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Research Report

Establishment of auditory discrimination and detection of tinnitus induced by salicylic acid and intense tone exposure in the rat

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ABSTRACT

Rats were trained in a two lever food reinforced operant procedure to discriminate a 8000 Hz pure tone stimulus from its absence. Responding on one lever was reinforced in the presence of the tone and responding on the other lever was reinforced when the tone was absent. Frequency generalization testing yielded an inverted U-shaped function, whereas sound pressure level generalization testing yielded a continuous decrease in responding on the tone associated lever with decreasing sound pressure levels. The administration of sodium salicylic acid (150–450 mg/kg) generated responding on the tone associated lever suggesting that salicylic acid induced an experience that had commonalities with the percept of the training tone stimulus. After exposure to intense sound, responding consistent with the presence of tinnitus was achieved and Lidocaine failed to reduce tinnitus behavior. The use of a two choice design helped avoid confounding factors induced by drug induced side effects. Further, since no auditory cues were employed in the test situation the model achieves resistance to potential bias due to hearing impairment and hyperacusis. We propose that this model may be useful in detecting tinnitus.

1. Introduction

Tinnitus is considered an auditory experience that lacks external source. Continuous tinnitus is found in about 5–15% of the general population and is commonly associated with hearing impairment (Eggermont and Roberts, 2004). However, tinnitus may also occur after drug medication (Eggermont, 2005). For example, prolonged treatment with salicylic acid (SA) can generate tinnitus with a dose dependent intensity (Day et al., 1989; Cazals, 2000). Also, the use of other anti-inflammatory drugs, antimalarials, amino glycosides, antineoplastics and loop diuretics are associated with the occurrence of ototoxicity and tinnitus (Alvan et al., 1991; Jung et al., 1993; Rybak, 1986; Seligmann et al., 1996). The pharmacological diversity of drugs that may cause tinnitus highlights the need for valid behavioral animal methods for the detection of tinnitus as a side effect in drug development, consistent with recommendations by the International Conference on Harmonization (ICH) guideline S7A (ICH S7A, 2000).

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Efforts to establish behavioral correlates of tinnitus have a relatively short history during which SA treatment (Jastreboff et al., 1988; Bauer et al., 1999; Guitton et al., 2003; Ruttiger et al., 2003; Lobarinas et al., 2004; Moody 2004; Yang et al., 2007; Ralli et al., 2010) or traumatizing sound exposure (Bauer and Brozoski, 2001; Brozoski et al., 2002; Heffner and Harrington, 2002; Heffner and Koay, 2005; Kraus et al., 2010; Turner et al., 2012) have been used to induce tinnitus in rats, hamsters, chinchillas, mice and guinea pigs (see further overviews in Kaltenbach, 2011; Eggermont, 2013). In the pioneering work of Jastreboff et al. (1988), bottle licking was conditioned by electric shock treatment to occur in periods of noise and withheld during silence. Salicylic acid treatment produced licking behavior in periods of silence indicating the presence of tinnitus. This type of approach represents a "*go/no-go*" type of model as the behavioral expression of tinnitus (e.g. bottle licking) is either produced or withheld (e.g. Burdick, 1979).

With two exceptions (Moody, 2004; Heffner and Koay, 2005) the above mentioned models represent the "go/no-go" category. In a brief description of ongoing work to develop an operant model (Moody, 2004), data from a guinea-pig trained to respond for food on one lever in the presence of a tone and on the other when the tone was absent, indicated that SA shifted the pattern of lever pressing behavior to the tone lever suggesting the presence of tinnitus. Heffner and Koay (2005) presented a model in which animals were trained to lick the left or right hand sipper tube according to the location of a sound source. Licks at the sipper tube positioned at the same side as the sound source were reinforced with water access whereas an electric shock followed licks at the other side. Subsequent exposure to a traumatizing tone in one ear generated licking behavior at the sipper tube located at the same side as the traumatized ear. This suggested that tinnitus was unilateral and localized at the side of injury. Hence, these methods (Moody, 2004; Heffner and Koay, 2005) represent efforts to measure tinnitus relying on the direction at which the animal displays a specific behavior and represent a two choice approach ("*go-left/go-right*"; e.g. Burdick, 1979).

