Hearing Research 290 (2012) 64–71

Contents lists available at SciVerse ScienceDirect

Hearing Research

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

Research paper

Isoflurane blocks temporary tinnitus

Madeleine Norman, Katherine Tomscha, Michael Wehr*

Institute of Neuroscience, Department of Psychology, 1254 University of Oregon, Eugene, OR 97403, USA

ARTICLE INFO

Article history:
Received 19 December 2011
Received in revised form
21 February 2012
Accepted 21 March 2012
Available online 22 May 2012

ABSTRACT

Temporary tinnitus is a common consequence of noise exposure, and may share important mechanisms with chronic tinnitus. Noise-induced hearing loss is the most prevalent cause of chronic tinnitus. The reversibility of temporary tinnitus offers some practical experimental advantages. We therefore adapted a behavioral method based on gap detection to measure temporary tinnitus following brief acoustic trauma. Although anesthesia is often used during acoustic trauma exposure, many anesthetics can protect against noise-induced hearing loss. Whether anesthesia during acoustic trauma affects temporary tinnitus therefore remains an open question that directly affects experimental design in tinnitus studies. Here we tested whether anesthetizing rats with isoflurane during trauma had any effect on tinnitus. We found that gap-detection deficits, a behavioral measure of tinnitus, were 5 times stronger and lasted 10 times longer when isoflurane was not used. This suggests that isoflurane largely prevents temporary noise-induced tinnitus.

1. Introduction

Chronic tinnitus is widespread and costly, with up to 10% of the U.S. population reporting severe tinnitus (Cooper, 1994; Nondahl et al., 2002; Shargorodsky et al., 2010), and $1.3 billion in Veterans Administration tinnitus-related disability compensation expended in 2010. Subjective tinnitus following noise-induced hearing loss is the most common form of chronic tinnitus (Vernon and Møller, 1995). Temporary tinnitus is far more common than chronic tinnitus – nearly everyone has experienced a temporary ringing in the ears after intense sound exposure such as a loud concert or a gunshot. Although noise-induced temporary tinnitus does not pose the debilitating health concerns that chronic tinnitus does, it may share critical mechanisms with chronic tinnitus caused by noise-induced hearing loss. The reversibility of temporary tinnitus offers practical experimental advantages, such as the ability to compare brain activity when the animal is or is not experiencing tinnitus. For example, temporary tinnitus produced by high doses of salicylate alters both driven and spontaneous activity levels and inhibitory function at multiple levels of the auditory system, including dorsal cochlear nucleus, inferior colliculus, and auditory cortex (Bauer et al., 2000; Holt et al., 2010; Ralli et al., 2010; Stolzberg et al., 2011; Zhang et al., 2011). However, because drug-induced tinnitus and noise-induced tinnitus may arise from distinct brain mechanisms (Eggermont, 2008; Sun et al., 2009; Norena et al., 2010), a method for inducing temporary noise-induced tinnitus is likely to be useful. This approach may be able to provide insight into the mechanisms underlying chronic tinnitus. We therefore sought to develop a rapid and robust method for inducing and behaviorally measuring temporary tinnitus.

Pure-tone acoustic trauma causes temporary tonal tinnitus in humans. Although relatively few studies have been performed (Loeb and Smith, 1967; Atherley et al., 1968; Chermak and Dengerink, 1987), it is clear that exposure to 5 min of a 110 dB sound pressure level (SPL) pure tone causes temporary, tonal tinnitus, lasting from 15 to 50 min, in most (70–90%) human subjects. The relationship between tinnitus pitch and trauma frequency appears to be variable, although tinnitus pitch tends to increase with increasing trauma frequency, and tinnitus pitch does not necessarily match the frequency of maximum temporary hearing threshold shift.

Most behavioral measures of tinnitus in rats, such as conditioned suppression of licking (Jastreboff et al., 1988) or lever pressing (Bauer et al., 1999; Bauer and Brozoski, 2001), or avoidance conditioning (Lobarinas et al., 2004; Yang et al., 2007), require extensive training (weeks to months) to achieve reliable performance. Here we used an alternative method based on gap detection, recently developed by Turner and colleagues (Turner et al., 2006; Yang et al., 2007). Because it takes advantage of the naturally occurring startle reflex, this gap-detection paradigm requires no training, and can be reliably measured in naive rats. The method is based on the detection of gaps in continuous narrow-band background noise that is designed to mimic a tinnitus percept.

