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Prepulse inhibition of the acoustic startle reflex vs. auditory brainstem response for hearing assessment

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ABSTRACT

The high prevalence of noise-induced and age-related hearing loss in the general population has warranted the use of animal models to study the etiology of these pathologies. Quick and accurate auditory threshold determination is a prerequisite for experimental manipulations targeting hearing loss in animal models. The standard auditory brainstem response (ABR) measurement is fairly quick and translational across species, but is limited by the need for anesthesia and a lack of perceptual assessment. The goal of this study was to develop a new method of hearing assessment utilizing prepulse inhibition (PPI) of the acoustic startle reflex, a commonly used tool that measures detection thresholds in awake animals, and can be performed on multiple animals simultaneously. We found that in control mice PPI audiometric functions are similar to both ABR and traditional operant conditioning audiograms. The hearing thresholds assessed with PPI audiometry in sound exposed mice were also similar to those detected by ABR thresholds one day after exposure. However, three months after exposure PPI threshold shifts were still evident at and near the frequency of exposure whereas ABR thresholds recovered to the pre-exposed level. In contrast, PPI audiometry and ABR wave one amplitudes detected similar losses. PPI audiometry provides a high throughput automated behavioral screening tool of hearing in awake animals. Overall, PPI audiometry and ABR assessments of the auditory system are robust techniques with distinct advantages and limitations, which when combined, can provide ample information about the functionality of the auditory system.

1. Introduction

Quick and accurate assessment of auditory thresholds is a prerequisite for experimental manipulations in the field of auditory research. To date, a variety of protocols have been used to determine audiometric thresholds. Auditory brainstem responses (review by Stapells and Oates, 1997), behavioral audiograms (Heffner and Masterson, 1980; Radziwon et al., 2009), and startle reflex audiometry (Young and Fechter, 1983; Walter et al., 2012) have each been used to assess hearing, yet each has limitations which should be taken into account when interpreting data related to threshold shifts and overall cochlear damage following sound or chemical lesions.

Perhaps the most ubiquitous test used to assess hearing performance is the auditory brainstem response. Rapid assessment of auditory brainstem circuitry makes ABR a good candidate for detecting gross changes in the auditory system. It is known that following an auditory insult, ABRs reliably identify elevated thresholds. This temporary threshold shift is thought to result from swelling of cochlear nerve terminals which is present for days after exposure. When measured with ABRs, thresholds return to baseline soon after noise exposure (Robertson, 1983). However, recent work has clearly demonstrated that this measure does not account for the trauma-induced damage to ribbon synapses (Kujawa and Liberman, 2009). ABR wave one amplitudes have been shown to more accurately represent suprathreshold hearing loss, as they correlate strongly with ribbon synapse denervation following sound exposure (Liberman and Liberman, 2015). However, the ABR methodology is not without some caveats. Immediately following sound exposure, ABR thresholds are often elevated past the point of detection where they cannot be accurately measured which also precludes wave one amplitudes from being assessed. Furthermore, ABR's are typically collected under anesthesia and are challenging to measure in animals with larger body mass (Chambers et al.,

Abbreviations: PPI, prepulse inhibition; ABR, auditory brainstem response; ASR, acoustic startle reflex; SO, startle only; ITI, inter-trial interval; ANOVA, analysis of variance; LSD, Least Significant Difference post hoc test; ANF, auditory nerve fiber

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2012; Cederholm et al., 2012). Lastly, some have contended that while ABRs thresholds are useful in detecting noise-induced damage to the auditory brainstem, they do not provide any perceptual indications of hearing loss (Davis, 1984). Alternatively, others have stated that ABRs can closely approximate behavioral thresholds in humans, yet ABRs are known to be less precise (Stapells, 2011). For these reasons, it is useful to explore alternatives for hearing assessment.

Many years ago it was found that prepulse modulation of the acoustic startle reflex (ASR) could be employed to assess behavioral response thresholds (Fechter et al., 1988). Prepulse inhibition, a decrease of ASR magnitude when a preceding weaker sound (prepulse) is presented before the startle, has been used for over half a century to objectively measure complex neurological systems (Hoffman and Searle, 1965; Hoffman and Wible, 1970; Graham, 1975; Gerrard and Ison, 1990). Much like behavioral audiograms collected by operant conditioning methods (Heffner and Masterson, 1980; Radziwon et al., 2009), the prepulse was varied in intensity and frequency to differentially modulate the startle response. This method has successfully identified behavioral correlates of cochlear damage due to ototoxic drugs (Young and Fechter, 1983) and temporary threshold shifts due to pure tone acoustic exposure (Walter et al., 2012). Advantages of this approach for assessing hearing thresholds are numerous. First, the measure is based on a reflex and does not require experience in animal behavior and months of animal training as in other commonly used behavioral paradigms. Second, in contrast to the ABR approach, PPI audiometric functions can be collected in awake animals, avoiding confounds of anesthesia. Finally, a key advantage is that many animals can be tested at once with a short preparation, allowing for high data throughput, and timely data collections at various experimental conditions.

