants completing the SH/SP study suggested poorer hearing sensitivity than was observed at the university lab and the military lab LA/LW study. Narrowband noise air conduction threshold results, obtained during the enrollment visit, were analyzed for participants who completed the study (Table 11) and for participants who did not take part in the study (Table 12). In general, participants who completed the study (n = 59) exhibited very good hearing levels, with the 50th percentile thresholds at or better than 10 dB HL in the left and right ear at 0.125, 0.25, 0.5, 1, 2, 4, and 8 kHz. However, the 90th percentile and maximum values of participant air conduction thresholds were often outside of the exclusion criteria used at the university lab and in the LA/LW studies, with maximum hearing levels reaching 30 dB. Typically, participants were included in the SH/SP portion of the study if air conduction thresholds were within the exclusion criteria range

at all but one frequency. Narrowband noise air conduction thresholds for participants who did not take part in the study (Table 12) were generally also very good (n = 15). The use of narrowband noise stimuli complicates direct comparison to the other portions of the study, where pure tone stimuli were used to obtain air conduction threshold results.

Table 11. Air Conduction Thresholds in Response to 1/3 Octave Bands of Noise Sentered Around the Frequency Listed, in dB HL, Obtained at the Enrollment Visit, for Participants Who Completed the SH/SP Study (n = 59). The -45 dB HL Minimum Threshold Level at 4 kHz in the Right Ear was an Artifact of Operator Error

kHz	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
Left ear							
min	-5	-10	-10	-10	-5	-10	-10
p10	-5	-10	0	-5	0	0	0
p25	0	-5	0	0	0	0	0
p50	0	-5	5	0	5	5	5
p75	5	0	5	5	10	10	10
p90	5	5	10	10	15	15	15
max	10	5	15	15	25	25	30
mean	2	-3	3	1	6	7	5
sd	4	5	5	5	6	7	7
Right ear							
min	-15	0	0	-20	-10	-45	-10
p10	-5	5	5	-5	-5	0	-5
p25	0	5	5	0	0	0	0
p50	0	5	10	0	0	5	5
p75	5	10	10	5	5	10	10
p90	10	15	20	10	10	10	15
max	15	15	30	10	20	20	20
mean	2	7	10	1	1	4	4
sd	5	4	6	5	6	10	7

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Table 12. Narrowband Noise (1/3 octave band) Thresholds in dB HL, Obtained at the Enrollment Visit, for Participants Who Did Not Complete the SH/SP Study

kHz	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
Left ear							
min	-5	-10	0	-5	0	-5	-5
p10	-5	-5	0	-5	0	0	-5
p25	0	-5	0	0	5	0	0
p50	5	-5	5	5	5	10	5
p75	5	0	5	5	10	15	20
p90	10	5	10	10	15	25	25
max	10	5	15	15	30	30	60
mean	4	-2	5	3	8	11	11
sd	5	4	4	6	7	10	16
Right ear							
min	0	5	0	0	0	-10	-5
p10	0	5	10	0	0	0	-5
p25	0	5	10	0	0	0	0
p50	5	10	10	0	0	5	5
p75	10	10	15	5	10	15	15
p90	10	15	20	5	15	20	25
max	15	20	20	10	15	20	45
mean	5	9	11	3	4	8	9
sd	5	4	5	3	6	8	13

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Tympanometry.

Results of conventional middle ear assessments (0.226 kHz tonal probe) indicated normal effective volume, static admittance, peak pressure, and tympanometric width for the participants completing the study (Table 13) with the exception of nine participants, whose results were slightly above the normative values for one measure, and within normative values for the remaining measures. Mean results among those not completing the study were also consistent with normal middle ear function, although outlier results showed considerable variability.

Table 13. Tympanometric Results, Including Ear Canal Volume, Compliance, Peak Pressure, and Tympanometric Width, for Participants Who Completed the Study, and for Those Who Did Not Complete the SH/SP Study

		Mean	***SD***	**p5**	**p95**	***n***
Participants completing the study, Left ear						
	Volume (cm^3)	1.30	0.36	0.71	1.86	58
	Admittance (mmho)	0.79	0.33	0.40	1.46	58
	Peak Pressure (daPa)	-10	23	-55	12	58
	Tympanic Width (daPa)	88	37	45	152	58
Participants completing the study, Right ear						
	Volume (cm^3)	1.27	0.37	0.73	2.00	58
	Admittance (mmho)	0.76	0.29	0.40	1.52	58
	Peak Pressure (daPa)	-10	27	-41	6	58
	Tympanic Width (daPa)	85	26	46	128	58
Participants not completing the study, Left ear						
	Volume (cm^3)	1.22	0.29	0.80	1.93	14
	Admittance (mmho)	1.35	1.39	0.46	4.99	14
	Peak Pressure (daPa)	-5	56	-145	128	14
	Tympanic Width (daPa)	70	25	19	109	14
Participants not completing the study, Right ear						
	Volume (cm^3)	1.16	0.26	0.73	1.48	14
	Admittance (mmho)	0.83	0.37	0.44	1.64	14
	Peak Pressure (daPa)	-2	44	-103	117	14
	Tympanic Width (daPa)	81	25	41	114	14

Video otoscopy.

Otoscopic video recording results were examined in the same way as in Study 1. Otoscopic results were unremarkable with the exception of four participants exhibiting cerumen allowing only a partial view of the tympanic membrane. Study investigators recommended that these participants seek medical attention to remove the cerumen. No attempts were made to remove the cerumen by the study investigators. All four of these participants exhibited immittance values within the normative range for both ears.

Participants (Live fire experiments).

Live fire – active / Live fire – waiting (LA/LW), Military lab.

A total of 34 participants completed the enrollment visit of the military lab live fire (LA/LW) study (USAMRMC IRB Protocol Number M-10588). The LA/LW tasks used the university lab inclusion and exclusion criteria (Table 3). Of those participants, 19 were eligible and completed the live fire range experiment visit. Of the 19 participants completing the live fire range experiment visit, only 17 completed the laboratory reflexive experiment visit and an additional participant who did not complete the live fire experiment visit completed the laboratory reflexive experiment visit for a total of 18 participants completing the laboratory experiment visit. Participant flowchart details are included in Figure 13.

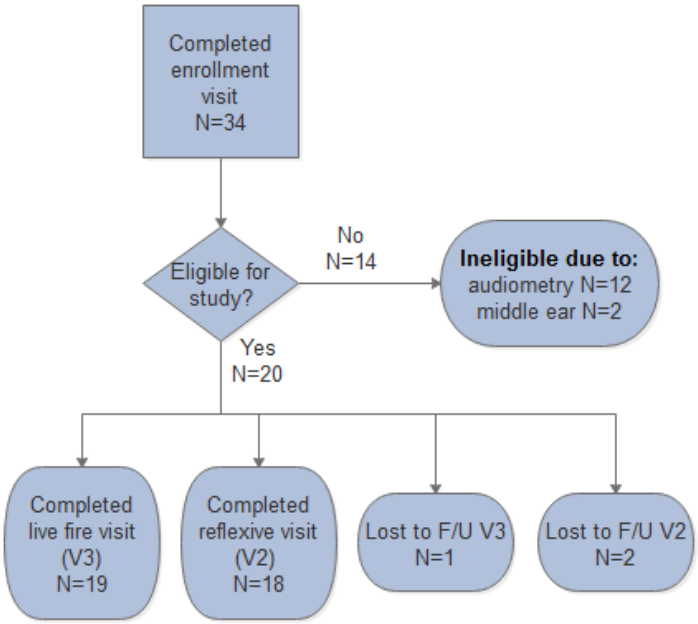

Figure 13. Live Fire (LF) study participant flowchart. In addition to the enrollment visit, participants completed a laboratory rMEMC experiment visit and a live fire experiment visit. V2=laboratory experiment visit, V3=range experiment visit (LA/LW participants).

Pure tone air conduction thresholds.

Pure tone threshold results, obtained during the enrollment visit, were analyzed for participants who completed the study (Table 14) and for participants who did not take part in the study (Table 15). In general, participants who completed the study exhibited excellent hearing levels, with all participants exhibiting hearing thresholds within study criteria in both ears and at all frequencies. Pure tone thresholds for participants who did not take part in the study (Table 15) were generally also very good with the 50th percentile at or better than 10 dB HL for both ears and all frequencies tested (N = 15).

Table 14. Pure Tone Air Conduction Thresholds in dB HL, Obtained at the Enrollment Visit, for Participants Who Completed the LA/LW Study

kHz	**0.125**	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**
Left ear									
Min	-5	-10	-10	-5	-10	-5	-10	-10	-5
p10	-5	-10	-5	-5	-5	-5	-5	0	0
p25	-5	-5	0	-5	-5	0	-5	0	0
p50	0	0	0	0	0	0	0	5	5
p75	0	5	0	5	0	5	5	10	10
p90	10	5	5	5	10	10	5	15	15
Max	10	10	10	10	10	20	15	20	20
Mean	0	-1	0	-1	-1	3	1	7	7
Sd	5	6	4	5	5	6	6	7	7
Right ear									
Min	-15	-10	-5	-15	-5	-10	-10	-10	-10
p10	-5	-10	-5	-10	-5	-5	-10	-10	-5
p25	0	-5	0	0	-5	0	-5	-5	0
p50	0	0	0	0	0	5	0	0	5
p75	5	0	5	5	0	10	10	5	10
p90	5	5	10	10	5	10	15	5	15
Max	10	5	10	10	10	15	15	10	15
Mean	0	-1	1	1	-1	4	1	1	6
Sd	5	4	5	7	4	6	8	6	7

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Table 15. Pure Tone Air Conduction Thresholds in dB HL, Obtained at the Enrollment Visit, for Participants Who Did Not Complete the LA/LW Study

kHz	**0.125**	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**
Left ear									
Min	-5	-5	-5	-5	-10	-5	0	-5	-5
p10	-5	-5	-5	-5	-5	0	0	0	0
p25	-5	-5	-5	-5	-5	0	0	5	5
p50	0	0	0	5	5	5	10	10	10
p75	0	5	5	10	10	15	15	20	15
p90	5	5	15	20	10	25	35	35	40
Max	10	20	20	20	20	50	45	50	55
Mean	-1	1	2	5	4	11	13	13	13
Sd	5	6	8	9	8	14	13	14	16
Right ear									
Min	-5	-10	-5	-5	-10	-5	-5	-10	0
p10	-5	-10	-5	-5	-5	0	-5	-5	0
p25	-5	-5	-5	0	-5	5	0	0	0
p50	0	0	-5	5	0	5	5	5	10
p75	5	5	5	10	10	15	10	15	10
p90	5	10	10	15	10	20	20	40	40
Max	10	15	20	15	15	30	20	40	45
Mean	1	0	1	5	2	10	5	7	11
Sd	5	7	8	7	7	9	8	15	14

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Tympanometry.

Results of conventional middle ear assessments indicated normal effective volume, static admittance, peak pressure, and tympanometric width for the participants completing the study (Table 16). Mean results among those not completing the live fire portion of the study were also consistent with normal middle ear function.

Table 16. Tympanometric Results, Including Ear Canal Volume, Compliance, Peak Pressure, and Tympanometric Width, for Participants Who Completed the Study, and for Those Who Did Not Complete the LA/LW Study

		min	**p10**	**p50**	**p95**	**max**	**mean**	***SD***
Participants completing live fire experiment visit, Left ear								
	Volume (cm^3)	0.86	0.96	1.21	1.93	1.93	1.26	0.26
	Admittance (mmho)	0.27	0.41	0.81	1.66	1.66	0.88	0.36
	Peak Pressure (daPa)	-25.00	-22.00	-4.00	9.00	9.00	-5.63	8.33
	Tympanic Width (daPa)	43.00	53.00	75.00	114.00	114.00	76.58	19.12
Participants completing live fire experiment visit, Right ear								
	Volume (cm^3)	0.78	0.90	1.27	1.84	1.84	1.28	0.31
	Admittance (mmho)	0.33	0.35	0.88	1.36	1.36	0.87	0.32
	Peak Pressure (daPa)	-28.00	-21.00	-7.00	17.00	17.00	-4.89	11.14
	Tympanic Width (daPa)	50.00	56.00	78.00	131.00	131.00	80.53	21.68
Participants not completing live fire experiment visit, Left ear								
	Volume (cm^3)	1.17	1.26	1.37	2.37	2.37	1.49	0.31
	Admittance (mmho)	0.52	0.54	0.89	2.53	2.53	1.04	0.56
	Peak Pressure (daPa)	-41.00	-35.00	-6.00	2.00	2.00	-10.85	12.58
	Tympanic Width (daPa)	26.00	54.00	73.00	99.00	99.00	71.62	20.86
Participants not completing live fire experiment visit, Right ear								
	Volume (cm^3)	1.06	1.11	1.34	2.34	2.34	1.46	0.38
	Admittance (mmho)	0.45	0.47	0.93	3.34	3.34	1.11	0.78
	Peak Pressure (daPa)	-26.00	-22.00	-8.50	4.00	4.00	-10.43	7.95
	Tympanic Width (daPa)	27.00	29.00	75.50	114.00	114.00	71.57	25.09

Video otoscopy.

Otoscopic video recording results were reviewed in .AVI format. Each video was examined for a variety of possible results as described in earlier portions of the study. Otoscopic results were unremarkable for participants who completed the experiment visits.

Stimuli.

Simulated trigger / dry fire – University lab (ST/DF).

Eligibility visit stimuli details were described in Study 1 Stimuli section.
During the experiment visit, changes in the middle ear were assessed using the same probe click stimuli discussed in Study 1. Briefly, broadband clicks with a passband from 0.2-8 kHz,

presented at a 20 Hz repetition rate, were presented in the test ear (contralateral to the participant's trigger finger). Although no other acoustic signals were presented during ST and DF tasks, ambient signals associated with the trigger pull and hammer fall were often audible and may have been at a level great enough to elicit rMEMCs. Probe sweeps, as discussed in Study 1, were presented before and after each portion of testing confirming ER-10X probe function.

Simulated shooter / simulated spotter – Military lab (SH/SP).

