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Research Paper

Addressing variability in the acoustic startle reflex for accurate gap detection assessment

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ABSTRACT

The acoustic startle reflex (ASR) is subject to substantial variability. This inherent variability consequently shapes the conclusions drawn from gap-induced prepulse inhibition of the acoustic startle reflex (GPIAS) assessments. Recent studies have cast doubt as to the efficacy of this methodology as it pertains to tinnitus assessment, partially, due to variability in and between data sets. The goal of this study was to examine the variance associated with several common data collection variables and data analyses with the aim to improve GPIAS reliability. To study this the GPIAS tests were conducted in adult male and female CBA/CaJ mice. Factors such as inter-trial interval, circadian rhythm, sex differences, and sensory adaptation were each evaluated. We then examined various data analysis factors which influence GPIAS assessment. Gap-induced facilitation, data processing options, and assessments of tinnitus were studied. We found that the startle reflex is highly variable in CBA/CaJ mice, but this can be minimized by certain data collection factors. We also found that careful consideration of temporal fluctuations of the ASR and controlling for facilitation can lead to more accurate GPIAS results. This study provides a guide for reducing variance in the GPIAS methodology – thereby improving the diagnostic power of the test.

1. Introduction

A reliable animal model of tinnitus is a prerequisite for tinnitus related therapies. Due to its relatively minor time commitment gap-induced prepulse inhibition of the acoustic startle reflex (GPIAS) has quickly become one of the predominant behavioral assessments for tinnitus (Turner et al., 2006) in several animal models (see Eggermont, 2013; Hayes et al., 2014; Galazyuk and Hébert, 2015). Many groups have used GPIAS to behaviorally assess tinnitus in rats (Turner et al., 2006; Lobarinas et al., 2013; Singer et al., 2013; Ropp et al., 2014), mice (Longenecker and Galazyuk, 2011; Middleton et al., 2011; Hickox and Liberman, 2014; Lowe and Walton, 2015; Yu et al., 2016), guinea pigs (Dehmel et al., 2012; Berger et al., 2013), and hamsters (Salloum et al., 2016). However, some uncertainty of this method is rooted in a lack of consistency of methodologies and data assessment strategies across labs (Galazyuk and Hébert, 2015). While the acoustic startle reflex (Landis and Hunt, 1939; Fleshler, 1965), prepulse inhibition (Hoffman and Searle, 1965; Ison and Hammond, 1971; Carlson and Willott, 1996; Swerdlow et al., 2001), and gap-induced prepulse inhibition (Ison, 1982) have been studied for decades, many of the specifics of stimuli, hardware, technical, and analytic information related to tinnitus detection have not yet been solidified. For this reason, we have previously addressed some hardware and stimulus presentation issues (Longenecker and Galazyuk, 2012), as well an in-depth analysis of the startle response (Grimsley et al., 2015). Although these initial steps have improved the confidence of GPIAS assessments, the finer details concerning data collection and data analysis, as they specifically relate to tinnitus assessment, need further attention.

GPIAS studies have largely neglected to provide details on data collection when assessing tinnitus in laboratory animals. However, many aspects of these approaches can dramatically affect conclusions of GPIAS experiments. This is especially true for animals that have high startle reflex variability (Berger et al., 2013; Longenecker and Galazyuk, 2016; Salloum et al., 2016). Limiting ASR variability is

Abbreviations: PPI, prepulse inhibition; ASR, acoustic startle reflex; ITI, inter trail interval

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critical in a repeated measure designs which compare an animal's performance before and after a tinnitus-inducing experimental treatment. Several factors influencing the ASR should be considered in order to control the inherent variability. These include issues that pertain to inter-trial intervals (ITI) (Ison and Hammond, 1971; Leitner et al., 1993; Willott and Carlson, 1995; Plappert et al., 2004), circadian rhythm (Chabot and Taylor, 1992a; Chabot and Taylor, 1992b), sex differences (Plappert et al., 2005; Koch, 1998), and sensory adaptation. A change to any of these factors can alter the startle response magnitude and startle response variability. These issues could be magnified when assessing the gap detection abilities in noise exposed animals, due to a suppressive effect of acoustic over-exposure on startle magnitude (Longenecker and Galazyuk, 2011; Lobarinas et al., 2013). Standardizing data collection efforts could decrease GPIAS data variability between animals, experiments, and lab groups.

