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A novel behavioral assay for the assessment of acute tinnitus in rats optimized for simultaneous recording of oscillatory neural activity

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HIGHLIGHTS

- A novel behavioral task was developed for detecting tinnitus perception in rats.
- The task was designed for simultaneous recording of neural activity.
- Tinnitus was identified following a single systemic dose of sodium salicylate.
- Significant changes in oscillatory neural activity were detected and discussed.

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ABSTRACT

Background: Human magneto/electrophysiology studies suggest that the phantom sound of tinnitus arises from spontaneous oscillatory neural activity in auditory cortex; however, in animal models, behavioral techniques suitable for testing this hypothesis in combination with electrophysiology recordings have yet to be evaluated. While electrophysiological studies of tinnitus have been reported in passive, awake animals, these studies fail to control for attentional mechanisms likely to play a role in the perception of tinnitus.
New method: A novel appetitive operant conditioning, two-alternative identification task was developed for detecting acute tinnitus in rats. The procedure optimizes conditions for simultaneously recording oscillatory neural activity while controlling for the attentional state of the animal.
Results: Tinnitus was detected in six of seven rats following systemic injection with sodium salicylate (200 mg/kg IP), a known inducer of tinnitus. Analysis of ongoing local field potentials recorded from chronically implanted electrodes in auditory cortex of a rat reporting tinnitus revealed changes in the spectrum of ongoing neural activity.
Comparison with existing method(s): Existing tinnitus-detection methods were not explicitly designed for the simultaneous recording of neural activity. The behavioral method reported here is the first to provide the conditions necessary for obtaining these recordings in chronically implanted rats.
Conclusions: The behavioral assay presented here will facilitate research into the neural mechanisms of tinnitus by allowing researchers to compare the electrophysiological data in animals with confirmed tinnitus.

Abbreviations: AC, auditory cortex; LFP, local field potential; NBN, narrow-band noise; AM, amplitude modulated noise.

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1. Introduction

Subjective tinnitus is the perception of a ringing or hissing sound with no acoustic source. In the United States, an estimated 50 million adults have experienced hearing tinnitus occasionally, and 16 million adults are estimated to experience frequent tinnitus perception (Shargorodsky et al., 2010). Despite decades of research, the neural circuits underlying tinnitus generation remain elusive. Recent human brain imaging studies have generated testable hypotheses regarding the relationships between abnormal patterns of neural activity in specific brain regions and tinnitus perception (see below). In order for these hypotheses to be more rigorously tested, behavioral models of tinnitus should be designed to allow for the simultaneous recording of neural activity during an auditory task.

Several magneto- and electroencephalographic studies in humans have reported abnormal patterns of ongoing neural activity in auditory and non-auditory brain regions of tinnitus subjects. Macroscopic fluctuations in brain activity are measured outside the cranium with magneto- or electroencephalography and reflect subthreshold currents generated within the brain which can be measured as voltage fluctuations called local field potentials (LFPs; Buzsaki et al., 2012). Oscillations of the LFP have been shown to modulate the probability and precise timing of neuron action potentials and have been demonstrated to play critical roles in various aspects of perceptual or cognitive function (for reviews on the topic, Basar et al., 2000; Buzsaki et al., 2012; Fries, 2009; Singer, 1999). Studies in human subjects suggest that specific changes in oscillatory activity are involved in tinnitus perception or an emotional response associated with the tinnitus percept (Adjamian et al., 2012; Ashton et al., 2007; Lorenz et al., 2009; Moazami-Goudarzi et al., 2010; Sedley et al., 2012; van der Loo et al., 2009; Weisz et al., 2005, 2007b; Wienbruch et al., 2006). In particular, tinnitus subjects exhibit a reduction in alpha band activity (~10 Hz; Lorenz et al., 2009; Weisz et al., 2005) with an increase in delta band (1.5–4 Hz; Weisz et al., 2005, 2007a) and gamma band (>30 Hz; Ashton et al., 2007; Llinas et al., 1999; Lorenz et al., 2009; Weisz et al., 2007a) activity compared to control subjects.