As in the enrollment visit at the university lab, data from the enrollment visit of the SH/SP study were used to determine eligibility into the experiment visit. Thresholds were measured using narrow bands of noise (1/3 octave band) from 0.125 to 8 kHz. Inadvertent playback at an incorrect sampling rate (51.2 kHz vs. 44.1 kHz) led to an actual bandwidth of 1.03 to 1.30 kHz (versus 0.891 to 1.123 kHz) for a stimulus nominally centered on 1 kHz. Thresholds were determined via the modified Hughson-Westlake procedure (Carhart & Jerger, 1959), and were defined as the lowest presentation level producing a 50% or greater likelihood of response on at least three ascending trials. Conventional ipsilateral and contralateral acoustic reflex (AR) traces were obtained in each ear using a conventional 0.226 kHz probe tone. Acoustic reflex decay was assessed using a 0.5 kHz ipsilateral 10 second tone presented at levels up to 100 dB HL. Middle ear status was examined via conventional and wideband tympanometry (pressure sweep from +200 to -300 daPa), as well as wideband absorbance.

Middle ear muscle activity was assessed during the SH/SP experiment visit tasks using the same probe click stimuli discussed in other portions of the study. Probe sweeps, confirming ER-10X probe function, were presented prior to each test. Here, subjects were involved in both firing a carbine simulator (an M4 carbine outfitted for use in the EST-2000 training system; note the EST-2000 system itself was not used), and spotting while the investigator fired the carbine simulator towards air-rifle targets. Probe click stimuli were presented in the test ear, while a recording of a gunshot (recorded in an indoor range), presented at 110 dB peak SPL, was played over the Etymõtic ER-4PT (contralateral to the test ear) each time the carbine was fired (in both conditions), thus providing UCS in addition to any participant-specific CS.
The trigger pull required real time detection in order to initialize the playback of a recorded gunshot via the Etymotic ER-4PT. The gunshot recording included a quiet period prior to the onset, thus the gunshot arrival at the ear occurred 62.2 ms after the trigger pull was detected.

Live fire – active / live fire – waiting (LA/LW).

The live fire enrollment visit utilized very similar stimuli to those used in most other study components (AV, AA, UA, ST, DF). Conventional ipsilateral and contralateral acoustic reflex (AR) traces were obtained in each ear using a conventional 0.226 kHz probe tone. Pure tone AR elicitors at 0.5, 1, 2, and 4 kHz, with levels ranging from 80 to 100 dB HL, were assessed. Acoustic reflex decay was assessed using a 1 kHz contralateral 10 second tone presented at 10 dB above the level at which a 0.05 mmho response was obtained, or at 115 dB HL if a response of that level was not obtained at the highest acoustic reflex stimuli level (100 dB HL). Middle ear status was examined via conventional and wideband tympanometry (pressure sweep from +200 to -300 daPa), as well as wideband absorbance.

During experiment laboratory and range visits, changes in the ear's response to the probe signal were measured using the same series of broadband clicks discussed throughout the report. Briefly, broadband clicks with a passband from 0.2-8 kHz, presented at a 20 Hz repetition rate, were presented at 93 dB SPL as measured in an IEC-60318-4 occluded ear simulator.
Live fire participants were instructed to aim and fire on various areas of a standard 25-meter target using recorded target call instructions. The standard deviation of the RMS dBFS (dB Full Scale) target call values was 1 dB (range: 3 dB), which is reasonable given the small number of phonemes in each target call and interphonemic differences in level at a fixed vocal effort. The levels of the target calls varied over a 3-dB range due to differing phonemic content. The target calls were presented to participants at a comfortable loudness.

Instrumentation.

Simulated trigger / dry fire – University lab (ST/DF).

Participants completing the ST/DF study used the same instrumentation, described in Study 1 Instrumentation – University lab section, for the enrollment and experiment visits. The ST task used the same toy gun as in the UA task described in Study 1. The DF task used a single-shot 16-gauge shotgun that was disabled specifically for use in this study. The toy gun and disabled gun were mounted on a rail during the ST and DF task, respectively, and movement centered on rotation around a fulcrum defined by a yoke system (see Figure 14).

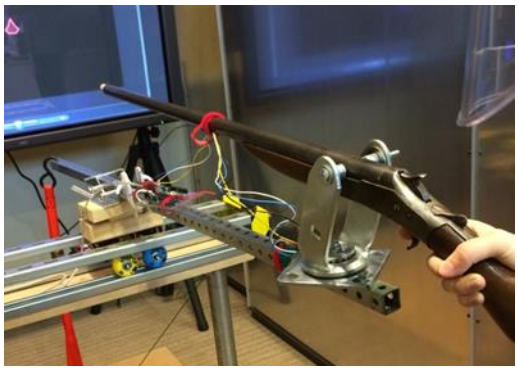

Figure 14. A photo of the distraction activity setup for the Dry Fire condition. The IMU is attached to the barrel. The FSR400 is attached to the gun trigger.

The ST and DF tasks involved the presence of a distractor activity, with the desire to control the level of attention to the task. The distractor activity was the same modified open source MATLAB-based video game activity used in the UA task. The distractor activity required the participant to track and trigger on a target moving randomly around a screen. The hardware used during this task included an IMU, with output routed to the Arduino Uno microprocessor and subsequent integration with the data from the NI modules. A force sensitive resistor (FSR400), attached to the trigger, was used to measure the force and timing associated with the trigger pull. The Delsys Bagnoli eight-channel electromyography (EMG) system, equipped with dry double-differential surface electrodes, was used to monitor the activity of the flexor digitorum superficialis (the largest muscle on the anterior forearm, used to flex the fingers),

which is activated during triggering. A field microphone (G.R.A.S. type 40AC) was used to record ambient noise levels and to determine trigger pull timing in conjunction with the FSR400 and the flexor digitorum superficialis muscle (FDS) EMG recordings.

Figure 5 is a screenshot of the distractor activity. The red marker is the target which randomly moves about the screen and the blue marker is the location of the gun barrel based on the IMU output. Greater shooting accuracy increases the speed of the red target and less accuracy decreases the target speed. Figure 15 displays the EMG recording (upper panel) and simultaneous acoustic response (lower panel), measured using the free field microphone, when pulling the toy gun trigger.

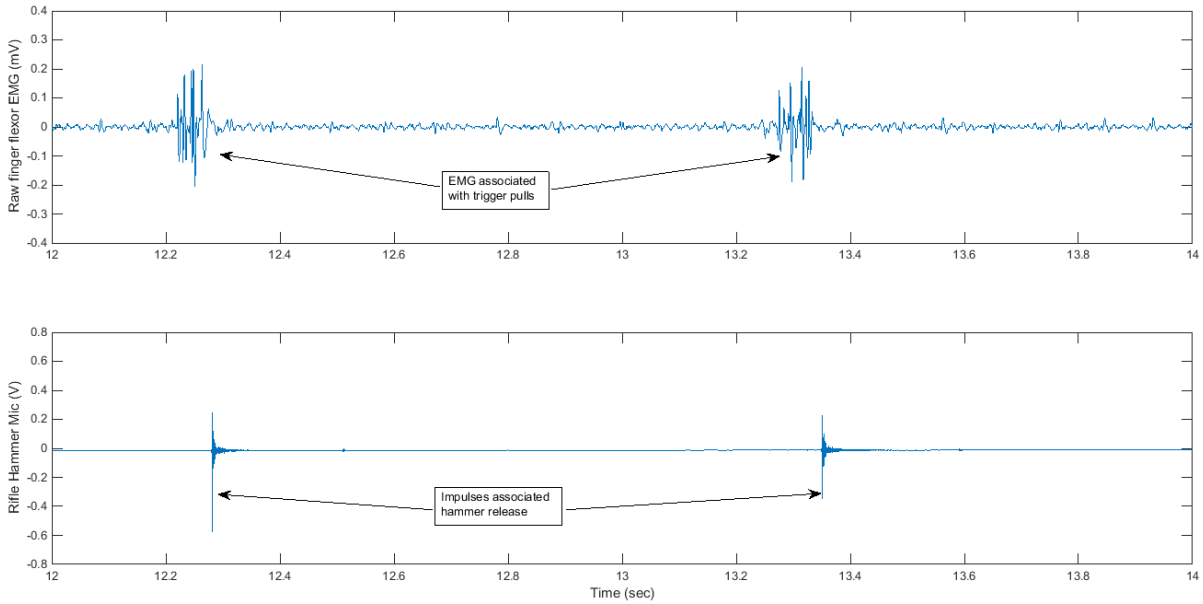

Figure 15. The upper plot represents an EMG recording associated with trigger pulls using a toy gun, and the lower plot represents the associated acoustic signal generated by the hammer release.

Simulated shooter / simulated spotter – Military lab (SH/SP).

The enrollment visit instrumentation was installed inside a double-walled, audiometric sound booth (Internal Dimensions: 4' x 5'), interfacing with a PC (Dell Precision™ tower 5810) installed at a control station outside of the booth. Data collection was controlled by a custom MATLAB script running on the control PC that interfaced with the experiment hardware and ensured consistent study procedures. Otoscopic examination and recording was conducted using a Welch-Allyn Digital MacroView video otoscope. Audiometric stimuli were delivered using the Nelson Acoustics ART automatic audiometry software utilizing NI Hybrid PXI/PXIe-4461 modules, with air conduction signals presented via Sennheiser HDA-200 circumaural headphones. Participant responses were tracked using a VIacoustics response switch box. Middle ear assessments were conducted using the Interacoustics Titan middle ear analyzer.

The experiment data collection visit occurred in the military lab indoor air rifle range, and instrumentation was controlled by a PC (Dell Precision™ Tower 7910) running MATLAB custom scripts. Data acquisition was performed via an NI PXI/PXIe system. Sound stimuli were digitally generated in MATLAB, and output via an NI PXI-4461 module. Analog inputs were recorded via an NI PXI-4498 module. A Tucker-Davis Technologies Real-Time Processor (TDT RP2.1) was used to deliver a recorded gunshot to the ear following trigger release on a simulated weapon.

Shooter (SH) participants were asked to aim and trigger on a pellet-range target using a carbine system from an Engagement Skills Trainer (EST2000). The EST2000 is an indoor small arms training simulator, employed in U.S. Army marksmanship training protocols (U.S. Department of the Army, 2011, 2012). The EST2000 M-4 carbine system (Figure 166) used in this study simulates the actual weight and recoil of a functional M-4 carbine, providing additional generalizability to range settings, but in a minimal risk laboratory setting. An FSR400 was used to monitor trigger force and timing. A Delsys Bagnoli® 8-channel electromyography system equipped with dry single-differential surface electrodes was used to monitor the activity of selected muscle groups.

Middle ear muscle activity was monitored via the ER-10X otoacoustic emissions system and disposable multi-lumen probe-tubes to allow the microphone and stimulus ports to align flush with the end of the eartip. The probe was designed to couple to the ear canal with a soft rubber ear tip; however, adapting a foam otoacoustic emissions eartip produced more stable placement in the ear canal, and more reliable results. The primary driver of the ER-10X was an analog output of the NI PXI-4461, and the microphone channel was recorded by an input on the NI PXI-4498. The ER-4PT high-output commercial insert earphone was used to present recorded gunshot stimuli as determined using the TDT RP2.1, during the SH/SP tasks.

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Figure 16. Custom hardware installed on a cap gun (used in another part of this study, results not discussed here), and carbine simulator (from the EST2000 system) used during the SH/SP tasks.

Live fire – active / live fire – waiting (LA/LW).

The military lab LA/LW enrollment visit instrumentation was unchanged from the military lab SH/SP enrollment visit instrumentation described above. An audiometric trailer (Figure 17) was stationed onsite at the range during the entirety of live fire LA/LW data collection (*Figure 18*). Equipment was powered by a Whisperwatt diesel powered AC Generator (approximately 65dB(A) at 7 meters), stationed behind the audiometric trailer to provide a noise barrier, and consistency of power was monitored and controlled via an Uninterruptible Power Supply. The range instrumentation was controlled using a Windows-based PC workstation (Dell model 7910) connected to an NI PXIe-1082 chassis and an NI PXIe-1062Q chassis. National Instrument PXI/PXIe (hybrid) 4461 dynamic signal analyzers, PXIe-4499 modules, NI M Series Multifunction DAQ (PXI-6220 or 6221), and PXIe-8360 MXI-Express (2) interface modules were used to produce probe clicks and simultaneously sample all input channels during live fire activities.

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Figure 17. The audiometric trailer, generator, and storage box at the unoccupied firing range.

Otoscopic inspection and recording was obtained via the Welch-Allyn Digital Macroview video otoscope, and the Interacoustics Titan middle ear analyzer was used to perform an abbreviated middle ear test battery. Audiometric stimuli were delivered via the Nelson Acoustics Audiometric Research Tool automatic audiometry software utilizing the PXIe-4461 modules. The signal was routed to a Sennheiser HDA-200 circumaural earphone, and participant responses were tracked via hand switch. Most of the instrumentation required for data collection (e.g., computers, data acquisition chassis and modules) was secured within the audiometric trailer to minimize the probability of damage and simplify logistics.

The remaining instrumentation was placed under canopies erected daily (*Figure 18*), and signals were transmitted into the data acquisition system using cables routed from the front of the audiometric trailer to the firing stations. The ER-10X otoacoustic emissions system was used to present click stimuli into the monitored ear and to transduce the signal in the ear canal. The Delsys Bagnoli 8-channel EMG system, equipped with dry single-differential surface electrodes, was used to monitor the activity of selected muscles. The ER-4PT high-output commercial insert earphone was used to deliver instructions and communication to each participant. Field microphones were used to monitor ambient noise and timing of each shot fired. An FSR400 was fitted to the trigger of each participant's M4 to monitor trigger pressure and timing.

Figure 18. Live fire staging area during trial equipment set up. (Left) Side view (Right) View looking down range.

Figure 19 and Figure 20 demonstrate two examples of simultaneous ear canal recordings and field microphone measurements, and Figure 21 shows simultaneous trigger force, orbicularis oculi (OO) activity, and field microphone measurements. Much of the instrumentation in the LA/LW study (e.g., audiometric headphones and hand switches, EMG systems, ER-4PT and ER-10X, FSR400) required duplication in order to allow for simultaneous data collection of both participants.