* Corresponding author.
E-mail address: wehr@uoregon.edu (M. Wehr).

0378-5955/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2012.03.015

Turner et al. (2006) showed that a 1 h acoustic trauma produced a gap-detection deficit in rats, and interpreted this as consistent with a tinnitus percept that fills in the silent gap and impairs its detection. Here we adapted this method to measure, with a temporal resolution of about 15 min, the time course of temporary tinnitus following a brief (2-min) acute acoustic trauma.

We initially used isoflurane anesthesia during trauma exposure in order to minimize the possibility of discomfort to the animal during the intense pure tone. Isoflurane offers the advantage of rapid onset and recovery. Because the animal is only anesthetized for 3–4 min, we can measure gap detection immediately before and directly after acoustic trauma. However, isoflurane has recently been reported to have a neuroprotective effect on noise-induced hearing loss in mice (Kim et al., 2005; Chung et al., 2007). This could block temporary tinnitus by preventing noise-induced hearing loss. Some experimental studies of chronic tinnitus have used anesthesia during exposure (isoflurane: Kraus et al., 2010; ketamine-xylazine: Bauer and Brozoski, 2001; Turner et al., 2006), but some do not (Milbrandt et al., 2000; Norena and Eggermont, 2005; Holt et al., 2010; Norena et al., 2003). Whether isoflurane anesthesia during acoustic trauma prevents temporary tinnitus therefore remains an open question that directly affects experimental design in tinnitus studies. Here we took advantage of the reversible nature of temporary tinnitus to directly test the effect of isoflurane on temporary tinnitus by comparing tinnitus in the same animals with and without the use of isoflurane. Animals were exposed to acoustic trauma while anesthetized on one day, and while unanesthetized on a different day (in counterbalanced order). We found that tinnitus, as measured by gap-detection deficits, was dramatically stronger and longer in duration when isoflurane was not used. This suggests that isoflurane blocks temporary noise-induced tinnitus.

2. Methods

All procedures were in accordance with the National Institutes of Health guidelines as approved by the University of Oregon Animal Care and Use Committee. Our sample size was 10 rats (Sprague–Dawley, Harlan Laboratories), both males and females, between 25 and 50 days old. Each animal underwent two experimental sessions: once with isoflurane anesthesia during acoustic trauma, and once without. The two sessions were separated by 2–12 days (median: 3 days). The design was counterbalanced such that 5 animals were anesthetized during trauma on the first experimental session, and the other 5 were anesthetized during trauma on the second session. Reported errors are standard deviations unless otherwise noted.

2.1. Gap detection

To measure tinnitus, we used a modified version of a behavioral gap-detection paradigm originally developed by Turner et al. (2006), who validated the method with an independent behavioral measure of tinnitus (Bauer and Brozoski, 2001). In this paradigm, we quantified perceptual gap detection using a variation of pre-pulse inhibition of the acoustic startle response. The method is illustrated in Fig. 1 with an example of behavior from one animal. A rat was placed in a custom wire-mesh and plexiglass chamber resting on a pressure sensor, inside an acoustic isolation chamber. Sounds were delivered free field with a Fountek NeoCD2.0 Ribbon Tweeter, located 10–12 cm in front of and facing the animal. Prior to each experiment, the speaker was calibrated with a Bruel & Kjaer 4939 ¼″ microphone placed where the rat's ear would be. Rats startled in response to a white noise burst (e.g., a 100 dB SPL, 25 ms white noise burst), producing a characteristic deflection of the pressure sensor output (black traces in Fig. 1a). We quantified the startle response as the peak deflection, averaged across trials. The experiment was conducted in the presence of continuous, narrow-band background noise ($^1/_3$ octave, center frequency 6 kHz). A 50 ms gap in this background noise, which preceded the startle stimulus by 50 ms (measured from gap offset), reduced the probability and amplitude of the startle response (Fig. 1a–b). This inhibition of the startle response served as a measure of perceptual gap detection. Because of individual differences in the parameters