One of the major confounds in GPIAS data analysis is gap-induced facilitation. A gap in a continuous background noise usually inhibits or reduces the startle response magnitude (Stitt et al., 1973; Ison, 1982). However, this is not always the case. Depending on stimulus conditions, a prepulse, gap, or alterations of the background noise can alternatively act as a facilitator of the startle reflex in mice (Plappert et al., 2004; Willott and Carlson, 1995), rats (Stitt et al., 1974; Ison et al., 1997), and humans (Aasen et al., 2005). While it is not fully understood why this dichotomy exists, both responses represent real sensory gating phenomenon but likely have separate complimentary biological circuit (Schmajuk and Larrauri, 2005). Thus, GPIAS assessments should be cognizant of this issue when examining “how well” an animal can detect a gap. If not appropriately addressed during data analysis, facilitation can limit the effectiveness of GPIAS as a tool to assess gap detection performance because of unnecessary variability. The exact details of data processing can also significantly affect GPIAS data interpretation. Unfortunately, this part of data analysis is typically neglected in method sections of the relevant papers. Nevertheless, details such as which data points are included/excluded, how many testing sessions/days were used in the control and experimental conditions, how ratios were calculated and/or averaged together, are critical to draw defendable conclusions based on GPIAS data.

Our results indicate that ITI, circadian cycle, sex, and sensory adaptation all play roles in the degree of variance present in GPIAS experiments. Our data also suggest that particular data analyses are critical to minimize the effect of variance in GPIAS data. Taken together, these findings suggest that the GPIAS method is more complicated than previously described, but can provide accurate assessments of gap detection performance.

2. Materials and methods

2.1. Subjects

A total of 62 CBA/CaJ mice were used in this study. Group A contained fourteen male mice which were used in the majority of experiments. Group B contained an additional 48 mice (24 males and 24 females) which were used in the sex-based variation experiments. In group A, mice were divided into two groups of seven and were housed in separate rooms: seven of these mice were housed in a regular light dark cycle (lights on 10 a.m. to 10 p.m.) (inactive mice) and other seven were kept in a reverse light dark cycle (lights off 10 a.m. to 10 p.m.) (active mice). Group B mice were housed in a regular 12-h light–dark cycle. Mice were obtained from Jackson Laboratories and were approximately 10 weeks old. Mice were housed in pairs within a colony room at 25 °C. Procedures used in this study were approved by the Institutional Animal Care and Use Committee at the Northeast Ohio Medical University.

2.2. Study design

Group A underwent baseline GPIAS testing for 21 days (one session per day) with 1–2 days between sessions. We tested seven inactive Group A mice using long ITIs during three sessions (ITI details are described in section 3.1.2). Following baseline assessment, all animals were sound exposed to induce tinnitus, as described below. Three and five months post exposure, behavioral evidence of tinnitus was assessed. Mice in Group B were not sound exposed and were tested for 10 sessions in 21days for sex differences.

2.3. Acoustic trauma

Mice were at least five months old at the time of sound exposure. 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 intramuscularly 30 min after the initial injection. Mice were exposed to a one octave band noise centered at 12.5 kHz (~8–17 kHz) unilaterally for one hour. This noise was generated using a waveform generator (Wavetek model 395), amplified (Sherwood RX-4109) to 116 dB SPL, and played through a loudspeaker (Fostex FT17H). The output of the loudspeaker was calibrated with a 0.25-in. microphone (Brüel and Kjaer 4135) and found to be ±4 dB between 10 and 60 kHz. The left external ear canal was obstructed with a cotton plug and a Kwik-Sil silicone elastomer plug (World Precision Instruments), a manipulation which reduces sound levels by 30–50 dB SPL (Turner et al., 2006; Ropp et al., 2014).

2.4. Acoustic startle hardware/software

The equipment used to collect all acoustic startle reflex (ASR) data has been described in detail previously (Longenecker and Galazyuk, 2012). Briefly, commercial hardware/software equipment from Kinder Scientific, Inc. was used. Each behavioral testing station was lined with anechoic foam to prevent sound reflection and wave cancelling sound echoes (Sonex foam from Pinta Acoustics). 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 40 kHz. Startle waveforms were recorded using load-cell platforms which measure actual force changes during an animal's startle. Each load cell was calibrated with a 100 g weight, corresponding approximately to 0.98 N of force in standard gravity.

2.5. Startle waveform identification and measurement

All waveforms collected during testing sessions were analyzed offline using a recently developed automatic method of startle waveform identification via a template matching paradigm (Grimsley et al., 2015). In that study we used high-speed video recordings (1000 frames/s) to visualize the animal's startles in order to identify stereotyped waveforms associated with a startle. This allowed us to develop custom software which automatically separates data into either startles or non-startle-related movements. Based on this separation, we only included trials that resulted in successful startle responses in our data analysis. We also used a mathematical approach to convert the force generated on a load cell plate to center of mass displacement (in mm) (Grimsley et al., 2015).

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