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Research Report

Methodological optimization of tinnitus assessment using prepulse inhibition of the acoustic startle reflex

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ABSTRACT

Recently prepulse inhibition of the acoustic startle reflex (ASR) became a popular technique for tinnitus assessment in laboratory animals. This method confers a significant advantage over the previously used time-consuming behavioral approaches utilizing basic mechanisms of conditioning. Although this technique has been successfully used to assess tinnitus in different laboratory animals, many of the finer details of this methodology have not been described enough to be replicated, but are critical for tinnitus assessment. Here we provide detail description of key procedures and methodological issues that provide guidance for newcomers with the process of learning to correctly apply gap detection techniques for tinnitus assessment in laboratory animals. The major categories of these issues include: refinement of hardware for best performance, optimization of stimulus parameters, behavioral considerations, and identification of optimal strategies for data analysis.

This article is part of a Special Issue entitled: Tinnitus Neuroscience.

1. Introduction

For several decades pre-pulse inhibition of the startle reflex has been successfully used as a powerful tool to identify various psychiatric disorders in humans and the deficiencies associated with sensorimotor gating in laboratory animals (Braff et al., 2001). The basic parameters of the stimulus paradigm used for this testing have been well studied. The stimulus parameters have been optimized for accurate assessment (Carlson and Willott, 1996; Hoffman and Searle, 1965, Ison et al., 2002, 2005). The neural circuits that relate to this phenomenon have also been identified and intensively studied (Koch and Schnitzler, 1997).

Recently prepulse inhibition of the acoustic startle reflex (ASR) has been adapted and successfully tested as a powerful technique for tinnitus assessment in laboratory animals (Turner et al., 2006). This method confers a significant advantage over the previously used time-consuming behavioral approaches utilizing basic mechanisms of conditioning (Bauer et al., 1999; Guitton et al. 2003; Heffner and Harrington, 2002; Heffner and Koay 2005; Lobarinas et al. 2004; Rüttiger et al., 2003). It does not require animal training. Tinnitus assessment can be done in animals within a single short testing session. This method relies on a reduction of the acoustic startle reflex by a preceding silent gap in an otherwise constant acoustic background. Animals with behavioral evidence of tinnitus cannot detect silence and therefore their reduction of the startle reflex is significantly less than in normal animals. This method has been successfully used to assess tinnitus induced by salicylate overdose or acoustic trauma in rats (Kraus et al. 2010; Turner et al., 2006; Wang et al., 2009; Yang et al., 2007; Zhang et al., 2011) and mice (Longenecker and Galazyuk, 2011;

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Middleton et al., 2011). Many of the finer details of this methodology, however, have not been described enough to be replicated, but are critical for tinnitus assessment. All these details can be roughly divided into four major categories: refinement of hardware for best performance, optimization of stimulus parameters, behavioral considerations, and identification of optimal strategies for data analysis. Thus, the purpose of this paper is to help newcomers with the typically painful process of learning to correctly apply gap detection techniques for tinnitus assessment in laboratory animals.

2. Results

2.1. *Hardware refinement*

Any lab equipment requires tuning for the best performance including those designed to measure the behavioral response of an animal to sensory stimuli. Here we focus on refining a system aimed to assess gap detection performance. This method has recently become popular for tinnitus assessment in laboratory animals. Currently, such systems are commercially available and several labs have designed their own systems. All these systems share the same principals but vary slightly in design. Here we want to emphasize the most general points of system refinement, which we found to be critical for better system performance.