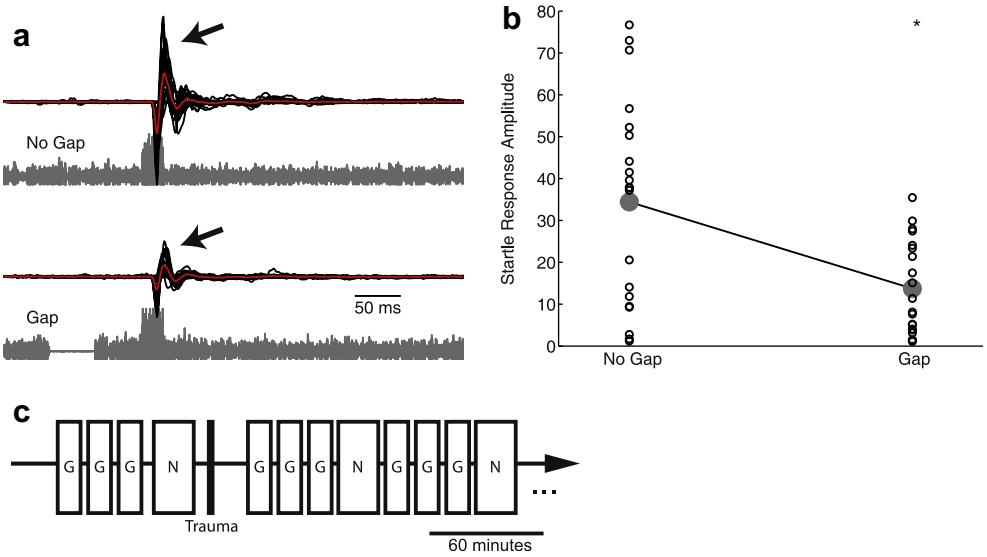

Fig. 1. Gap detection measure of temporary tinnitus. a) Example of startle responses (arrows) of an animal to a white noise burst embedded in background narrow-band noise. Top panel shows the startle response to the noise burst presented in isolation (black lines: 20 individual trials; red line: mean across trials; grey line: stimulus). Stimulus is clipped. Bottom panel shows that the startle response is reduced when the white noise burst is preceded by a 50 ms gap in the background noise. Startle response amplitude is in arbitrary units. b) Peak startle response amplitudes for the raw data shown in (a). Black circles: 20 individual trials; grey dots: mean across trials; *indicates that the gap caused a significant decrease in peak startle amplitude ($p < 10^{-2}$). The decreased startle response demonstrates successful gap detection by the animal, with a tinnitus index (see Methods) of 0.0002 (i.e., no tinnitus). c) Schematic of time course for a typical experiment (G: gap detection task, N: noise detection task. Blocks of tasks were repeated (indicated by…) until performance returned to baseline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

required to obtain good gap detection, startle pulse amplitude and background noise amplitude were tailored to each animal prior to each experimental session (range: 90–100 and 70–85 dB SPL, respectively). The interval between successive startle stimuli was randomized (uniformly distributed between 10 and 20 s, with a mean interval of 15 s).

A deficit in this gap-detection measure is consistent with the presence of tinnitus that is perceptually similar to the background noise, filling in the gap and making it less salient (Turner et al., 2006). We therefore quantified gap-detection deficits using a measure we termed the tinnitus index (TI), which varies from 0 (no tinnitus, i.e., successful gap detection) to 1 (tinnitus, i.e., a gap-detection deficit):

$$\mathrm{TI} = 1 - \int_{-\infty}^{t} \frac{\Gamma\left(\frac{n}{2}\right)}{\frac{n-1}{2}} \frac{1}{\pi\sqrt{n-1}} \frac{1}{\left(1+\frac{t^2}{n-1}\right)^{\frac{n}{2}}} \mathrm{d}t,$$

where,

$$t = \frac{\overline{X}_{\mathrm{NoGap}} - \overline{X}_{\mathrm{Gap}}}{s \cdot d \cdot (X_{\mathrm{NoGap}} - X_{\mathrm{Gap}})/\sqrt{n}},$$

