Journal of Neuroscience Methods 213 (2013) 188–195

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

A novel behavioural approach to detecting tinnitus in the guinea pig

Joel I. Berger*, Ben Coomber, Trevor M. Shackleton, Alan R. Palmer, Mark N. Wallace

MRC Institute of Hearing Research, University Park, Nottingham, UK

HIGHLIGHTS

- Prepulse inhibition can be reliably and robustly measured using the Preyer reflex.
- The Preyer reflex is a more reliable response than the whole-body startle in guinea pigs.
- Salicylate impairs gap detection at specific background noise frequencies, indicating the presence of tinnitus.
- Measuring gap detection using the Preyer reflex is a suitable method for identifying tinnitus.

ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form
30 November 2012
Accepted 22 December 2012

Keywords:
Tinnitus
Guinea pig
Preyer reflex
Pinna reflex
Gap detection
Prepulse inhibition
Sodium salicylate
Whole-body startle

ABSTRACT

Tinnitus, the perception of sound in the absence of an external stimulus, is a particularly challenging condition to demonstrate in animals. In any animal model, objective confirmation of tinnitus is essential before we can study the neural changes that produce it. A gap detection method, based on prepulse inhibition of the whole-body startle reflex, is often used as a behavioural test for tinnitus in rodents. However, in the guinea pig the whole-body startle reflex is subject to rapid habituation and hence is not an ideal behavioural measure. By contrast, in this species the Preyer or pinna reflex is a very reliable indicator of the startle response and is much less subject to habituation. We have developed a novel adaptation of the gap detection paradigm, which uses the Preyer reflex to measure the startle response, rather than whole-body movement. Using this method, we have demonstrated changes in gap detection, in guinea pigs where tinnitus had been induced by the administration of a high dose of salicylate. Our data indicate that the Preyer reflex gap detection method is a reliable test for tinnitus in guinea pigs.

1. Introduction

Tinnitus is estimated to chronically affect 10–15% of the UK population at some point in their life, and in severe cases is linked to depression and even suicide (Heller, 2003). No effective cure for tinnitus has been developed to date, in part because the pathophysiological mechanisms responsible are still not understood. The development of animal models of tinnitus has played an essential role in furthering our knowledge of the physiological and molecular mechanisms that underlie this condition (Kaltenbach, 2011). The most common trigger for the condition is repeated exposure to excessive loud noise (Eggermont and Roberts, 2004), but among others it can also occur as a result of the effects of ototoxic drugs such as sodium salicylate or quinine (Ralli et al., 2010). Sodium salicylate is frequently the cause of tinnitus when patients receive high doses of aspirin (Cazals, 2000). Salicylate has been shown to reliably induce transient tinnitus in an animal model, often with less variability than noise exposure (Stolzberg et al., 2012) and over a much shorter time-scale (5 h as opposed to 8 weeks). The neural mechanisms are likely to be different to those observed following noise exposure, but it is still a useful experimental model for tinnitus.

In 1988, Jastreboff et al. developed the first behavioural test for tinnitus in rats that relied on a conditioned response; this test has since been refined (Bauer and Brozoski, 2001; Heffner and Harrington, 2002). In 2006, Turner et al. devised a new test for tinnitus that did not rely on a conditioned response, but used the whole-body startle (WBS) reflex which did not require time-consuming training periods. Moreover, this test did not rely on memory formation, a potential confound in studies of the origins and treatment of tinnitus, and enabled tinnitus-related changes to be tracked over time as behavioural extinction is not a factor. As previous work demonstrated a lag between acoustic trauma and tinnitus manifestation, in some cases as much as 8–9 weeks (Turner et al., 2006), this is a useful characteristic of the model.

* Corresponding author at: MRC Institute of Hearing Research, Nottingham University Section, University Park, Nottingham, NG7 2RD, UK.
Tel.: +44 115 9518108x253; fax: +44 115 9518503.
E-mail address: joel@ihr.mrc.ac.uk (J.I. Berger).

0165-0270 © 2013 Elsevier B.V. Open access under CC BY license.
http://dx.doi.org/10.1016/j.jneumeth.2012.12.023

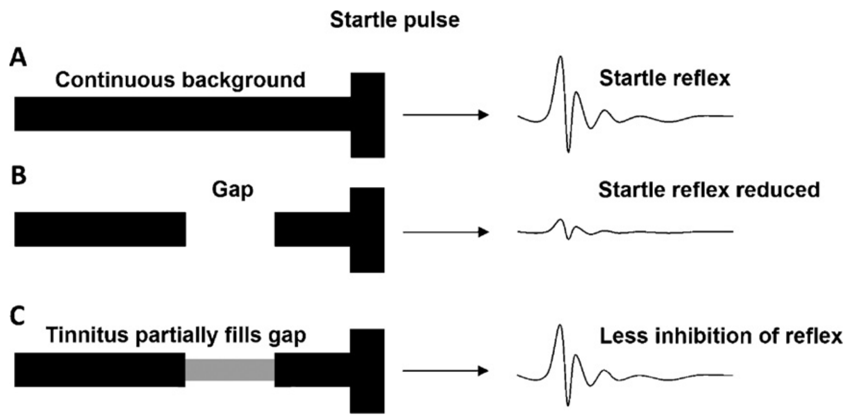

Fig. 1. Schematic of the gap detection test, adapted from Turner and Parrish (2008). A startling pulse in continuous background noise elicits a startle reaction (A). When a gap (50 ms) is presented before the startling pulse, this reduces the amplitude of the startle response (B). When an animal is experiencing tinnitus, it will have difficulty detecting the gap, as this will be partially filled in by the tinnitus, and will show less inhibition of the startle response (C).