2.1.1. *Addressing an issue of speakers' nonlinearity*

Depending on the design, prepulse inhibition testing stations (boxes) have either one (Sun et al., 2009) or two loud speakers (Turner et al., 2006). In a two speaker system one speaker presents a startle stimulus while another presents either a continuous background interrupted by a gap of silence or a prepulse. Typically, speakers at a given input have nonlinear frequency transfer functions. As a result, such a speaker may have very dissimilar (sometimes more than 15 dB) sound intensities at different frequencies. Fig. 1A shows frequency transfer functions of seven Fostex FT17H speakers calibrated with a 1/4-inch microphone (Brüel and Kjaer 4135). It is important to make adjustments (typically via software) in order to make all frequency outputs roughly equal in amplitude. Such an adjustment is much more critical for a speaker presenting a narrow band noise centered at different frequencies than for a wide band noise startle speaker. Some systems might have the ability to adjust speakers at different testing stations independently. If this option is available, it should be used. However, if your system consists of multiple testing stations but only one correction can be used, you may face a problem. This problem arises from the fact that each speaker may have a different frequency transfer function even though each speaker is the same make and model (Fig. 1A). The question then becomes how to make all speakers' frequency transfer function linear by using just one correction file. The solution is very simple: a set of speakers for all testing stations (possibly even a few extra, for future possible repairs) should be purchased from the same manufacturer with a particular emphasis that the speakers are made during the same manufacturing cycle time (the same batch). Typically, these speakers have very similar frequency transfer functions

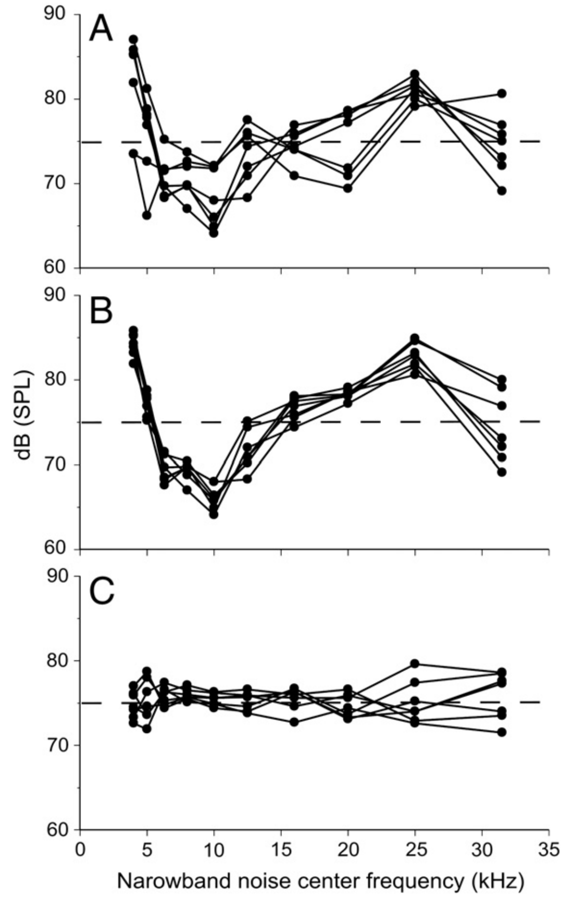

Fig. 1 – Frequency/amplitude output functions for 7 loudspeakers. Dotted line represents the target calibrated amplitude (75 dB) for all frequencies tested. A) Loudspeakers which were made at different manufacturing cycles (batches). B) Loudspeakers which were made at the same manufacturing cycle. C) Loudspeakers shown in B after software adjustment for speaker nonlinearity using one correction file applied to all speakers.

(Fig. 1B). Installation of these speakers will allow for one correction file to adjust the frequency/amplitude output in a linear manner (Fig. 1C). In this case, any given background frequency for the gap detection paradigm of all simultaneously tested animals, in different testing stations, will receive equal sound intensity. This will allow comparisons of results collected from different animals and different testing stations.

2.1.2. *Restrainer issues*

Animal restrainers come in all shapes and sizes. The design of the restrainer is critical in several respects to how an animal will respond to sound stimuli.

First, it is important that the space between the restrainer and the animal should be minimal in an effort to minimize undesired movements during the startle stimulus. The animal should not be able to move freely and should not be able to rear up (Turner et al., 2006; Walton et al., 1997). On the other hand, the animal should not be hindered completely