X are peak startle response deflections for Gap and No-Gap conditions, n is the number of trials, s.d. indicates standard deviation, Γ indicates the gamma function, and $-$ indicates averaging across trials. Note that TI as computed here is mathematically equivalent to the p-value returned by a 1-sided paired-t-test, which is a convenient way to calculate it. Intuitively, TI (like the p-value of a t-test) should be close to zero when perceptual gap detection occurs, since there is a large difference in the startle responses between the Gap and No-Gap conditions. When the animal cannot detect the gap, the responses are equivalent in the two conditions and the TI increases in value. TI is an attractive measure of gap-detection deficits because it takes into account both the mean and the variance of startle responses, and increases in value with increasing behavioral evidence for tinnitus. It is important to note that TI should not be literally interpreted as a p-value, since we measured TI repeatedly over the time course of an experiment. Moreover, any choice of a cutoff TI value to indicate the presence or absence of tinnitus is arbitrary. In Fig. 2d, we operationally defined the duration of tinnitus as the amount of time TI exceeded 0.05, but varying this cutoff over a wide range (0.001–0.25) did not change the significance of the result in Fig. 2d. More generally, the use of an arbitrary cutoff can be avoided by comparison of TI with baseline levels, as in Fig. 2a–b. A commonly used measure of gap detection deficits is the percentage by which the startle response is inhibited by the presence of the gap (percent gap-induced pre-pulse inhibition of acoustic startle, or %GPIAS, Turner et al., 2006; Yang et al., 2007). We computed %GPIAS as

$$\%\mathrm{GPIAS} = \frac{\overline{X}_{\mathrm{NoGap}} - \overline{X}_{\mathrm{Gap}}}{\overline{X}_{\mathrm{NoGap}}} \cdot 100$$

We computed the maximal change in %GPIAS by subtracting the mean of the three baseline measurements from each value, and taking the maximal change.

We used 6 kHz background noise based on the outcome of pilot studies. In preliminary experiments (data not shown), we observed that 17 kHz pure-tone trauma produced a strong gap-detection deficit when we used narrow-band background noise with center frequency of 6 kHz, but not when other center frequencies were used, consistent with a previous study that reported tonal tinnitus below the trauma frequency (Turner et al., 2006).

2.2. Acoustic trauma

We exposed animals monaurally to a 115 dB SPL, 17 kHz pure-tone for 2 min. All animals were briefly (~5 min) anesthetized with isoflurane (2.5–3%) while the left ear was plugged with silicone elastomer. Before plugging the ear, a very light coat of mineral oil was applied to the pinnae to ensure easy removal of the plug after acoustic trauma. Animals were then either allowed to recover in their home cage until bright, alert, and responsive (usually <5 min), or maintained on isoflurane anesthesia during acoustic trauma. The tone was delivered with a PO-55T piezo high frequency tweeter located ~2 cm from the right ear. Tone level was measured during exposure with a Bruel & Kjaer 4939 ¼″ microphone placed next to the ear. After trauma, anesthesia was discontinued (if present) and the plug was removed. Thus both groups of animals were briefly anethetized with isoflurane, and animals that were anesthetized during trauma were only under anesthesia 3–4 min longer than animals that were only anesthetized for ear-plugging. After trauma, animals were returned to the acoustic startle chamber. Animals often showed a brief (<10 min) period of elevated activity after recovery from anesthesia, and to ensure that this did not interfere with startle response measurements we waited until activity levels (as measured by pressure sensor output) returned to baseline (usually 10–20 min) before resuming data collection. Note that this applied both to animals anesthetized during trauma and to animals anesthetized only for ear-plugging. The time elapsed between trauma and the first startle response measurement was not different for animals anesthetized during trauma (26.2 ± 9.9 min) or not anesthetized during trauma (33.7 ± 20.9 min; n.s., $p > 0.3$).

2.3. Narrow-band pre-pulse inhibition

To control for the possibility that hearing loss, rather than tinnitus, accounted for gap-detection deficits, we also assessed perceptual hearing loss with a narrow-band pre-pulse inhibition paradigm in each animal. In this noise-detection paradigm, there was no continuous background noise. The startle stimulus was preceded by a narrow-band pre-pulse, which reduced the startle response. The bandwidth and center frequency ($^1/_3$ octave, 6 kHz) of the pre-pulse were identical to those of the background noise used in the gap-detection paradigm. We included a range of pre-pulse levels, in order to test not just the level used in the gap-detection paradigm, but lower levels as well. As with the gap-detection paradigm, the interval between successive startle stimuli was randomized (uniformly distributed between 10 and 20 s, with a mean interval of 15 s).