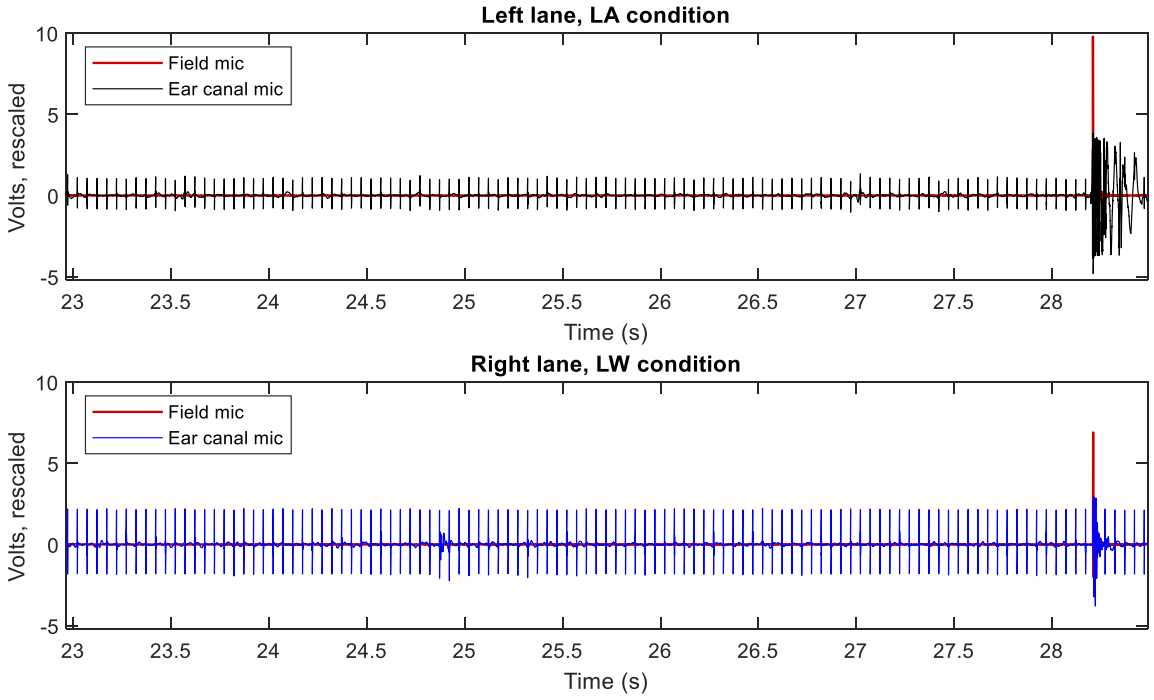

Figure 19. Example of field microphone and ear canal recordings. Participant in left lane active (LA); right lane waiting (LW). The red trace represents the field microphone signal nearest the selected firing lane. The black trace represents the recording taken from the participant in the left firing lane. The blue trace represents the recording taken from the participant in the right firing lane. Click signals (repeated every 50 ms) appear as vertical bars in the black and blue traces.

The gunshot noise appears as the spike in the red traces (Time approximately 28.25 s). The disturbance in ear canal recordings following the arrival of the gunshot indicates overload of the ER-10X system and reflection of the gunshot in the ear canal.

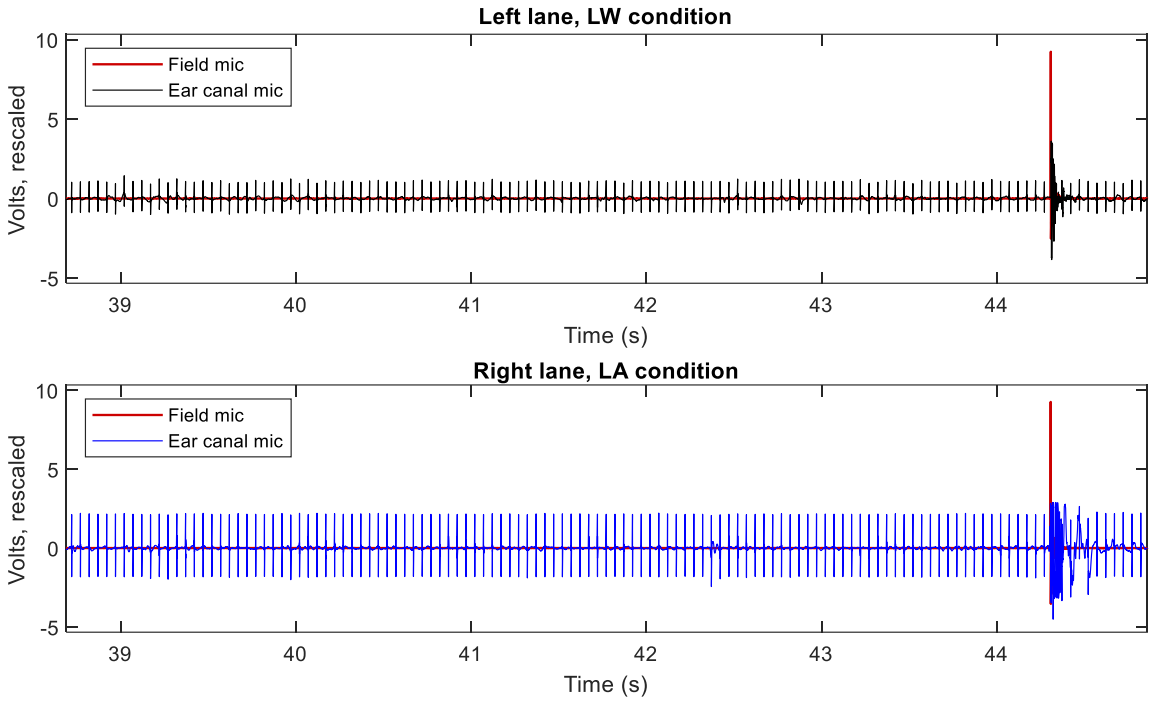

Figure 20. Example of field microphone and ear canal recordings. Participant in left lane waiting (LW); right lane active (LA). Details similar to preceding figure.

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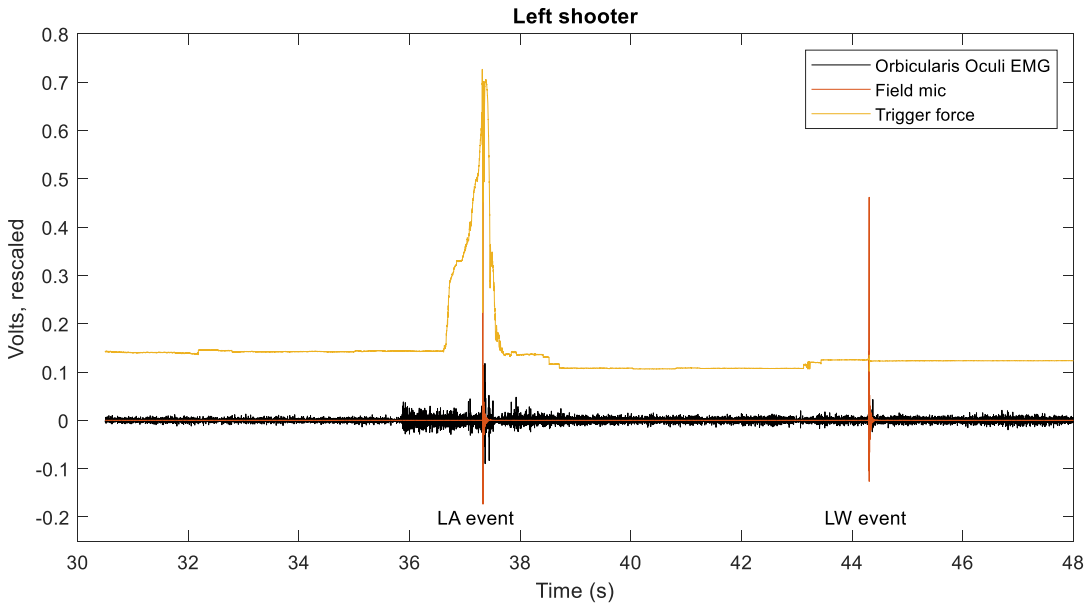

Figure 21. Example of consecutive LA and LW events from the same participant. The black trace represents the EMG signal from the orbicularis oculi (OO) muscle. The red trace represents the waveform recorded at the field microphone. The yellow trace represents the force applied to the gun trigger by the participant. The LA event region begins with activation of the OO muscle, followed by increasing trigger force leading to discharge, which is detected using the field microphone. The LW event region shows only the discharge of the weapon operated by the right shooter, with no significant change in OO EMG or trigger force during the event period, except a small startle response on the OO muscle immediately following blast arrival. Participants fired a standard M4 (5.56 x 45 NATO) service carbine with standard (M193 ball) ammunition on a 25-meter firing range (Figure 22).

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Figure 22. Participants in left and right shooter positions, supervised by the Range Safety Officer.

Calibration procedure.

Western Michigan University lab.

All acoustic stimuli and transducers were calibrated and the function of all other devices (buttons, sensors, etc.) was confirmed at the beginning and end of each test day. Calibration procedures conducted at the university lab were described in Study 1 Calibration procedure section above.

Fort Rucker, USAARL Military lab SH/SP.

Acoustic stimuli were calibrated at the beginning and end of each test day. Calibration procedures were executed using MATLAB software developed at the University lab and modified slightly for the military lab. Instrumentation used for daily calibration procedures included a G.R.A.S. type 43AA ear simulator (IEC 60318-1), Bruel &Kjaer Nexus microphone power supply, utilizing narrowband noise stimuli .WAV files (51.2 kHz sampling rate).

Fort Rucker, USAARL Military lab LA/LW.

Acoustic stimuli were calibrated at the beginning and end of each test day. Calibration procedures were executed using MATLAB software. Instrumentation used for daily calibration procedures included a G.R.A.S. type 43AA ear simulator (IEC 60318-1), GRAS 12AA power supply, a B&K Type 4231 calibrator, and a G.R.A.S. Type 42AP 0.25 kHz pistonphone calibrator was used as a central calibration reference for the military lab study conditions.

Data collection procedure.

Simulated trigger/dry fire – University lab.

The general data collection procedure for participants completing the ST or DF task was outlined in Study 1, Figure 7. Participants were asked to complete an enrollment visit, and if eligible, were invited to complete an experiment visit. Participants who were regular firearm users (i.e., who had fired at least 10 rounds within 30 days of the enrollment visit) completed either the ST or the DF conditioned task (i.e., Middle Ear Test Part II in Figure 7). However, unlike AA, UA, and AV, where conditioning procedures were conducted as part of the protocol, the ST and DF tasks did not include conditioning procedures. Regular firearm users were assumed to have been previously conditioned during the act of firing their own weapons within the 30 days prior to their enrollment visit.

Participants were asked to aim and trigger either a toy gun (ST) or a modified gun (DF, Figure 14), on the same moving targets (Figure 5) involved in the Study 1 distractor activity. The procedure duration was set at 250 seconds for both tasks, and resulted in a variable number of shots based on participant triggering pace. Increased targeting accuracy resulted in increased target speed and difficulty. Study staff were responsible for resetting the DF single shot weapon between shots. The toy gun used in the ST task was able to continuously trigger, and participants were asked to pause between shots to allow for an adequate baseline between shots. During these tasks, only the probe clicks were presented in the participant's test ear. Participants wore the ER4PT in the ear contralateral to the test ear, although no stimulus was presented via the ER4PT during these tasks.

The onset of the CS was assumed to be embedded in the process of firing a weapon although, as previously discussed, this likely varies by participant based on their prior experience with firearms, training, and individual differences. The onset of triggering was determined by the participant and measured by tracking the force applied to the trigger on the toy gun or modified gun, EMG activity on the flexor digitorum superficialis (FDS) muscle, and the output of a field microphone recording of the impact of the trigger mechanism upon completion of the trigger action. The trigger action timing was easily identified from the output of the FSR400, field microphone recordings of the hammer impact, and recordings of EMG activity of the FDS muscle.

Simulated shooter/simulated spotter – Military lab.

The SH/SP data collection procedure (Figure *23*) was similar to the Study 1 procedure, with participants completing an enrollment visit and, if eligible, an invitation to complete an experiment visit. The SH/SP participants did not complete bone conduction threshold testing, and completed acoustic reflex decay testing in the ipsilateral ear (participants completing AV, AA, UA, ST, DF, and LA/LW completed contralateral acoustic reflex decay testing). There were additional differences in signal types and levels, as discussed in the Stimuli section. Reflexive tasks were conducted during the experimental visit as potential covariates in the event of substantial eMEMC responses, but are not discussed in this report. Compensation was provided only to participants volunteering during off-duty periods.

During the experiment visit, participants were asked to complete a set of tasks involving the act of triggering on a target indoors and subsequently acting as spotter while a study staff member released the trigger while pointing the simulated weapon downrange. The EST2000 M4 weapon was used during both portions of the procedure. Similar to the ST/DF tasks, each procedure was set to 250 seconds. Additionally, the act of conditioning was not completed during the experiment visit and any conditioning of an eMEMC was assumed to have occurred during prior firearm use. The ear contralateral to the test ear was presented with the recorded gunshot following the trigger pull, and changes in response to the probe signal were measured in the test ear.

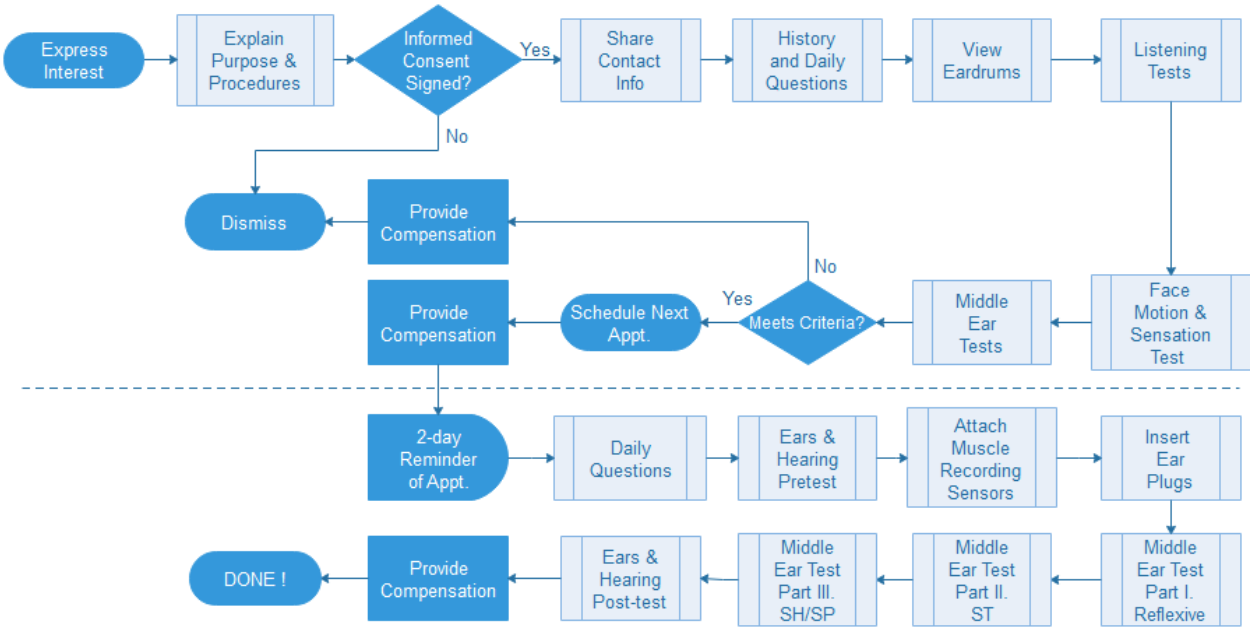

Figure 23. The SH/SP data collection procedure.