Although PPI audiometry has several advantages over other currently used hearing assessment methodologies, several important questions need to be explored before it can be widely applied. First, it is still unknown whether it is sensitive enough to assess hearing in individual animals, which would be much more beneficial than group averages. Second, the extent of PPI threshold reliability from day to day is also unknown. Third, it is important to know whether PPI audiometry can be used to detect permanent threshold shifts caused by the most common hearing insult, noise-induced hearing loss. The goal of this study was to address these questions and compare PPI audiometry with the traditional ABR approach.

2. Methods

2.1. Animals

A total of 16 male CBA/CaJ mice obtained from Jackson Laboratories were used. To avoid startle variability which is known to result from hormone fluctuations of the estrous cycle, female mice were not used in this study (Plappert et al., 2005; Ison and Allen, 2007). This phenomenon has also been shown in human subjects (Kumari et al., 2004). Mice were 12 weeks old at the beginning of the experimental procedures. They were housed in pairs within a colony room with a 12-h light–dark cycle at 25 °C.

Ten mice were sound exposed as described below while six unexposed mice were used as controls. The exposed mice (depicted throughout all figures in color: Blue: unexposed, Purple: one day after exposure, Orange: three months after exposure) were tested during a 3 month period to detect permanent threshold shifts. The 6 control mice were used to test for the consistency of PPI measurements across time. Procedures used in this study were approved by the Institutional Animal Care and Use Committee at the Northeast Ohio Medical University.

2.2. Acoustic trauma

Mice were anesthetized with an intramuscular injection of a ketamine/xylazine mixture (100/10 mg/kg). An additional injection (50% of the initial dose) was given 30 min after the initial injection. Mice were unilaterally exposed to a one octave narrow-band noise centered at 12.5 kHz (~8–17 kHz). This noise was generated using a waveform generator (Tektronix AFG 3021B), amplified (QSC RMX 2450) to 116 dB SPL, and played through a speaker (Fostex T925A Horn Tweeter). The output of the loudspeaker was calibrated with a 0.25-in. microphone (Brüel and Kjaer 4135) attached to a measuring amplifier (Brüel and Kjaer 2525) and found to be ±4 dB between 4 and 60 kHz. During exposure the speaker was located ~5 cm from the animal's right ear. The left external ear canal was obstructed with a cotton plug and a Kwik-Sil silicone elastomer plug (World Precision Instruments), a manipulation which typically reduces sound levels by 30–50 dB SPL (Turner et al., 2006; Ropp et al., 2014).

2.3. Auditory brainstem response testing

Mice were anesthetized with ketamine/xylazine as during the acoustic trauma. Sterile, stainless-steel recording electrodes (connected to a Tucker Davis Technologies (TDT) RA4LI Low Impedance Headstage) were placed subdermally, one behind each pinna with the reference electrode along the vertex. Tone bursts at 4, 12.5, 16, 20, 25, and 31.5 kHz were presented at increasing sound intensities ranging from 10 to 80 dB SPL in 10 dB steps. Tones were 5 ms duration, 0.5 ms rise/fall time and delivered at the rate of 50/s. ABRs were averaged over 300 repetitions. These waveforms were amplified (TDT RA4PA Medusa Preamplifier), digitized (TDT RZ6 Multi-I/O Processor), and analyzed offline using a customized program within OpenEx Software (TDT). Thresholds, the smallest sound amplitude that evoked a visible ABR, were determined by visually examining the ABR waveforms in response to every sound frequency presented at different sound levels. ABR wave one amplitudes (μV peak to peak) were measured at each intensity/frequency combination for all exposed mice at all time points tested (prior to exposure (control), one day after exposure, and three months after exposure).

2.4. PPI audiometry

2.4.1. Acoustic startle hardware/software

The equipment used to collect all acoustic startle data has been described in detail previously (Longenecker and Galazyuk, 2012). Commercial hardware/software equipment from Kinder Scientific, Inc. was used in behavioral experiments. Each behavioral testing station was lined with anechoic foam to prevent sound reflection and wave cancelling sound echoes (Sonex foam from Pinta Acoustics). A small customization of the hardware's startle stimulus system was made by adding SLA-4 (ART) power amplifiers to adjust sound levels to correct for variations in speaker loudness between testing station. Mice restrainers were open walled to allow for maximum sound penetration (Fig. 3 in Longenecker and Galazyuk, 2012). Background sound levels within each testing chamber were calibrated with a 0.25-in. microphone (Brüel and Kjaer 4135) attached to a measuring amplifier (Brüel and Kjaer 2525) and found to be less than 40 dB SPL between 4 and 60 kHz. Startle waveforms were recorded using load cell platforms which measure actual force changes during an animal's jump. Each load cell was calibrated with a 100 g weight which corresponds to 1 N of force. Offline waveform analysis converted these forces into center of mass