While human studies on the neural mechanisms of tinnitus have provided important insights, animal models allow for a more precise and controlled manner of investigation, specifically the induction of tinnitus and its potential reversal. However, a major limitation of most animal studies on tinnitus is that the electrophysiological experiments were performed under anesthesia which significantly alters both spontaneous and sound-evoked neural activity in the auditory system (Kisley and Gerstein, 1999). Since anesthesia abolishes perception, electrophysiological recordings in conscious animals with behaviorally confirmed tinnitus are needed to provide insight into the precise aberrant mechanisms responsible for generating the tinnitus percept. To date, only a handful of electrophysiological studies following manipulations intended to induce tinnitus perception have been reported in conscious, but passive animals (Norena et al., 2010; Yang et al., 2007; Zhang et al., 2011). Electrophysiological studies with animals actively reporting the presence of tinnitus, which would more appropriately model the human studies, have yet to be reported. Thus we endeavored to control for the effect of attention on neural activity by performing electrophysiological recordings when an animal was attending to its acoustic environment and actively reporting the presence of tinnitus via behavioral testing. To our knowledge, this study represents the first behavioral paradigm in animals that has been optimized for the simultaneous recording of neural activity during behaviorally confirmed tinnitus perception.

Since Jastreboff and colleagues first established that tinnitus could be detected in rats (Jastreboff et al., 1988), a variety of behavioral paradigms have been developed for rats and other laboratory animals. Existing behavioral paradigms for tinnitus screening fall into roughly three main categories (for a review on tinnitus behavior paradigms see Turner, 2007): shock avoidance (Bauer et al., 1999; Guitton et al., 2003; Heffner, 2011; Heffner and Harrington, 2002; Jastreboff et al., 1988; Lobarinas et al., 2004), appetitive operant conditioning (Rüttiger et al., 2003), and gap pre-pulse inhibition of the acoustic startle reflex (Berger et al., 2013; Dehmel et al., 2012; Longenecker and Galazyuk, 2012; Turner et al., 2006; Turner and Parrish, 2008; Tzounopoulos, 2008; Yang et al., 2007). It is important to note that most of these behavioral paradigms include features that would make them incompatible for simultaneously measuring ongoing oscillatory neural activity while screening for tinnitus. Specifically, in order to properly assess oscillatory components of the LFP, neural activity needs to be sampled continuously over several seconds and therefore the behavioral paradigm requires relatively long and stable epochs. This requirement excludes most existing behavioral paradigms which detect tinnitus using discrete stimuli such as brief silent gaps in noise or tone-burst detection. Furthermore, behavioral paradigms that rely on sound-evoked responses would likely interfere with tinnitus-related neural activity that occurs spontaneously in the absence of sound. To overcome these limitations, we developed a novel two-alternative choice identification task for the detection of tinnitus in rats. This method optimized conditions for acquiring electrophysiological data during several seconds while performing a behavioral task in which the rat reports hearing a steady sound during quiet intervals; i.e. tinnitus.

In order to evaluate the efficacy of our paradigm, temporary tinnitus was induced in trained rats by systemic injection of sodium salicylate which reliably induces temporary tinnitus in normal hearing humans (Mongan et al., 1973) and rats (Jastreboff et al., 1988; Lobarinas et al., 2004). To illustrate its utility, we include a subset of electrophysiological recordings from a chronically implanted electrode in auditory cortex (AC) of a rat concurrently reporting tinnitus.

2. Materials and methods

2.1. Objective

To obtain estimates of ongoing oscillatory neural activity from the AC during tinnitus, we developed a novel two-alternative choice identification task for the detection of tinnitus in rats. The use of rats in this project was approved by the Institutional Animal Care and Use Committee at the University of Buffalo and was carried out in accordance with National Institutes of Health guidelines.

2.2. Behavior apparatus

The behavioral apparatus was constructed of transparent acrylic walls and equipped with 3 infrared (IR) detectors; one located at the center nose poke and one at the right feeder trough and one at the left feeder trough (Vulintus, Dallas, TX, USA or MedAssociates Inc., St. Albans, VT, USA). A bright light (green or white light-emitting diode) was located directly above the center nose poke and served as the GO cue (Fig. 1). The behavior apparatus was illuminated by an ambient house light and housed in a double-walled, electrically shielded sound-attenuating cubicle constructed of 2 layers of dense fiber board.

The behavior apparatus was computer-controlled with custom software written in Matlab (MathWorks, Nattick, MA, USA) interfaced with real-time processing hardware (RX6-5, Tucker-Davis Technologies, Alachua, FL, USA). The speaker (FT28D, Fostex, Tokyo, Japan) was located on the roof of the cage. The intensity of the acoustic stimuli used was calibrated with a microphone (1/4″ mic