We quantified noise detection by comparing the peak startle responses for trials with a pre-pulse to the responses on trials without a pre-pulse (using a paired, one-sided t-test). We reasoned that if a given pre-pulse level significantly reduced the startle response, then the animal's detection threshold must be below the amplitude of the pre-pulse for that center frequency and bandwidth. This provides an upper bound for the hearing threshold, allowing us to test for the possibility that hearing loss accounts for a gap-detection deficit.

2.4. Experimental design

The sequence of trials is shown in Fig. 1c. Each gap-detection trial (G in Fig. 1c) consisted of 40 repetitions of the startle stimulus (20 with a gap, and 20 without, randomly interleaved) and took 10–13 min. This means that the temporal resolution with which we could measure the time course of tinnitus is limited to 10–13 min. Each narrow-band noise-detection trial (N in Fig. 1c)

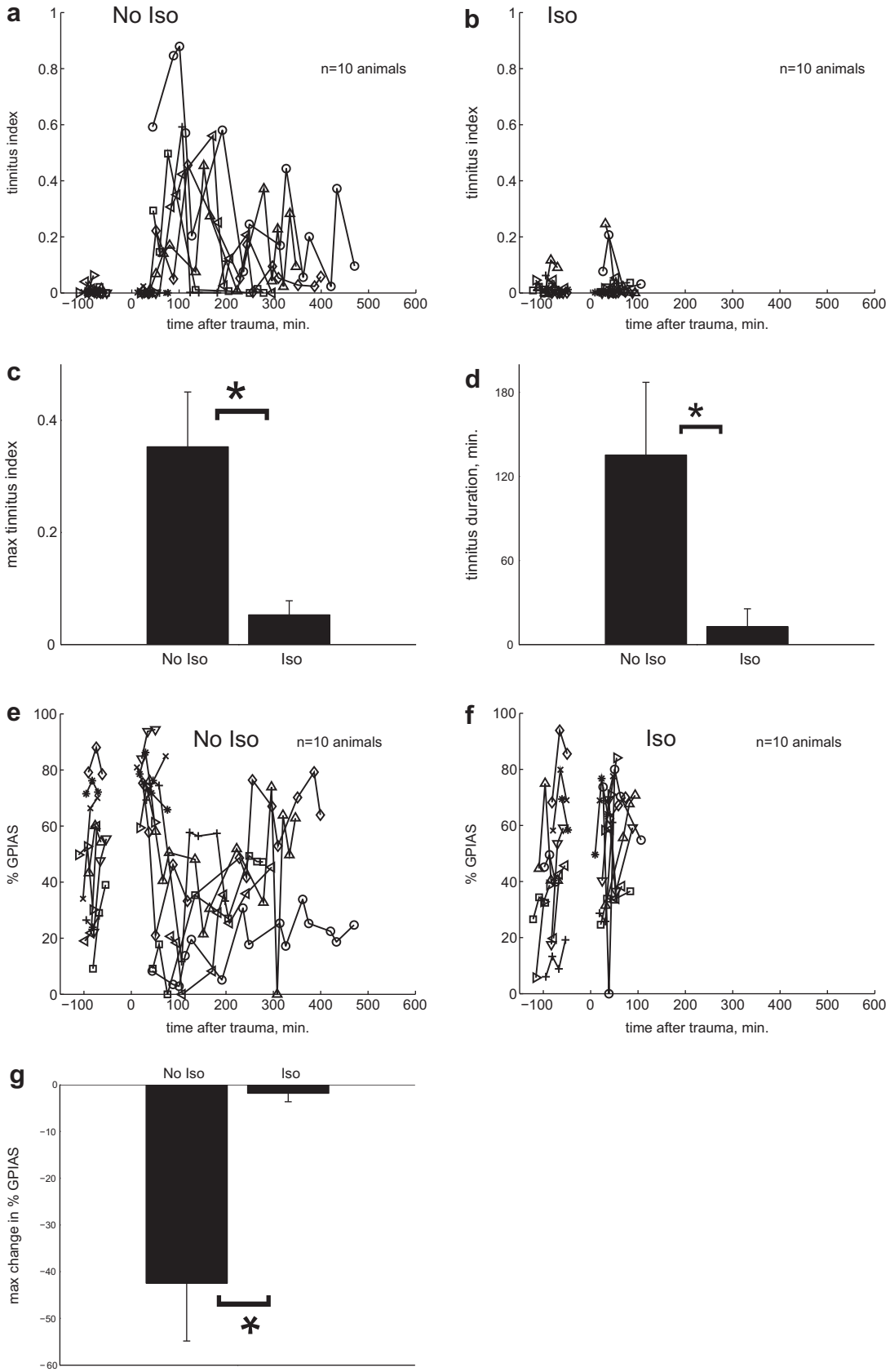

Fig. 2. Isoflurane blocks temporary tinnitus. a) Time course of temporary tinnitus following brief noise trauma. Animals ($n = 10$) were not anesthetized with isoflurane during pure-tone trauma. Note that tinnitus index rose sharply after trauma and remained elevated for hours. Symbols in a, b, e, f indicate different animals. b) Time course of tinnitus when animals were anesthetized with isoflurane during noise trauma. These are the same 10 animals as in (a), but tested at least 2 days apart. c) The maximum tinnitus index was significantly greater when isoflurane was not used ($p < 10^{-2}$). d) Tinnitus duration was significantly longer when isoflurane was not used ($p < 0.05$). Tinnitus duration was defined as the amount of time that the tinnitus index exceeded 0.05. e) A reduction in %GPIAS (consistent with presence of tinnitus) showed a similar time course as the increase in tinnitus index in (a) for animals not anesthetized with isoflurane during trauma. f) Animals anesthetized during trauma showed no reduction in %GPIAS. g) The maximal change in %GPIAS from baseline was significantly greater when isoflurane was not used ($p < 10^{-2}$). Error bars in c, d, g indicate standard errors of the mean.

consisted of 20 repetitions of the startle stimulus at each of 4 different pre-pulse levels (including no pre-pulse) and took 20–25 min. We collected a block of 3 gap-detection trials followed by 1 noise-detection trial as a baseline before acoustic trauma, and then collected similar blocks (3 gap-detection trials followed by 1 noise-detection trial) continuously after trauma until TI had returned to baseline (defined as at least three consecutive gap-detection