Live fire – active/live fire – waiting – Military lab.

Participants volunteering for the LA/LW study were active-duty Soldiers who were invited to complete three visits (Figure *24*). The enrollment visit, completed to determine eligibility (Table 3), was similar to Study 1 enrollment visit details, including otoscopic examination, pure tone air conduction threshold testing, cranial nerve V and VII screening, middle ear test battery, and questionnaires. Pure tone air conduction thresholds from 0.125 to 8 kHz, including inter-octave frequencies of 3 and 6 kHz, were obtained via the same modified Hughson-Westlake procedure (Carhart & Jerger, 1959). The laboratory experiment included an examination of acoustic and non-acoustic rMEMCs, included as potential covariates in the event of substantial eMEMC responses, and details are not discussed in the current report. The range experiment visit, examining conditioned eMEMCs, is described below. The laboratory and range experiment visits both began and ended with an examination including otoscopic video recording, air conduction thresholds, and an abbreviated middle ear battery (i.e., conventional and wideband tympanometry and wideband absorbance at ambient pressure). Participants always

completed the enrollment visit first, but the remaining two visits were not always done in the same order. Compensation was only provided to participants volunteering during off-duty hours.

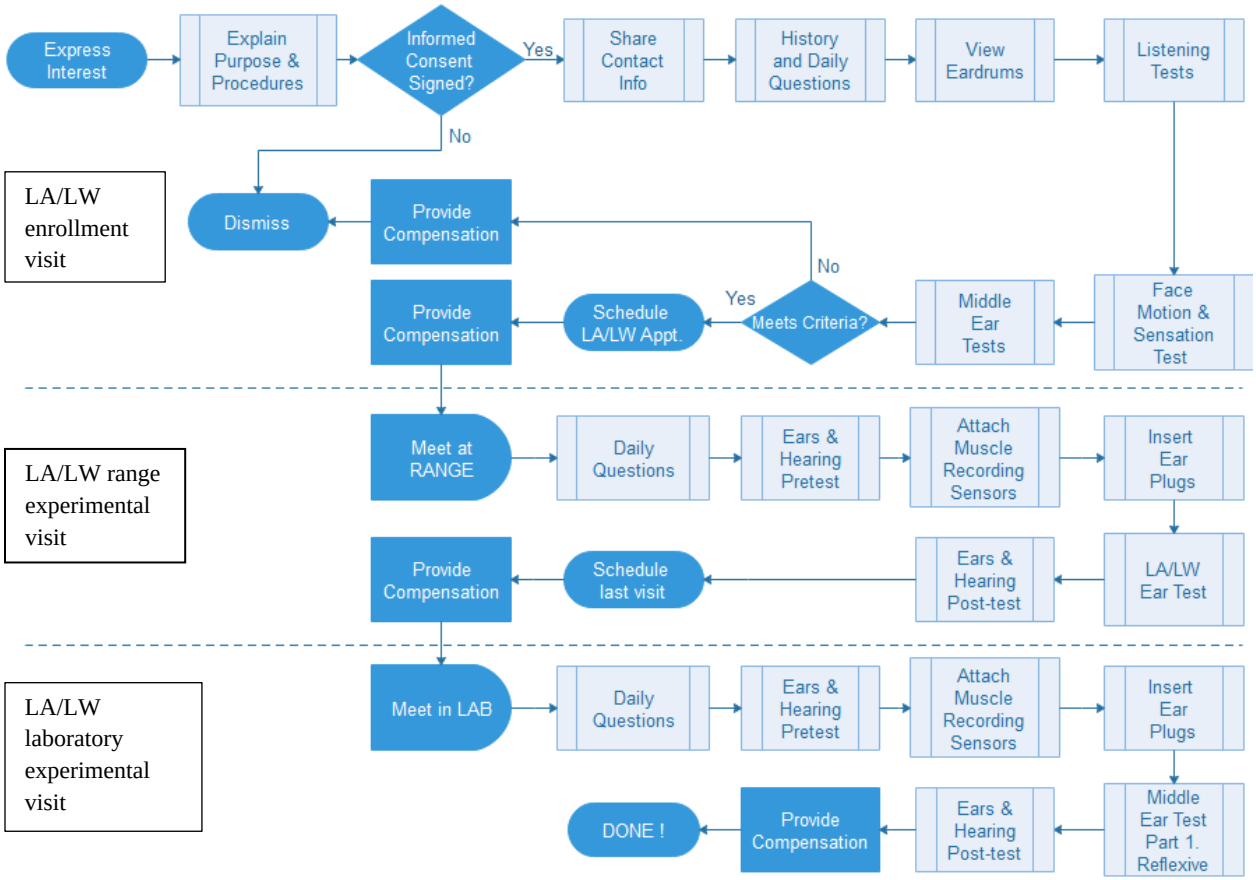

Figure 24. The LA/LW data collection procedure is similar to the university lab conditioned eMEMC procedure for two visits (enrollment visit and laboratory experimental visit). Differences include an additional visit (range experimental visit), where live fire activities were completed.

The final set of conditioned tasks assessing the presence of eMEMCs involved simultaneous data collection from two participants using M4 military weapons on a military firing range. Participants engaged in an active firing (LA) and waiting (LW) pattern, wherein one participant actively triggered on a target while the other participant waited and vice versa. Data collection for the LA/LW tasks was conducted near firing lane 38 at the Ft. Rucker, Alabama, North Range (Figure 17 and Figure 18). Pre- and post-testing and set-up occurred in an audiometric trailer that was on site for the duration of data collection. A number of military support personnel were on site during data collection (e.g., Officer in Charge, Range Safety Officer, ammunition and weapons handlers, combat life savers). The Officer in Charge had range authority at all times that weapons and ammunition were present. The Range Safety Officer monitored the participants, simulated tower instructions to the participants, and responded according to standard procedures in the event of a jammed weapon or other malfunction. Each shot series was initiated on the command of the Range Safety Officer.

Data collection for the LA/LW range task was divided into four series with six shots each, three shots fired by each participant during a series. Participants were provided with the M4 carbine and 3 rounds of ammunition per recording interval, and loaded the magazines with live rounds into their M4 at the beginning of each shot series. Participants were in prone position in neighboring firing lanes. A frame with an opaque fabric was located between the participants to block visual contact (see Figure 22). Participants attended to standard M16-A1 25-meter targets (Figure 25) downrange while awaiting simulated tower instructions played into an earphone in their non-test ear (Figure 26). The ER-10x probe assembly was placed in the test ear, which was contralateral to the shoulder against which the weapon rested.

In order to stagger the order of fire, each participant received firing instructions separately, permitting the assessment of eMEMCs for the participant actively firing their weapon (LA) and the participant waiting to be instructed to fire their weapon (LW). The LA and LW conditions parallel to the Warned and Unwarned conditions implemented in the AHAAH damage-risk criterion. Shooting order, target and distance (e.g., Left100, Center100, Right100), and the gap between shots were randomly chosen. The minimum gap interval between instructions was 2 seconds. The initial shot was preceded by a Ready command and a 6-second gap to permit two probe sweeps confirming ER-10X probe function. Data were monitored visually during acquisition and swept-sine measurements of the ER-10X transducers were conducted before and after each shot series. The final 3-second gap was followed by a notification that the series was over.

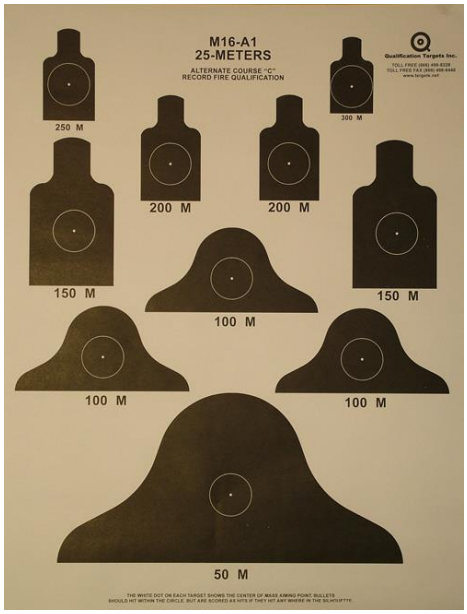

Figure 25. Standard 25-meter targets placed downrange from participants during LA/LW activities.

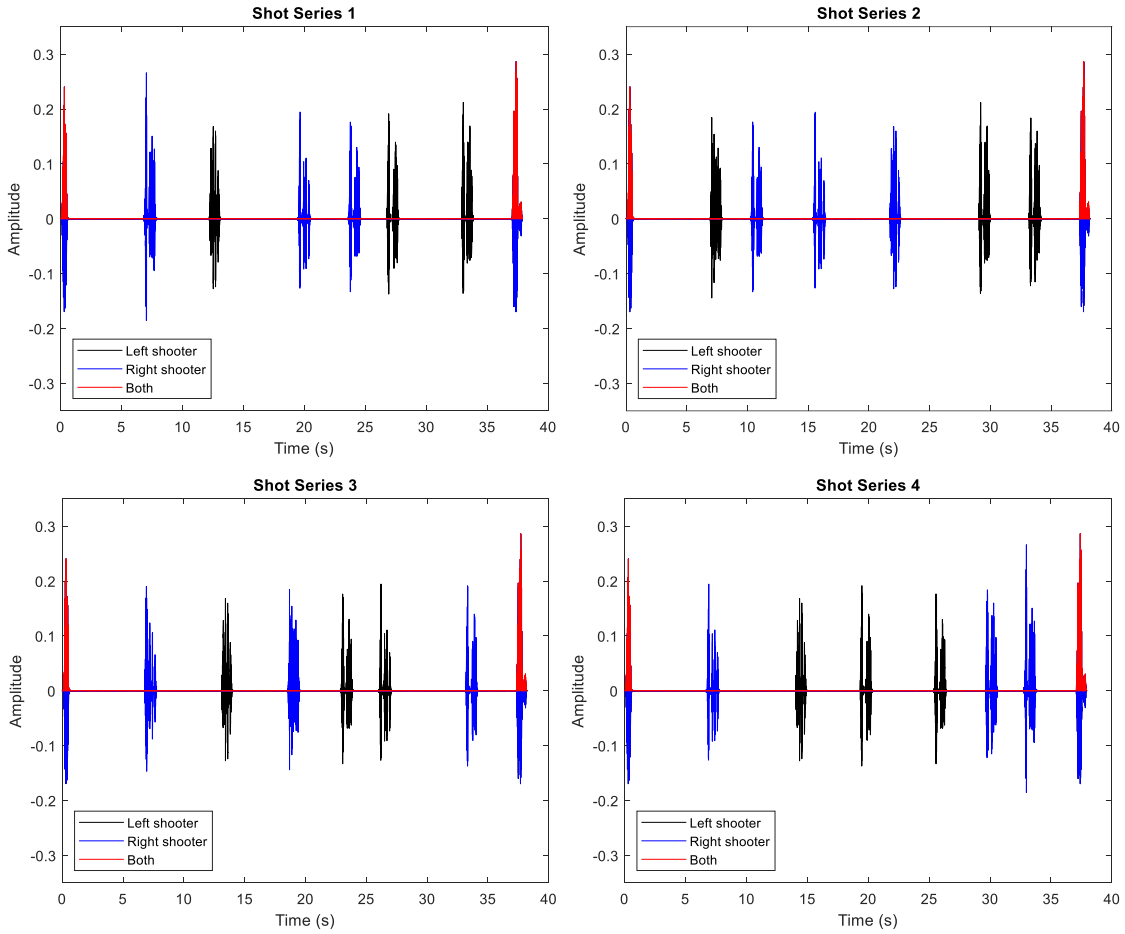

Figure 26. Time-staggered simulated tower instructions were presented to each participant in the ear contralateral to their test ear. Black traces represent the signal routed to the left shooter's ER-4PT, blue traces represent the signal routed to the right shooter's ER-4PT, and the red trace represents the minimum absolute value of both channels, which indicates the signals routed to both shooters (i.e., the Ready and Relax commands).

Data management and analyses.

Multiple levels of data review and analysis were completed using custom MATLAB functions and Stata statistical software. The stimulus and method used to detect the presence of an eMEMC was identical to the process described in Study 1 (eMEMC probe stimulus and Evaluation of eMEMCs sections). The significant difference between Study 1 and Study 2 procedures was the timing of events, as Study 1 timing was controlled by study protocol and automated scripts. The timing of events in Study 2, although grossly controlled by study staff, was largely dictated by study participants. The timing of baseline intervals and presentation of any UCS (i.e., gunfire) was determined using a combination of ambient microphone recordings (i.e., precise timing of gunshot), EMG recording of the FDS muscle (i.e., trigger pull finger muscle measurement), and the FSR400 recording (i.e., force of the finger on the trigger pull and release). The combination of these three measurements provided a precise timeline for measuring changes in the response of the probe signal during baseline and non-baseline periods. The period prior to firing of the weapon was examined in Study 2, and the precise period of time examined

varied by task, as described below. The presence of an rMEMC in response to the UCS (i.e., gunfire) was not examined, as that does not indicate presence of an eMEMC response and because the test ear measurement equipment was typically infiltrated with the gunfire signal resulting in inaccurate measurements after the weapon was fired.

Simulated trigger/dry fire – University and simulated shooter/simulated spotter – Military.

As mentioned in Study 1, the eMEMC detection paradigm was based on changes in acoustic energy in the ear canal during baseline and CS or UCS intervals. Those changes could occur due to a change in middle ear admittance or a change in the noise (i.e., the toy gun or disabled gun hammer impact) infiltrating the ER-10x probe. According to the time constants of the AHAAH Warned MEMC, the eMEMC onset must occur within, or earlier than, the -100 to -50 ms stairstep in order to reach maximum magnitude just prior to the stimulus onset (Figure 27). The sync error allowance accounts for individual variations, operational circumstances, and a slower eMEMC time course (Jones et al., 2018; Repp, 2005) During these analyses, some stairsteps appeared to rise at the –50 to 0 ms (re: trigger release) stairstep, which is likely associated with the infiltration of noise into the prior click interval.