The paradigm devised by Turner et al. (2006) was based on the phenomenon of prepulse inhibition (PPI). A loud sound causes an animal to jump: the whole body startle (WBS) reflex. When the startling sound is cued by a preceding sound, the magnitude of the startle response is reduced (Hoffman and Searle, 1965). In the tinnitus test (Turner et al., 2006), the startle pulse was always preceded by a quiet narrow-band continuous noise (Fig. 1A). This does not alter the magnitude of the WBS, but introducing a short silent gap provides a cue for the startle pulse and PPI occurs (Fig. 1B). They assessed PPI in rats with acoustic trauma and, by varying the centre frequency of the narrow-band background noise, demonstrated selective deficits in PPI that they reasoned were due to tinnitus – with a similar spectrum to the background noise – filling the gap preceding the startle stimulus and obscuring the preceding cue (Fig. 1C); this method was validated through comparison with an operant conditioning model (as used by Bauer and Brozoski, 2001).

Although the rat has been widely used as an animal model of tinnitus, introducing further animal models will provide a broader understanding of the mechanisms leading to tinnitus, across a range of mammals, including humans. The auditory system of guinea pigs is quite typical of most mammals and their low frequency hearing sensitivity is very similar to that of humans (Prosen et al., 1978; West, 1985) providing a good species for comparison (Harrison et al., 1981).

Guinea pigs are difficult to train in complex behavioural tasks, rendering the use of the tinnitus test developed by Jastreboff et al. (1988), or that of Bauer and Brozoski (2001) or Heffner and Harrington (2002), highly problematic in this species. Although a number of studies have demonstrated WBS PPI deficits in rats, only two studies have thus far used the method for measuring deficits in guinea pigs (Dehmel et al., 2012a,b). Like the WBS, the auditory-evoked pinna or Preyer reflex is an unconscious response (Bohmer, 1988). The neural circuitry mediating the Preyer reflex has also been defined; the cochlear nucleus (CN), superior olivary complex, and the inferior colliculus (IC) all play a role, as well as regions of the reticular nucleus and facial motor nuclei (Li and Frost, 1996). Furthermore, the variables that can change the reflex are well-understood, *e.g.*, the length of the gap, delay between gap and startle stimulus, or drug intervention (Davis et al., 1993; Leitner et al., 1993; Swerdlow et al., 2001). We hypothesised that utilising the Preyer reflex to quantify startle response and gap detection may provide a useful alternative to the WBS in this behavioural test for tinnitus. Often used as a gross measure of hearing in rodents (Jero et al., 2001), the parameters involved in modulating this response are well-established. Furthermore, the Preyer reflex is susceptible to change induced by PPI (Cassella and Davis, 1986). We demonstrate that in guinea pigs the Preyer reflex is a more reliable and consistent measure of PPI than WBS.

2. Materials and methods

2.1. Animals

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986, UK and the approval of the ethical review committee at the University of Nottingham, UK. Experiments were conducted on a total of 24 male and female pigmented guinea pigs (GPs) from an in-house colony weighing 300–500 g at onset of behavioural testing. GPs were group-housed on a 12:12 h light:dark cycle, and food and water were freely available.

2.2. Measuring whole-body startle

The ability of a GP to detect a gap in background noise – and consequently produce PPI – was assessed by quantifying whole-body movement in response to the startling stimulus. GPs were placed in a wire cage (310 mm × 155 mm × 155 mm) on a custom-made startle platform in a sound proof booth; GPs were not restrained and were free to move around the cage. The startle platform was connected to a load-cell (3 kg capacity; Model 1022, Vishay Tedea-Huntleigh, Basingstoke, UK) to measure the downward force applied to the platform following a startling acoustic stimulus. The output from the load-cell was amplified by a factor of 1000 and recorded in Adobe Audition (Adobe Systems Incorporated, San Jose, CA) *via* a Tascam US-122 external sound card (44.1 kHz sampling rate, 16-bit resolution; TEAC Professional Division, USA). Synchronisation pulses were recorded simultaneously with the signal from the load-cell; pulses of different size denoted either a 'gap' or 'no gap' presentation. The signal was low-pass filtered at 200 Hz *post hoc*.

2.3. Preyer reflex

In addition to measuring the WBS response, we used a motion-tracking camera system to monitor flexion of the pinna. The motion tracking system (Vicon Motion Systems, Oxford, UK) consisted of four infrared cameras. A reflective marker (4 mm diameter) was attached to each pinna using cyanoacrylate adhesive (Fig. 2), and an additional marker was attached to a central point, usually in the middle of the back, to determine the orientation of the animal. The motion-tracking system used these markers to triangulate the position of the ears, and subsequently to track pinna movement during