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Research paper

The gap-startle paradigm for tinnitus screening in animal models: Limitations and optimization

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ABSTRACT

In 2006, Turner and colleagues (Behav. Neurosci., 120:188–195) introduced the gap-startle paradigm as a high-throughput method for tinnitus screening in rats. Under this paradigm, gap detection ability was assessed by determining the level of inhibition of the acoustic startle reflex produced by a short silent gap inserted in an otherwise continuous background sound prior to a loud startling stimulus. Animals with tinnitus were expected to show impaired gap detection ability (i.e., lack of inhibition of the acoustic startle reflex) if the background sound containing the gap was qualitatively similar to the tinnitus pitch. Thus, for the gap-startle paradigm to be a valid tool to screen for tinnitus, a robust startle response from which to inhibit must be present. Because recent studies have demonstrated that the acoustic startle reflex could be dramatically reduced following noise exposure, we endeavored to 1) modify the gap-startle paradigm to be more resilient in the presence of hearing loss, and 2) evaluate whether a reduction in startle reactivity could confound the interpretation of gap prepulse inhibition and lead to errors in screening for tinnitus. In the first experiment, the traditional broadband noise (BBN) startle stimulus was replaced by a bandpass noise in which the sound energy was concentrated in the lower frequencies (5–10 kHz) in order to maintain audibility of the startle stimulus after unilateral high-frequency noise exposure (16 kHz). However, rats still showed a 57% reduction in startle amplitude to the bandpass noise post-noise exposure. A follow-up experiment on a separate group of rats with transiently-induced conductive hearing loss revealed that startle reactivity was better preserved when the BBN startle stimulus was replaced by a rapid airpuff to the back of the rat's neck. Furthermore, it was found that transient unilateral conductive hearing loss, which was not likely to induce tinnitus, caused an impairment in gap prepulse inhibition as assessed with the traditional BBN gap-startle paradigm, resulting in a false-positive screening for tinnitus. Thus, the present study identifies significant caveats of the traditional gap-startle paradigm, and describes experimental parameters using an airpuff startle stimulus which may help to limit the negative consequences of reduced startle reactivity following noise exposure, thereby allowing researchers to better screen for tinnitus in animals with hearing loss.

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1. Introduction

Subjective tinnitus is often described as a ringing or buzzing sensation in one or both ears or emanating from inside the head in the absence of an external sound. In the United States, approximately 25% of the adult population has experienced tinnitus, with nearly 8% reporting frequent bouts (Shargorodsky et al., 2010) and an estimated 1–2% suffering from severe, chronic, debilitating tinnitus (McCombe et al., 2001). According to a recent study, tinnitus is also a significant concern for members of the military; 49% of personnel exposed to blast trauma reported tinnitus as the primary audiologic complaint (Cave et al., 2007). In the general population, aging and noise exposure continue to be leading causes of hearing loss and tinnitus. Unfortunately for patients who develop persistent tinnitus, there are no widely accepted or FDA-approved treatments that completely abolish the phantom auditory perception.

In an effort to study the putative brain regions and neural mechanisms underlying tinnitus, several animal models have been developed, many of which require extensive animal training prior to tinnitus assessment (Bauer and Brozoski, 2001; Bauer et al., 1999; Guitton et al., 2003; Heffner, 2011; Heffner and Harrington,

Abbreviations: ABR, auditory brainstem response; BBN, broadband noise; ITI, inter-trial interval; SPL, sound pressure level.

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2002; Heffner and Koay, 2005; Jastreboff et al., 1988; Lobarinas et al., 2004, 2006; Ruttiger et al., 2003; Yang et al., 2011). In 2006, Turner and colleagues introduced a high-throughput method for screening tinnitus in rats based on gap detection ability and its effect on the acoustic startle reflex (i.e., the large motoric response to a sudden, loud sound) (Turner et al., 2006). The dependent measure in this traditional gap-startle paradigm is the amplitude of the acoustic startle reflex elicited by a broadband noise (BBN), a reflex that can be suppressed when a silent gap inserted in an otherwise continuous background sound is detected prior to the presentation of the startle stimulus (Ison, 1982; Ison et al., 1991). Turner and colleagues hypothesized that animals would have poorer gap detection ability (as measured by a lack of gap prepulse inhibition of the acoustic startle reflex) if the background sound in which the gap was embedded was qualitatively similar to their tinnitus (i.e., tinnitus would effectively 'fill in' the gap). Several research groups, including our own, have since adopted the gap-startle paradigm to test for behavioral evidence of noise-induced tinnitus in rodents (Dehmel et al., 2012; Engineer et al., 2011; Holt et al., 2010; Kraus et al., 2010; Longenecker and Galazyuk, 2011; Middleton et al., 2011; Turner et al., 2006; Wang et al., 2009; Zhang et al., 2011). In each of these studies, gap detection ability was determined based on the assessment of gap prepulse inhibition, which involved a calculation of the ratio of the startle amplitude generated during trials that contain a brief silent gap versus the amplitude of the startle response in trials without the preceding gap.