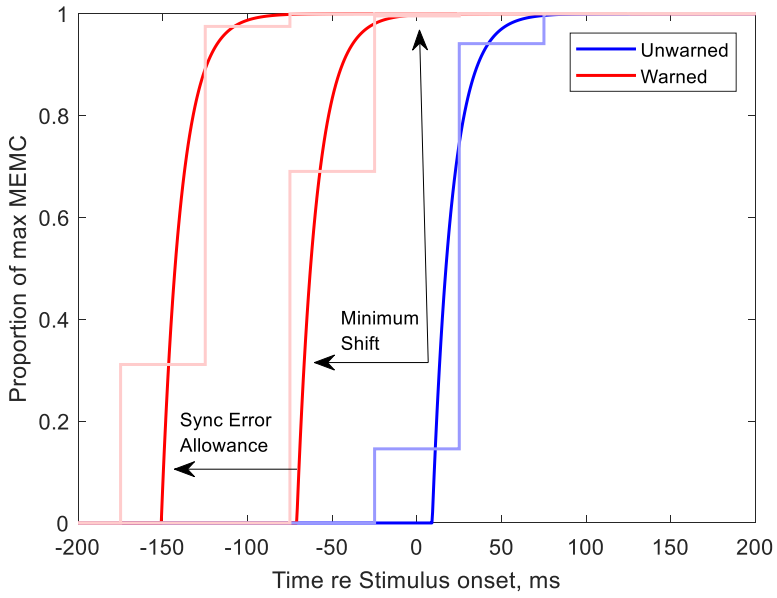

Figure 27. In AHAAH, the unwarned MEMC (blue) is expected to follow the onset of the impulse by 9 ms and then rise to a maximum within about 50 ms, with a morphology similar to a capacitive system that follows time constant of 11.7 ms. The warned MEMC (red) follows the same parameters as the unwarned response, with maximum MEMC occurring prior to stimulus onset (Price, 2007).

An eMEMC would be represented by a change in the energy developed in the ear canal prior to the arrival of the impact noise. Thus, one would expect to observe a reliable change from

baseline prior to the stairstep including time=0 (dashed line in Figure 28). The interval from about -200 to -100 ms would be a reasonable time to see this. Figure 28 shows inconsistent trial level response changes (colored lines) during the -200 to -100 ms time window, which return toward baseline prior to the onset of the elicitor and are not seen when examining the 25th percentile of response changes (bold black line). It is also reasonable to expect that the ear canal response would be time-linked with (and possibly precede) the increase in trigger force. Figure 29 shows triggering force increase beginning at -450 ms, with no consistent increase or change in RMS ear canal activity occurring before or during triggering activity.

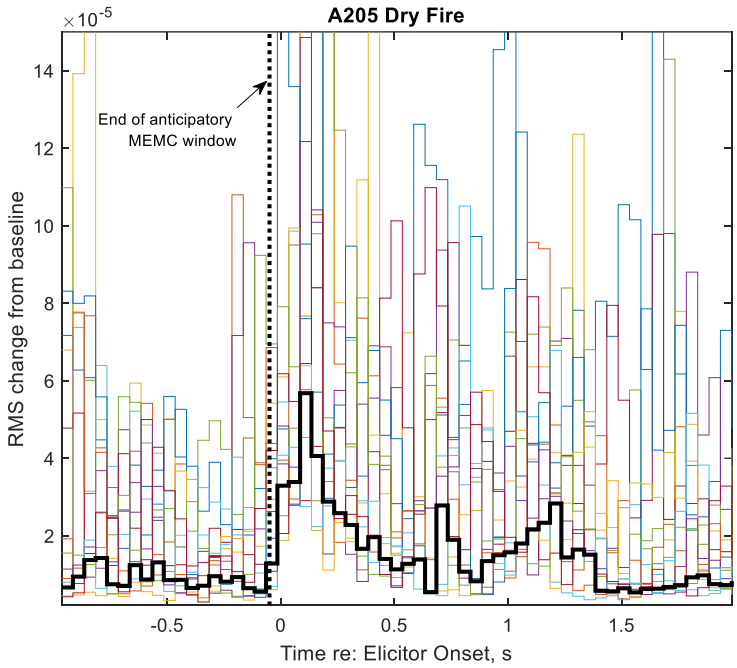

Figure 28. Example of conditioned eMEMC outcome for one participant assigned to the dry fire (DF) task. Thin non-black stairstep functions represent individual trials (i.e., trigger pulls on the disabled gun) and the thick black step function represents the 25th percentile of the distribution within each 50 ms interval. The vertical dotted line represents the earliest onset of the click interval associated with any trial. The deflection of the 25th percentile after the vertical dotted line cannot be interpreted easily because it could represent an rMEMC, infiltration of the impact noise from the disabled gun hammer, or a combination of both. An upward deflection of the 25th percentile prior to the end of the anticipatory eMEMC window would be consistent with an anticipatory eMEMC. Evidence of an anticipatory eMEMC was not seen in this example.

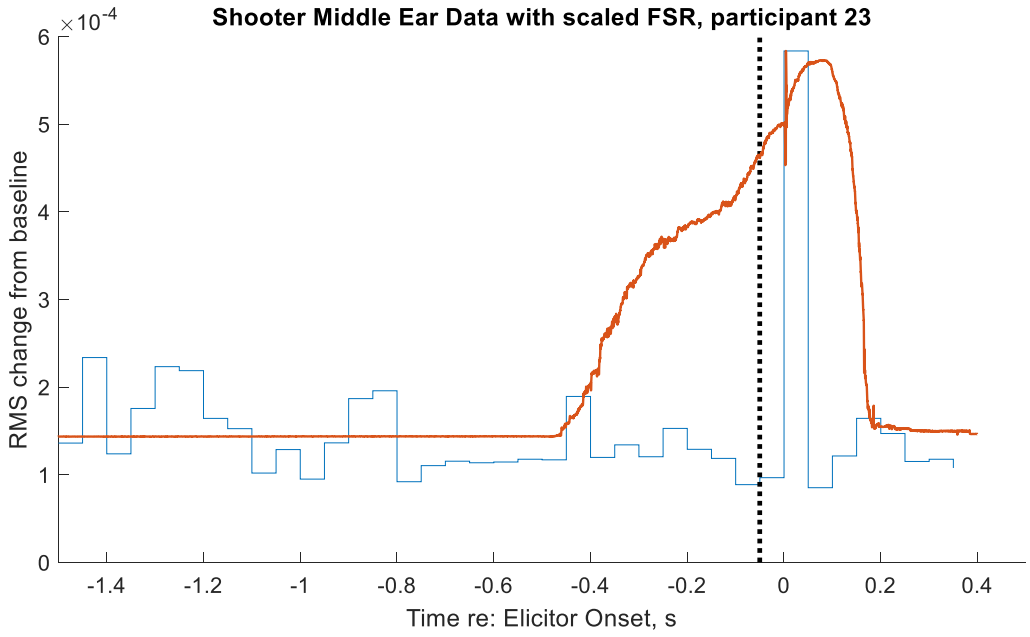

Figure 29. Example of 25th percentile RMS differences from baseline for participant 23 during the simulated shooter (SH) task with participant ear canal activity (blue) and scaled trigger force activity measured using the FSR400 (red). Trigger force begins to increase approximately 0.45 seconds prior to trigger pull and hammer fall, shown as the red notch at time=0. Ear canal activity remains unchanged from baseline prior to time=0, and the change occurring during the trigger pull/hammer fall may be an rMEMC (UCR), but more likely demonstrates infiltration of the hammer fall signal into the measurement equipment. The acoustic gunfire recording playback occurred 62 ms after time=0, and would not influence the RMS change occurring between 0 and 50 ms.

As in Study 1, rater judgments in ST, DF, and SH/SP were based on the relaxed eMEMC definition. Any stimulus-linked eMEMC response was considered, regardless of potential protection at the time of UCS arrival. Proportions of eMEMC in these analyses are also likely overestimates of the presence of protective eMEMCs.

Live fire – active/live fire – waiting interim data analyses.

The protocol for the LA/LW study involved participants discharging a service weapon (M4 carbine) loaded with live ammunition, and this task carried greater than minimal risk to the research participants. In a study that poses greater than minimal risk to the participants, it is in the participant's best interest to stop enrollment if the value of additional data is outweighed by the risk to the participants. Continued data collection would be futile. For example, additional data collection would be futile if it were known with reasonable confidence (e.g., 95%) that the prevalence of an eMEMC is less than 95%. This criterion differs from the pervasiveness criterion because it is determined by whether the upper boundary of the 90% confidence interval exceeds a proportion of 0.95. If the upper boundary is below 0.95, the probability is less than 5% that the population parameter falls at or above 0.95, and therefore additional exposures to participants would be unwarranted in 19 of 20 cases.

In an interim review plan, it was determined that periodic data review would take place, including the identification of eMEMC for each participant. Interim evaluations of the percentage of participants exhibiting eMEMC were to be examined first at a sample size of 10 participants and each time data review had been completed with an additional 6 participants since the prior interim analysis. For the first review (N = 10), a decision to halt data collection would be made if no participant exhibited an eMEMC. Other minimum counts and minimum percentages, assuming fixed 6-participant intervals, are presented in Table 17.

Beginning with the interim analysis of 16 or more participants, the end of data collection would be initiated if it was known with greater than 95% confidence that fewer than 95% of the sample population exhibited eMEMCs (i.e., data collection would be stopped once it was known that the upper limit of the 90% confidence interval for the proportion of eMEMCs was less than 95%).

The protocol for the end of data collection was as follows:

1. Participants not scheduled for live fire data collection were notified that data collection had been postponed.
2. Participants currently scheduled for live fire data collection were asked for an in-person meeting at the Fort Rucker, USAARL. During this meeting, participants were notified that previously-collected data have provided an answer to the primary research question and that any data they might contribute would provide only supplementary information. Participants were asked to provide an informed decision about whether to continue their involvement in the study. Only those participants re-affirming informed consent to participate were permitted to complete live fire data collection.
3. Potential participants (i.e., those who had expressed interest but who had not provided informed consent to participate) were notified that enrollment had been postponed.
4. Future potential participants expressing an interest in the study were notified that the study was no longer in active data collection.
5. Participants who had completed live fire data collection but had not yet completed the laboratory experiment visit would complete this visit according to the ordinary protocol.

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Table 17. Minimum eMEMC Counts and Percentages for Continued Participant Enrollment in the Less Than Minimal Risk Portion of the Study at Planned Interim Analysis Points

N	Minimum eMEMC Count	Minimum eMEMC Percent
16	14	87.5
22	19	86.4
28	25	89.3
34	30	88.2
40	36	90.0
46	41	89.1
52	47	90.4
58	52	89.7

Live fire – active/live fire – waiting eMEMC detection approaches.

The principal question in the LA/LW study was the detection of an eMEMC. The sound generated by the gunshot was sufficiently intense that it infiltrated the hearing protector and acoustic probe assembly. The signal passed through the system was not a cause for concern for the hearing of the participant, but it was sufficiently intense to interfere with the measurement of middle ear status. The exact onset of each gunshot was determined by the shooter, which meant that the arrival of the gunshot noise was not synchronous with the 50 ms probe click intervals. As expected, the implication of this timing was that changes in the ear canal signal could not be interpreted after the –50 ms interval (re: trigger release).

The LA/LW results were examined according to three different approaches. This was done to strengthen the ability to detect an eMEMC upon interim analyses, and because the exact mechanisms of conditioned or anticipatory eMEMC are largely speculative. The eMEMC definition applied to the LA and LW tasks required that disturbances in the 25th percentile RMS difference traces persist or grow until the onset of the UCS (i.e., rigid definition). The relaxed eMEMC definition used on tasks that did not involve discharging live ammunition (AV, AA, UA, ST, DF, SH, SP) was justified because it was plausible that a CR might have been interrupted due to the participant's knowledge of the task (e.g., knowledge that combustion was impossible with a disabled weapon). Participants in the LA and LW tasks were active-duty Service Members and were therefore intimately familiar with the M4 carbine. Participants also knew they were discharging live ammunition, therefore there was no reason to expect that an eMEMC would be inhibited. It would also have been misleading to describe a response that decayed to baseline prior to the arrival of a potentially hazardous impulse as conveying any protection to the ear.

Routine window approach.

First, raters independently identified the presence of a significant change in RMS differences within the standard window while using the rigid eMEMC definition (Figure 30). The change under evaluation was the 25th percentile RMS differences across all LA or LW conditions for a given participant. This method was designed to be sensitive to consistent changes in the middle ear response to the probe signals during the last 1.5 seconds leading up to the arrival of the gunshot noise.

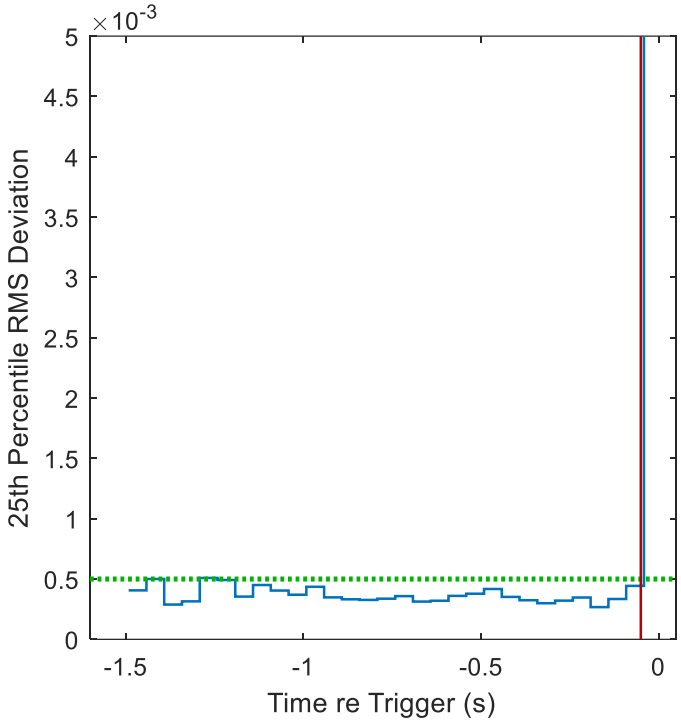

Figure 30. Example of a routine representation of the ear canal trace leading up to the arrival of the gunshot noise. The participant in this example was firing the weapon. Time relative to the arrival of the gunshot is represented on the horizontal axis. The 25th percentile deviation from the baseline click is represented in on the vertical axis. The red vertical line represents the time after which the trace is likely to be affected by the noise of the gunshot. The green dotted line represents the upper bound of the confidence interval of RMS differences between the times of -1500 and -500 ms (-1.5 to -0.5 seconds) prior to the arrival of the gunshot noise. No evidence of an eMEMC is shown in this figure.