The first aim of the present work was to develop and characterize an operant procedure in rats to detect the presence of drug induced tinnitus with the intention of being able to identify tinnitus as a drug induced side effect. For this purpose, designing a method resistant to bias due to other drug side effects such as hearing loss, hyperacusis, motor impairment or loss of motivation was essential. Further, these potentially confounding factors need to be controlled simultaneously with the actual measurement of tinnitus. For this task, a two choice food reinforced approach was selected. This approach is commonly used in the drug discrimination paradigm (Colpaert and Slangen, 1982) in which animals are trained to discriminate a drug stimulus from its absence in a two lever choice procedure. To earn food pellets, the animals are required to respond on one lever in the presence of a specific dose of a specific drug stimulus, and on the other lever in the absence of the drug stimulus. A key advantage of this approach lies in its potential to provide control over drug influence on factors that may influence the ability to respond on the levers or the motivation to work for the reinforcer. For example, a drug that impairs motor performance or reduces the motivation to eat will affect responding on either lever and not one of the levers specifically. Therefore, it will not affect the choice of levers in itself since the two stimulus conditions (drug/no drug) are programmed to require an equal work task to result in the reinforcer. As a result, drug side effects are unlikely to affect the discriminative measure itself and consequently this approach would improve the possibility to attain an unambiguous measure of tinnitus.

To establish auditory discriminative behavior, we modified the drug discrimination methodology on the assumption that interoceptive stimuli such as drug stimuli and sensory stimuli such as auditory stimuli share some common features that may be used as discriminative cues for rats (Heinemann and Chase, 1975; Colpaert et al., 1978; Swedberg and Järbe, 1986; Järbe and Swedberg, 1998). The characteristics of externally produced stimuli have previously been compared to drug stimuli and it has been suggested that the principal difference between drugs and conventional sensory stimuli lie in their route of administration (Overton, 1971). Further, in the drug discrimination literature the characteristics of drug stimuli have been described in terms of cue sensitivity and relative salience (Colpaert et al., 1980; Colpaert and Janssen, 1982a,b; Swedberg and Järbe, 1985, 1986). Recently, we described the utility of an operant discrimination technique to study visual cues and the utility of a visual discrimination model for studying drug side effects on the visual system (Carlsson and Swedberg, 2010). We demonstrated that stimulus control was maintained and that the visual effects could be separated from motor and motivational side effects.

Tinnitus induced by SA is generally described as a tone (Cazals, 2000; Day et al., 1989). Therefore, a pure tone and its absence were used as discriminative stimuli signaling the availability of reinforcement by pressing the correct (left or right) lever. After establishing auditory discriminative behavior, rats were tested for generalization to other frequencies and sound pressure levels (SPLs) to investigate the pitch and intensity characteristics of the auditory discrimination. Salicylic acid was subsequently employed to investigate to what extent this model could be employed to study the occurrence of drug induced tinnitus.

Further, these studies addressed the detection of tinnitus occurring as a consequence of exposure to intense sound. Tinnitus originating from intense sound exposure is common and there is a need to develop specific behavioral methods to advance its understanding (Kaltenbach, 2011; Eggermont, 2013). We trained rats to discriminate a tone from silence, exposed them to intense sound and tested for tone lever responding in a quiet test chamber. We subsequently explored the efficacy of Lidocaine treatment to reverse the tinnitus related behavior.

2. Results

2.1. *Experiment 1: effects of frequency and sound pressure variations on tone lever responding*

Three groups of rats were trained in this experiment. In a representative group, rats were shaped, trained and tested daily during a period of 2–3 months with a mean of 430 sessions