Ultimately, for the gap-startle paradigm to be a valid tool to screen for evidence of tinnitus, animals must not only be able to hear the background sound in which the gap is present but also react robustly to the acoustic startle stimulus. Therefore, to help preserve audibility, many researchers have elected to induce tinnitus by exposing animals to loud noise in only one ear (Dehmel et al., 2012; Kraus et al., 2010; Longenecker and Galazyuk, 2011; Middleton et al., 2011; Turner et al., 2006; Wang et al., 2009; Zhang et al., 2011). However, despite using a unilateral noise exposure, a recent study on mice showed a considerable reduction (52%) in the acoustic startle reflex that persisted three months after exposure, even when hearing levels had recovered completely (Longenecker and Galazyuk, 2011). Similar to a recent study (Engineer et al., 2011), our pilot testing revealed that some animals failed to startle following unilateral noise exposure, requiring that they be excluded from further tinnitus assessment, as any attempt to measure gap prepulse inhibition is rendered moot if the animals fail to generate a startle response. Excluding animals not only limits the high-throughput nature of the gap-startle paradigm, but could serve to eliminate animals that may indeed be experiencing tinnitus yet fail to startle. To avoid having to exclude animals, we sought to optimize the gap-startle paradigm to be more resilient to hearing loss.

In the present study, we conducted two separate experiments in which the startle stimulus of the gap-startle paradigm was modified in an effort to better preserve the startle response in rats with unilateral hearing loss. First, in rats exposed unilaterally to loud, high-frequency noise, the traditional BBN startle stimulus was replaced with a bandpass noise (5–10 kHz) in which the sound energy was concentrated at frequencies below the noise exposure (16 kHz) so that the startle stimulus would be more audible to the noise-exposed ear. In a follow-up experiment on a separate group of rats with unilateral conductive hearing loss via an earplug, the acoustic startle stimulus was replaced with a rapid airpuff delivered to the back of the rat's neck to determine if the multimodal (auditory + tactile) nature of the airpuff would help preserve the startle reflex following unilateral hearing loss. Moreover, because a temporary earplug does not produce tinnitus in rats (Bauer and Brozoski, 2001), it was possible to evaluate whether unilateral hearing loss alone could confound the measures of gap prepulse inhibition and lead to a false-positive screening for tinnitus. Ultimately, this study reports experimental parameters which may help to optimize the gap-startle paradigm for tinnitus assessment in animal models, and alerts other investigators to the caveats we have discovered using this common tinnitus screening tool. Preliminary findings of this work were presented in abstract form at the annual meeting of the Association for Research in Otolaryngology (Lobarinas et al., 2012).

2. Materials and methods

2.1. Subjects

At total of 32 adult, male, albino Sprague Dawley SASCO rats (3–5 months, 325–450 g) were used in this study; 26 animals in Experiment 1 and six animals in Experiment 2. Rats were housed in Plexiglas cages, allowed free access to food and water, and were maintained on a normal 12 h light/dark cycle in a temperature-controlled room. All experimental procedures used in the present study were approved by the University at Buffalo- Institutional Animal Care and Use Committee (IACUC).

2.2. Testing apparatus and general procedures

Startle reflex testing was performed by placing each rat in an acoustically-transparent, wire-mesh (0.5 cm × 0.5 cm) cage (20 cm L, 7 cm W, 6 cm H) mounted on a Plexiglas base (20 cm × 10 cm) which rested on a pressure sensitive 35 mm piezoelectric transducer (MCM 28-745) that generated a voltage proportional to the magnitude of the startle response. Prior to animal testing, the baseline noise floor and waveform output of the startle platform were inspected using an oscilloscope and various weights (10–40 g) dropped from a fixed distance (3 cm). The startle platform was placed inside a custom-built, medium density fiber (MDF), sound-attenuating cubicle (57 cm L, 46 cm W, 46 cm H) that was lined with acoustic foam (noise floor <20 dB SPL at frequencies >4000 Hz). Sound stimuli were generated (TDT RX6, ~100 kHz sampling rate), amplified, and delivered via a free-field speaker (Fostex FT17H) placed above the startle platform (25 cm). The sound within the cubicle was calibrated using a Larson Davis sound level meter (SLM 824) and a ½ or ¼ inch condenser microphone. The output of the startle platform was amplified (Behringer ADA8000), digitized and low-pass filtered by an A/D converter (TDT RX8, ~6 kHz sampling rate), and stored on a computer for offline analysis.

2.3. Experiment 1

In pilot experiments using the gap-startle paradigm, a broadband noise (BBN) burst was used to elicit the acoustic startle reflex, as this is the startle stimulus that has been used in all previous reports (Dehmel et al., 2012; Engineer et al., 2011; Holt et al., 2010; Kraus et al., 2010; Longenecker and Galazyuk, 2011; Middleton et al., 2011; Turner and Parrish, 2008; Turner et al., 2006; Wang et al., 2009; Yang et al., 2007; Zhang et al., 2011). However, following unilateral high-frequency noise exposure, it was observed that, although hearing was preserved in the non-exposed ear, the startle response was abolished in many animals, requiring them to be excluded from any tinnitus assessment. We suspected that this impairment in startle reactivity occurred because the saliency of the high-frequency component of the BBN was reduced due to the profile of the hearing loss in the noise-exposed ear. Therefore, we replaced the traditional BBN with a bandpass noise