Extended window approach.

The developers of the AHAAH have implemented an eMEMC that reaches maximum strength prior to the arrival of the gunshot noise, but no clear statement has been made by the developers about the expected onset time for this eMEMC. The time constant for the eMEMC implemented in the AHAAH model implies that the contraction would develop over a 60-80 ms interval, and it has been demonstrated that the time constant assumed in the AHAAH model is unrealistically fast, based on laser doppler measurements of reflexive and early MEMCs (Jones

et al., 2018). Taken together, these data suggest that an eMEMC could begin at least 50 ms, and more likely 150 ms before the arrival of the gunshot impulse (Figure 27*).* The latest possible MEMC onset must occur during the -100 to -50 ms time window in order to reach the maximum contraction just prior to stimulus onset. A sync error allowance was included as it would be reasonable to expect the accuracy of the onset to vary depending on the individual, operational circumstances, the CS, and human limitations in sensorimotor synchronization (Repp, 2005).

Direct supporting evidence for the timing would be contingent on the observation of eMEMC in a sufficiently large number of samples to permit generalization. The purpose of this study was to determine whether such a response could be found, so a situation existed wherein a precise expectation could not be developed without first having observed the phenomenon. In recognition that the eMEMC might be initiated earlier than 1.5 seconds (1500 ms) before the gun was fired, we also examined the ear canal signals over the maximum time frame - beginning shortly after the end of the prior discharge, or the start of the recording for the first shot (Figure 31). Note that the varying intervals between shots lead to sparse information for the earliest time points in the figure and increasing information (and trace stability) with time.

Judgments of the presence of eMEMCs over the extended time window were made by three investigators (authors GAF, SMT, KKD) for all LALW participants. The order of presentation across shots and shooter versus non-shooter status was randomized separately for each rater, and raters were not aware of the judgments of other raters when making their judgments.

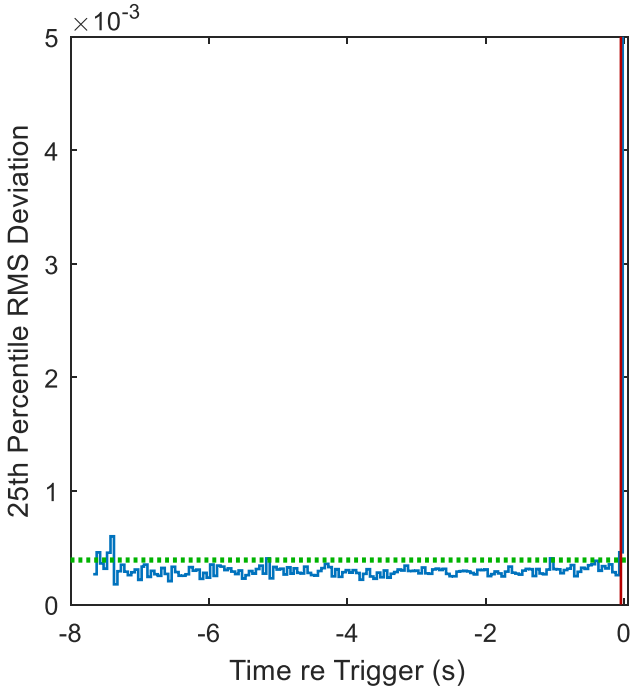

Figure 31. Example of the ear canal trace over an extended time window leading up to the gunshot. The analysis windows began shortly after the prior shot. Figure details similar to the prior figure. This figure provides no evidence of an eMEMC in the seconds prior to the arrival of the gunshot noise.

EMG-adjusted approach.

Finally, we considered the possibility that concomitant/incidental motor activity might mask the existence of an eMEMC. A multivariable regression approach was used to control the influence of concomitant/incidental muscle activity, as measured by EMG. The regression model extracts from the RMS differences the influence of this muscle activity, leaving a trace that would contain any evidence of an eMEMC embedded in the vector of residuals. Unlike the prior two analysis methods, which relied on a percentile of the distribution of RMS differences across shots, this approach can be applied at the level of individual shots, thus making it consistent with the time frame of DRCs assuming that each imminent impulse produces an eMEMC. An example of the EMG-adjusted trace for a single shot from one discharge of a shooter is represented in Figure 32. In this example, there was no evidence of a change in the response prior to the arrival of the gunshot noise.

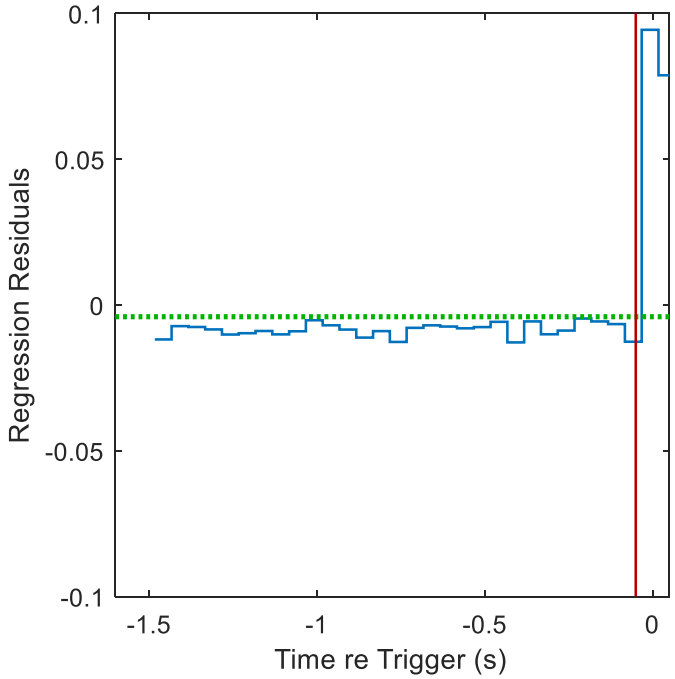

Figure 32. Example of the EMG-adjusted ear canal trace for a single shot. Time relative to the arrival of the gunshot is represented on the horizontal axis. The vertical axis represents the changes in the ear canal after the influence of concomitant/incidental muscle activity has been controlled. This figure provides no evidence of a residual change in the ear canal signal after the effects of concomitant/incidental muscle activity has been controlled.

Results

The series of tasks conducted in the current study were completed to determine the likelihood of observing conditioned or early MEMCs among varying levels of generalizability to the warfighter. For each task in this project, the level for recommending the adoption of a conditioned eMEMC in a damage-risk criterion was 95% confidence of 95% or greater

prevalence of exhibiting a response (i.e., pervasiveness). This criterion is equivalent to a question of whether the lower bound of the 90% confidence interval for the prevalence of observed eMEMCs exceeds 0.95. The requirement for adoption would not be met with one clear failure to exhibit an eMEMC in a group of 59 participants (Patterson et al., 1985).

Analyses of the conditioning tasks (AV, AA, UA) from the laboratory environment indicated that the probability of observing an eMEMC (i.e., evidence of an energy change in the ear after the CS and prior to the arrival of the UCS) depended heavily on the sensory modality of the CS and the participant's attention to or distraction from the CS (Figure 33).

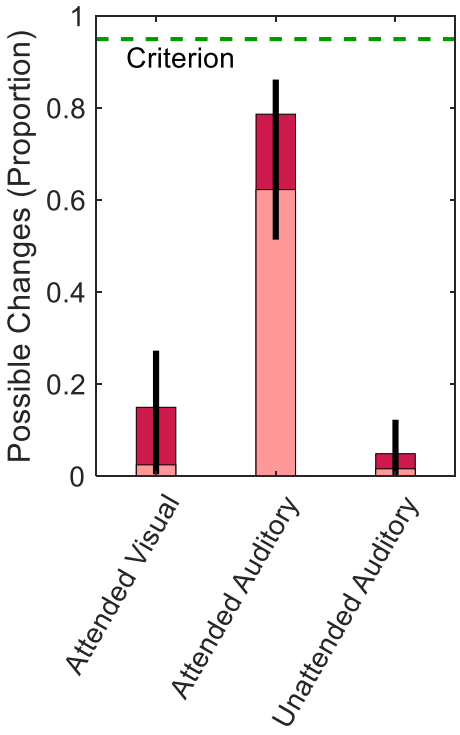

Figure 33. Conditioned task results from Study 1, completed using classical conditioning in a laboratory setting. Light colored bars represent the proportions of conditioned eMEMCs detected by consensus across three raters. Dark colored bars represent the proportions of conditioned eMEMCs detected by a majority of three raters. Black bars represent the range from the low bound of the 90% confidence interval for consensus ratings to the upper bound of the 90% confidence interval for majority ratings.

Attended Visual Task

Few participants assigned to the AV task exhibited a tendency toward an eMEMC. Approximately 15% of participants exhibited changes using the majority judgment (3% using consensus judgment) and the relaxed eMEMC definition applied to tasks not involving live ammunition. These results suggest that there is a 0.95 probability that more than 0% of people with normal hearing and acoustic reflexes has a tendency toward eMEMC. Conversely, there is a 0.95 probability that fewer than 27% of this population has a tendency toward eMEMCs developed using this CS. These percentages fail to meet the pervasiveness criterion.

Attended Auditory Task

Participants completing the AA task were most likely to exhibit tendencies toward eMEMCs. By majority judgments, nearly 80% (62% by consensus judgment) were observed using the relaxed eMEMC definition. This indicates that there is a 0.95 probability that more than 51% of people with normal hearing and acoustic reflexes has a tendency toward eMEMC and a 0.95 probability that fewer than 86% of this population has this tendency. These percentages fail to meet the pervasiveness criterion.

Unattended Auditory Task

Perhaps the most surprising aspect of the lab-based conditioning results comes in the contrast between the AA and UA tasks. The proportions of eMEMCs for the UA task were 5% or less, regardless of consensus or majority rater judgments, which is considerably lower than the AA task. The AA and UA tasks shared the same auditory CS and UCS and differed only in the degree of attention available. In the AA task, participants were instructed to press a response button as soon as they heard the CS. In the UA task, participants were presented with a distractor activity during the presentation of CS and UCS. There is a 0.95 probability that more than 0% of people with normal hearing and acoustic reflexes has a tendency toward eMEMC and fewer than 12% of this population has this tendency. These percentages fail to meet the pervasiveness criterion.

Comparison to DRC Hypothesis

The AV, AA, and UA results were compared to the AHAAH model hypothesis (DRC hypothesis) of a pervasive protective eMEMC. The pervasiveness criterion was not met at any time during the data collection phase of Study 1 (Figure 34, left plot). The right plot in Figure 34 represents the number of additional consecutive eMEMC responses required to support the hypothesis and warrant inclusion as a protective mechanism in the warned AHAAH model.

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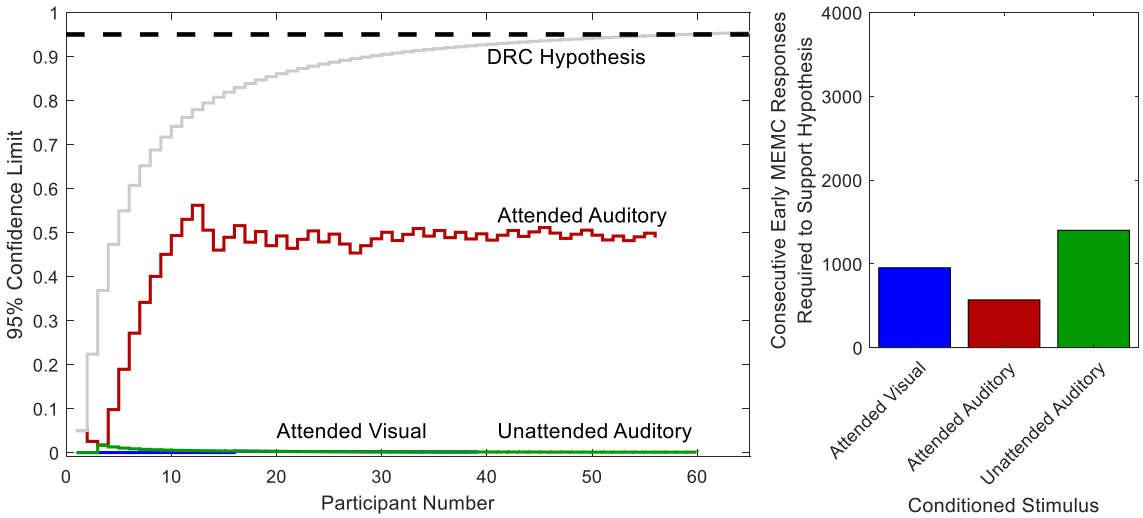

Figure 34. Consensus ratings of eMEMCs for AA, AV, and UA laboratory tasks, as compared to the AHAAH warned hypothesis (DRC Hypothesis) across numbers of participants. The figure on the right demonstrates the number of consecutive eMEMC responses required to support the AHAAH warned hypothesis.

Simulated Trigger / Dry Fire

Few regular shooters meeting the inclusion criteria were recruited into the university lab. The relatively small number of participants eligible for assignment to the ST and DF tasks (n = 6 and n = 9, respectively) results in broad confidence intervals for the estimates. The percentages of participants exhibiting a tendency toward eMEMC ranged from 33% (consensus ratings on ST) to 67% (majority ratings on ST and DF, Figure 35), using the relaxed definition of an eMEMC. There is a 0.95 probability that more than 7 and 18 % of regular shooters with normal hearing and acoustic reflexes would have a tendency to exhibit eMEMC on the ST and DF tasks, respectively. There is also a 0.95 probability that fewer than 93 and 89% of this population would exhibit a tendency toward eMEMC on these tasks, respectively. These percentages fail to meet the pervasiveness criterion, and this finding would be unlikely to change without at least 176 consecutive participants demonstrating a tendency toward eMEMC (Figure 36).

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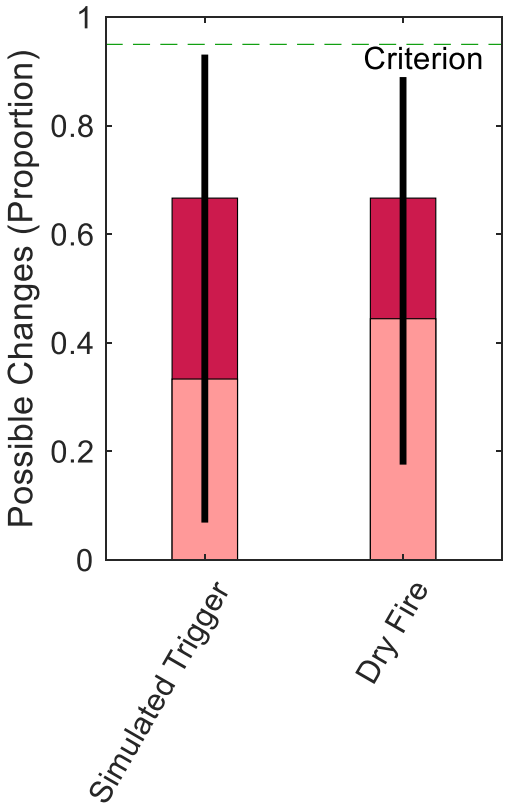

Figure 35. Simulated trigger and dry fire tasks, completed in a laboratory setting. Other details are the same as Figure 33.

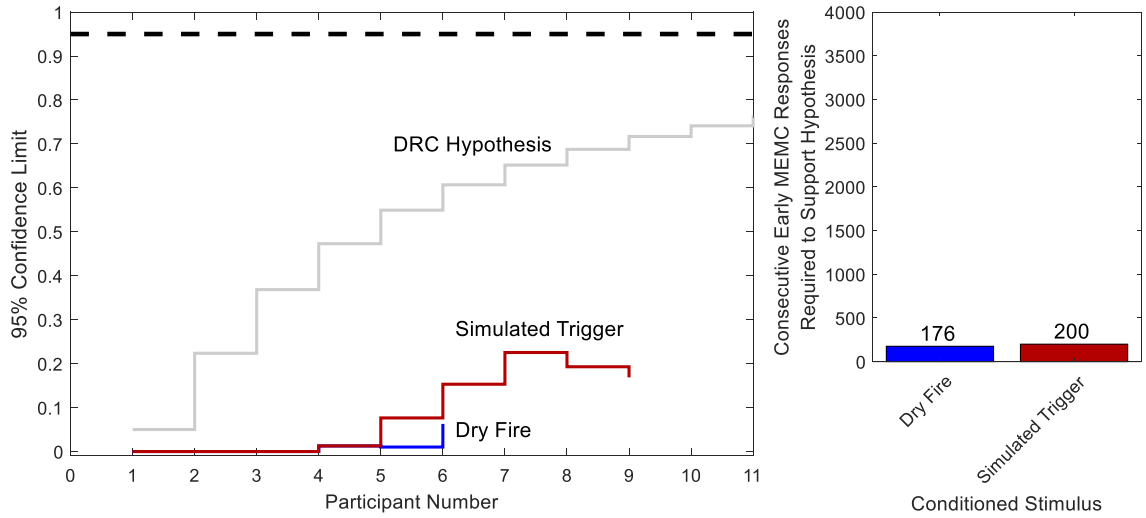

Figure 36. Consensus ratings of eMEMCs for ST and DF laboratory tasks, as compared to the AHAAH warned hypothesis (DRC Hypothesis) across numbers of participants. The figure on the right demonstrates the number of consecutive eMEMC responses required to support the AHAAH warned hypothesis.

Simulated Shooter / Simulated Spotter

Only 4 and 11% of firearm users and Service Members with near-normal hearing exhibited a tendency toward an eMEMC on the SH and SP tasks, respectively, using majority ratings (2% each using consensus ratings) and the relaxed eMEMC definition (Figure 37). There is a 0.95 probability that some portion (> 0%) of regular shooters and Service Members with normal hearing and acoustic reflexes would have a tendency to exhibit eMEMC on either task. There is also a 0.95 probability that fewer than 8 and 21% of this population would exhibit a tendency toward eMEMC on the SH and SP tasks, respectively. These percentages fail to meet the pervasiveness criterion, and this finding would be unlikely to change without at least 1200 consecutive participants demonstrating a tendency toward eMEMC (Figure 38).

Due to the differences in exclusion criteria used to evaluate study participation, results were also divided into subgroups for further examination: those with excellent hearing and recent firearm usage (n = 19), those with excellent hearing and less recent firearm usage (n = 9), and those with very good hearing and recent or less recent firearm usage (n = 25). No conditions or subgroups demonstrated significantly greater likelihood of an eMEMC.

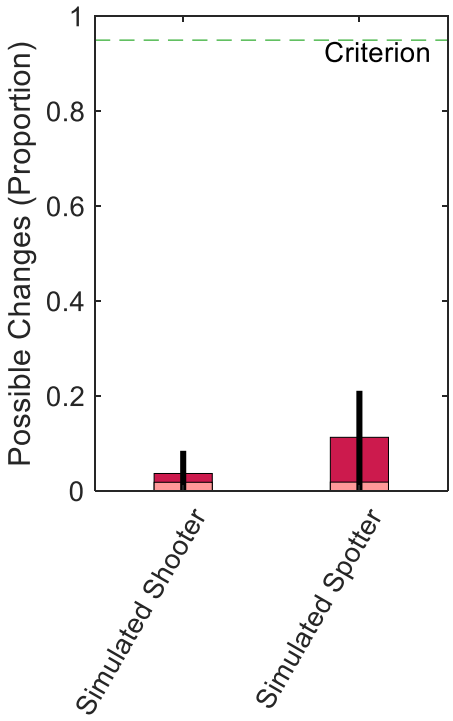

Figure 37. Simulated shooter and spotter tasks, completed using an EST2000 M4 carbine in a laboratory setting. Other details are the same as Figure 33.

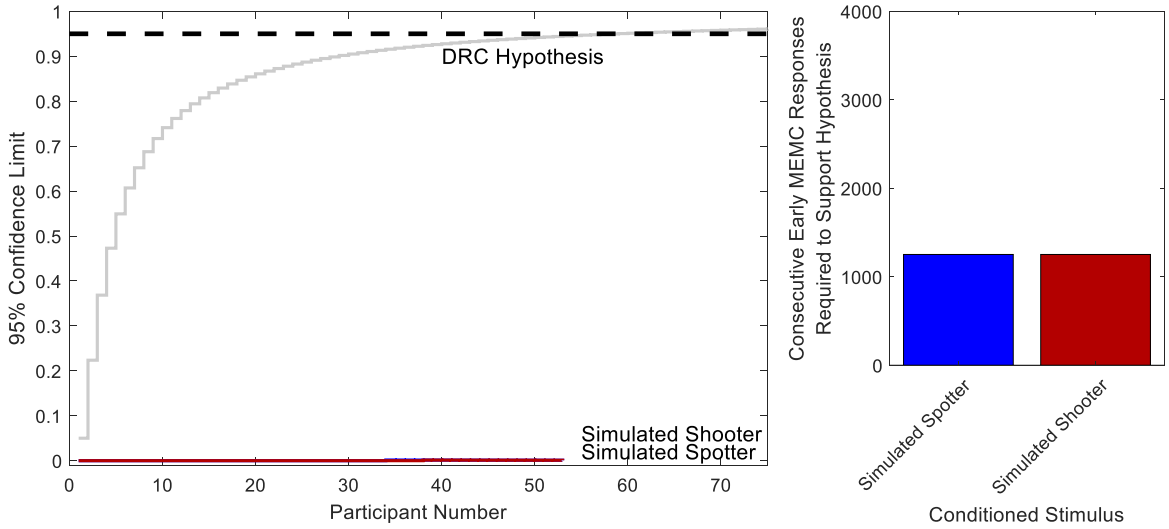

Figure 38. Consensus ratings of eMEMCs for SH/SP laboratory tasks, as compared to the AHAAH warned hypothesis (DRC Hypothesis) across numbers of participants. The figure on the right demonstrates the number of consecutive eMEMC responses required to support the AHAAH warned hypothesis.

Live Fire – Waiting / Live Fire – Active

Judgments of the presence of eMEMCs, using the rigid eMEMC definition, were made for all 19 participants using the routine (-1.5 second) window analysis. Instead of three raters as was used for the studies conducted at Western Michigan University, a fourth rater was included for the live fire studies at Fort Rucker, USAARL. Using majority judgments, 5% of the participants exhibited a tendency toward an eMEMC under the LW task, while approximately 11% exhibited a tendency toward an eMEMC in the LA task. Consensus agreement on the presence of an eMEMC was found with two participants and only in the LA task (11%). One case was identified as having an eMEMC by three of the four raters of these tasks. Three cases were identified as having an eMEMC by only one of the four raters, and all raters agreed that there was no evidence of eMEMCs on the remaining cases. Based on the results of the routine (-1.5 second) window analysis, there is a 0.95 probability that more than 0% and 2% of Service Members with normal hearing and acoustic reflexes would have a tendency to exhibit eMEMC on both LA and LW tasks, respectively. There is also a 0.95 probability that fewer than 23% and 30% of this population would exhibit a tendency toward eMEMC on these tasks, respectively.

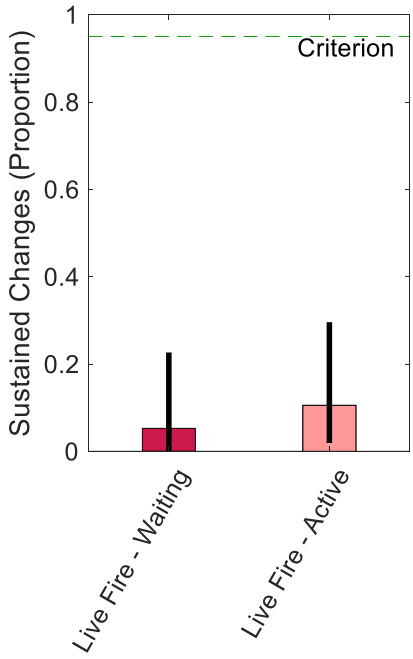

Figure 39. Live fire- waiting and active tasks, completed by active duty Soldiers using an M4 with standard ammunition on a firing range. Other details are similar to Figure 33.

The average detection rate for the extended window approach was approximately 11% for the non-shooter conditions and approximately 21% for the shooter conditions. Further, complete agreement across raters was found for one participant in the LW task and two participants in the LA task. Three participants were judged to have evidence of eMEMCs by two raters, and one participant was judged to have an eMEMC by one rater. The remaining 31 participants were judged by no raters to have evidence of eMEMCs. Based on the results of the extended window analysis, there is a 0.95 probability that more than 2 and 8% of Service Members with normal hearing and acoustic reflexes would have a tendency to exhibit eMEMC on the LW and LA tasks, respectively. There is also a 0.95 probability that fewer than 23% and 30% of this population would exhibit a tendency toward eMEMC on these tasks, respectively.

Judgments of the presence of changes in the EMG-adjusted residuals prior to the arrival of the discharge noise were made at the level of individual weapon discharges independently by four investigators (authors GAF, SMT, KKD, MVS) for all participants. Across shooter and non-shooter conditions, majority judgments indicating the presence of an early response was observed in zero of the 503 shots. Two participant weapon discharges were judged to have evidence of eMEMCs by two raters, and these shots occurred while participants were involved in the LW task, which suggests that these judgments were false-positives rather than bona fide eMEMCs. An additional 18 weapon discharges were judged to have evidence of an eMEMC by only one rater.

The proportions of participants exhibiting a tendency toward eMEMC and the proportions of eMEMCs at the level of individual weapon discharges were far below the criteria for declaring that eMEMCs are pervasive among Service Members firing the M4 carbine (Figure

40, left plot). Evidence of eMEMCs would need to be exhibited in more than 400 consecutive participants to change this conclusion (Figure 40, right plot).

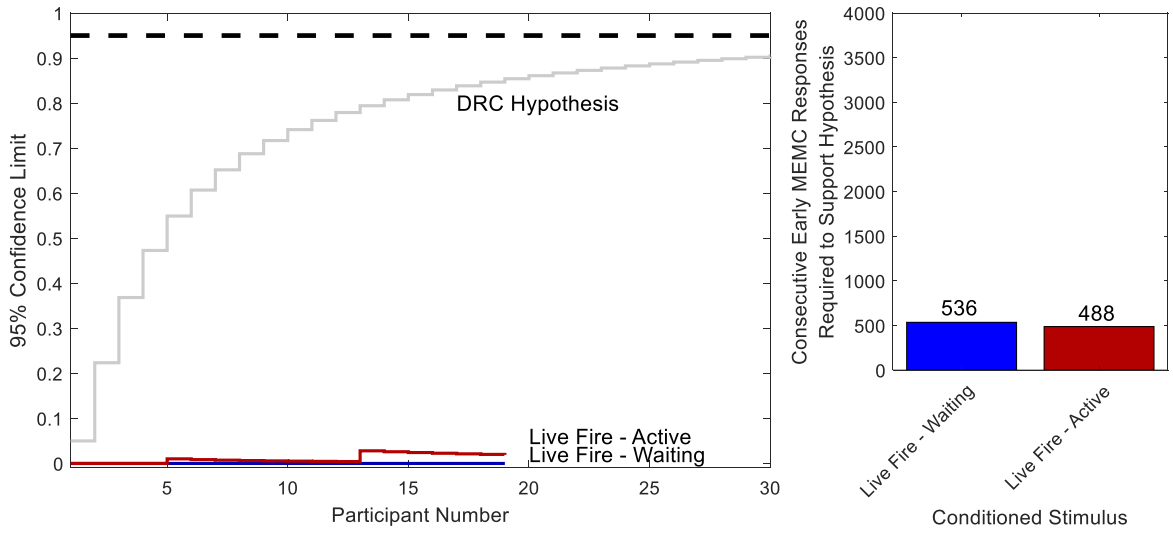

Figure 40. Consensus ratings of eMEMCs during LA/LW field tasks using the routine window, compared to the AHAAH warned hypothesis (DRC Hypothesis) across numbers of participants. The figure on the right shows the number of consecutive eMEMC responses required to support the AHAAH warned hypothesis.

Discussion

The present study was conducted to determine whether conditioned or early MEMCs are pervasive, in either laboratory or field settings, with an overall goal to inform decisions about the proper role of eMEMCs and the evaluation of DRCs that might include eMEMCs as a protective factor. Fidelity to military tasks ranged from a series of laboratory activities with non-firearm users to active duty Soldiers firing M4 service carbines on a military target range. Early MEMCs were not pervasive under any of the nine tasks included in this study. Starting with the data collected from these tasks, the numbers of consecutive participants exhibiting eMEMC required to demonstrate pervasiveness indicate that no reasonable study size could yield support for the hypothesis of a pervasive eMEMC (Table 18). The eMEMC was rarely present during laboratory conditioning tasks, even when participants were known to have normal clinical acoustic reflexes. Regular firearm users did not yield evidence of prior conditioning of eMEMC based on previous firearm use and training. Using the graded certainty categories defined by Rosenblith (1958) (e.g., “this we know,” “this is reasonably certain,” “of this we have considerable doubt,” and “on this we still cannot make an intelligent guess.”), there is, at best, *considerable doubt* that an eMEMC might be sufficiently prevalent to be protective.

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Table 18. Percentage of eMEMC Expected to be Exceeded with 95% Confidence in an Exposed Population, by Task. The Number of Consecutive eMEMC Responses Required to Support Inclusion as a Protective Measure in DRCs for Impulsive Noise are also Included. No Tasks Provide Support for Inclusion of eMEMCs in DRCs for Impulsive Noise.

Task	% Early/Conditioned – Person Level	# of consecutive eMEMC responses required for support	Support for inclusion of early/conditioned MEMC?
AV	0	953	No
AA	51	569	No
UA	0	1400	No
ST	7	176	No
DF	18	200	No
SH	0	1252	No
SP	0	1252	No
LA	0	488	No
LW	0	536	No

Conditioned eMEMCs

Results from laboratory conditioning tasks with participants who are not regular firearm users suggested that eMEMCs are very unlikely if the user cannot attend to the CS or if the CS is visual (Figure 33). The vast difference in rates in eMEMCs across the AA and UA tasks, which differ only in the level of attention to the CS, suggests that the eMEMC depends heavily on attention for encoding the association between the CS and UCS, recognition of the CS in producing a CR, or both. The dependence of CRs on attention is problematic for any DRC intended to be applied to warfighters, who are expected to maintain situational awareness and bear substantial cognitive loads (Hollands et al., 2019) during weapons fire. Attempts to overcome the decline in eMEMC by directing warfighter attention to eMEMCs would increase cognitive load, which could decrease the survivability and lethality of fire teams and increase the risk of fratricide (Scribner, 2002).

Sensory modality played an apparent role in conditioned eMEMCs. Early MEMCs were observed most frequently for the AA task (Figure 33). Shifting from an auditory to visual sensory modality, as in the AV task, produced much lower rates of eMEMCs. This suggests that CRs are less likely when the CS and UCS do not share a sensory modality. The need for the CS to be delivered via the auditory sensory modality is also problematic when DRCs are to be applied during warfighter training or operational scenarios because Service Members already use the auditory modality for task-critical information (e.g., command communication).
The laboratory-based conditioning tasks examined in Study 1 failed to produce rates of CRs sufficiently high to recommend assumptions of conditioned eMEMCs in DRCs for impulsive noise. Further, the proportion of participants with protective conditioned eMEMCs would be lower than the proportion showing any evidence of a CR. Taken together, the analyses of the laboratory conditioning eMEMC tasks provide no support for an assumption of conditioned eMEMCs as a protective phenomenon.

eMEMCs Associated with Prior Experience

Regular firearm users are unlikely to produce an eMEMC. This finding spans across varying levels of generalizability, from a toy gun in a laboratory setting to an M4 used by active duty Soldiers on a firing range. These results were consistent among participants who were aware of an impending impulse (i.e., Simulated Shooter, Live Fire- Active) because they were firing their own weapon, and in cases where the participant was unaware of the precise timing of an impending impulse (i.e. Simulated Spotter, Live Fire- Waiting). Furthermore, the eMEMC, if present, can be expected to attenuate only the frequencies below 1 kHz, can increase energy transfer above 1 kHz, and eMEMC magnitude, latency, and morphology varies significantly across people (Feeney & Keefe, 1999; Jones et al., 2017; Rabinowitz, 1977).

The results of the current study illustrate the problem of assuming that operational demands (e.g., engaging on a target, proper breathing, triggering techniques) are irrelevant to the development or execution of a CR. Sufficient attention and/or cognitive resources seem unlikely to be allocated to eMEMC precursors or the firearm's impulsive acoustic signal to produce a predictable eMEMC, especially given the modest cognitive demands associated with the tasks included in this study. During initial training, recruits focus on marksmanship fundamentals, and advanced training and military operations include many cognitive burdens (e.g., target identification, situational awareness, and communication) that could be expected to intrude on any conditioned or learned response leading to an eMEMC. The attention required to obtain the underlying marksmanship processes could also interfere with the process that creates the exposure-eMEMC association, and the allocation of processing resources away from a learned exposure-eMEMC association might attenuate any conditioned eMEMC response (Buser, 2006; Grillon & Baas, 2003) that could otherwise have been developed. It is unclear from these tasks whether the absence of eMEMCs is a consequence of a failure of associative learning, of inability to develop a response to the variety of potential CSs, inability to recall and execute the MEMC motor procedure, etc. It is also possible that any eMEMC response is inhibited by cognitive loading. Regardless of the reason for the absence, the results of this project are in accord with prior investigators who concluded that eMEMC were fragile and undependable (Bates et al., 1970).

The presence of possible elicitors of an eMEMC (i.e., facial reflexes, acoustic stimuli) cannot be relied upon as proof that a common eMEMC exists. Voluntary maximum eye closure is more likely to elicit MEMCs than brief acoustic stimuli (Tasko, Deiters, et al., 2020); however, operational settings do not allow the warfighter maximal eye closure during firing activities. The presence of an acoustic reflex in response to conventional acoustic reflex stimuli does not ensure an early or reflexive MEMC will be present for more brief acoustic signals (Deiters et al., 2019). Eyeblink startle activity in response to pneumotactile stimuli can occur reflexively, but does not lead to pervasive MEMC or eyeblink startle responses. Startle responses are prone to habituation/inhibition (Grillon & Baas, 2003), and are not shown to occur in anticipation of the pneumotactile stimuli (Deiters et al., 2020).

Laboratory results during the AA task showed a greater likelihood of producing eMEMCs than other tasks that were more generalizable to the warfighter. There are a number of

possible reasons for this. It could be related to the level of attention afforded to the auditory signal, because the AA task required participant attention. An analytical difference could also have contributed. All tasks except LA and LW were evaluated in terms of *potential* early MEMC (i.e., relaxed eMEMC definition; granting the possibility of an initial early response that was inhibited prior to impulse arrival), while the LA/LW tasks were evaluated in terms of an early response that was required to persist until impulse arrival (i.e., rigid eMEMC definition). Finally, it is also possible that the motor behaviors and learning acquired during marksmanship training interfered with the development or retention of an eMEMC (Holland & Schiffino, 2016).

Limitations

The series of nine tasks summarized in this report show unequivocally that eMEMC are observed too infrequently to justify inclusion of eMEMC as a protective factor in DRCs for impulsive noise. Nonetheless, the traits under consideration in this study are represented imperfectly by the research operations producing the variables analyzed in this project. For example, the measurement technique used to identify eMEMC was an indirect measure of muscle activity that detects changes in energy transfer through the middle ear system, which would have been the critical quantity to measure if eMEMC were pervasive. Invasive measurement techniques (e.g., near-field EMG) were not feasible in the current study due to the significant increased participant risk and impracticality when applied at the level required to demonstrate pervasiveness (i.e., N > = 59 per task). The MEMC measurement approach used in this study was developed over a period of 35 years (Allen, 1985; Feeney & Keefe, 1999, 2001; Keefe et al., 1992). This method has been demonstrated to be 12-14 dB more sensitive than conventional (226 Hz probe tone) methods (Feeney et al., 2003), suggesting that the low proportions of participants exhibiting eMEMC were not due to an insensitive measurement approach.

It is plausible that a different MEMC detection method developed in the future could be applied and yield a different outcome. But one cannot be sanguine about this prospect, given that pervasiveness has yet to be demonstrated in any of the studies over the last 60 years that have addressed this general topic. A variety of methods have been used to detect eMEMC, ranging from psychoacoustic (Gerhardt & Hepler, 1983), conventional 220-226 Hz tonal probes (Wilson, 1979), click-based acoustic probes (current study), and optical laser doppler vibrometry measurements (Jones et al., 2018). Although these studies have been conducted primarily in laboratory environments, the current study extended the environments and activities from well-controlled lab environments to an outdoor military firing range, and if any trend across study environments were to be inferred from these results, it is in the direction of a lower likelihood of eMEMC in more realistic settings. The convergent answers (re: pervasive eMEMC) returned across multiple research methods suggest that monomethod bias (Shadish et al., 2002) is unlikely to have biased the overall conclusion that eMEMC are not pervasive.

In this study, detection of the eMEMC was based on changes in the sound levels developed in the ear canal by a band-limited click stimulus. This stimulus was repeated every 50 ms, and the click analysis windows were not necessarily synchronous with UCS onset because the UCS was initiated by the participant or investigator (ST, DF, SH, SP, LA, LW tasks) or due to synchronization limits imposed by the operating system (AV task). Further, the timing of the

UCS was estimated empirically, and this estimate could lag the actual onset by a few milliseconds. As a consequence, responses to the click immediately prior to the interval including the UCS onset (i.e., time > = -50 ms re: UCS onset) were not interpretable as an eMEMC because they result from the infiltration of the UCS into the ear canal signal. The last 50 ms prior to UCS onset cannot be considered a likely time for eMEMC onset for any of the tasks included in this study because it implies a degree of knowledge and motor control that is likely beyond the participant's capacity, particularly if the participant is denied access to a pacing stimulus (Goebl & Palmer, 2008; Wing, 2002) to aid the planning and execution of the motor action. Further, there is reason to suspect that attention to trigger force and displacement is minimized during shooting. Targeting accuracy is improved and flinching is minimized when the moment of weapon discharge is unknown (U.S. Department of the Army, 2012).

Accurate marksmanship requires a slow continuous trigger squeeze rather than a rapid trigger pull that would be required to minimize the latency between the introduction of force on the trigger and the discharge of the weapon (U.S. Department of the Army, 2011). It is also important to recognize that a shooter's knowledge of the trigger release depends on ongoing comparison of the force the shooter is applying to the trigger and memory of the force required for discharge. In addition to the likely errors in estimating these quantities, gloves, sensitivity changes associated with ambient temperature, and the cognitive demands on the shooter will likely interfere with the accuracy of this comparison. The timing complexities involved in the triggering process may explain the poor specification of the conditions under which the eMEMC is expected (e.g., the "warned" hypothesis in AHAAH, described above). The force sensors used in the LA and SH tasks revealed that participants applied increased force to the weapon trigger well before the final click preceding discharge (Figure 29). Increased force on the M4 trigger provides a clear indication of the intent to discharge the weapon, and the lack of a coordinated eMEMC casts doubt on the proposition that a preparatory eMEMC is present. Further doubt arises with the lack of a common eMEMC observed using laser doppler vibrometry methods (Jones et al., 2018). The current study focused on changes in the ear prior to triggering onset. Prior publications by this team presented rMEMC prevalence estimates following elicitor onset (Deiters et al., 2019) and eMEMC results measured via laser doppler vibrometry (Jones et al., 2018), which has near-instantaneous temporal resolution, and both have shown results that are consistent with those found in the present study.

The underlying physiology and mechanisms that might lead to eMEMC are beyond the scope of this study, which had the purpose of determining whether such investigations might be justified. The sample sizes required to demonstrate pervasiveness (Table 18) show clearly that studies of eMEMC as a primary research question would not be an efficient use of public resources in the interest of DRCs. Additionally, the small numbers of people exhibiting tendencies toward eMEMC imply that the statistical power to examine correlates of eMEMCs would be quite poor. It is, however, important to monitor for MEMCs in studies evaluating the link between exposure and auditory outcomes because they could operate as a nuisance variable that contributes systematic error to the analyses. Particular attention should be given to non-acoustic elicitors of MEMC, based on the results of Tasko, Flamme, et al. (2020), Tasko, Deiters, et al. (2020), and Tasko et al. (2018).

The Microsoft Windows operating system driving the data acquisition system applied a hierarchy of tasks, which occasionally led to variable CS-UCS time lags during the AV task. So, although the lag between the visual CS and auditory UCS was intended to be constant, there was some variation (<43 ms) in the lag within a given participant. The range of lags is not sufficiently large to interfere with the development of a CR (Bangasser et al., 2006; Connor & Gould, 2016; Kalmbach et al., 2010; Woodruff-Pak & Disterhoft, 2008), suggesting that this variability was inconsequential.

There were differences in participant characteristics, including hearing characteristics, middle ear characteristics, recent self-reported firearm use, and test procedures for participants completing the SH and SP tasks. Interpretation of differences between these tasks and the other tasks in this project must be tempered with these differences in mind. Despite the differences in participant characteristics across studies, the likelihoods of observing eMEMC in the SH and SP tasks were comparable to the other tasks, and therefore suggest that the findings are robust across these demographic categories.

Recommendations

These findings provide strong evidence that DRCs used for military acquisition, or the development of training and doctrine, should not include a protective role for eMEMC. Developers of DRCs for impulsive noises should remove assumptions of protective eMEMCs from models intended to protect against hearing loss from impulsive noise exposure.

Conclusion

In this project, eMEMCs were measured in response to conditioning stimuli and in more generalizable warfighter training scenarios. The results of the tasks included in this project demonstrate that eMEMC are not sufficiently prevalent to be a protective factor in DRCs for impulsive noise. There was no evidence of a tendency toward eMEMC in the vast majority of firearm users. The strongest evidence for developing a CR was found when attention was directed to an auditory CS (i.e., the AA task, Table 18), and this evidence was insufficient to support the eMEMC as a protective factor. The likelihood of developing a CR to the auditory CS declined precipitously when participant attention was directed elsewhere. It is unreasonable to expect that the attention required to develop an eMEMC would be available during any military training or operations. Fewer than 11% of participants showed a tendency toward eMEMC when discharging live ammunition (M4 carbine). This project finds minimal evidence of the presence of eMEMCs and cannot support including eMEMCs in any DRC for impulsive noise.

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