trials with TI < 0.05). This means that the temporal resolution with which we could measure noise-detection was roughly once per hour. Because our end-point for each experiment was functional (i.e., TI at baseline for 3 measurements in a row) rather than a fixed time duration, the amount of time in the testing booth varied for each animal. The total duration of each experiment ranged from 109 to 831 min (mean $\pm$ s.d.: 258 $\pm$ 174 min). Note that because some animals showed no evidence of tinnitus (i.e., TI was at baseline) for three consecutive measurements directly following trauma, testing in some animals was completed in as little as 100 min after trauma. We therefore could have missed tinnitus that did not begin to develop until after 100 min following trauma. In 3 cases, testing was discontinued after 6–8 h because the animal became too restless (i.e., spontaneous activity interfered with measurement of startle responses); in these cases our tinnitus duration estimates are lower bounds. The animals did not have access to food and water inside the testing chamber during data collection, but were removed from the chamber every 1–2 h for a 10–20 min break which included access to food and water. Uninterrupted time spent in the chamber ranged from 40 to 192 min (mean $\pm$ s.d.: 97 $\pm$ 37 min).

3. Results

We induced temporary tinnitus in 10 rats with a brief (2-min) 17 kHz pure-tone acoustic trauma. We measured tinnitus behaviorally using a gap-detection task based on the startle reflex (Turner et al., 2006). The task is similar to conventional pre-pulse inhibition, in which the startle response is reduced when the startle stimulus is preceded by a cue. In the gap-detection task, the cue is a gap in continuous narrow-band noise (Fig. 1a). A significant decrease in the probability and amplitude of the startle response indicated gap detection by the animal (Fig. 1a–b). The reasoning underlying the task is that if the animal is experiencing tinnitus that is perceptually similar to the narrow-band background noise, it may fill in the gap, making the gap less salient. This decreased salience should lead to a gap-detection deficit. Our tinnitus index (TI), which varies from 0 (no tinnitus) to 1 (maximal tinnitus, see 2) is therefore a measure of the gap-detection deficit. Because one ear was plugged during trauma, afterward the animal should be able to use that good ear to hear the stimuli and perform the task, suggesting that task deficits are not due to hearing loss, but rather due to specific temporary tinnitus generated by monaural trauma to the other ear.

To measure the time course of temporary tinnitus after a brief pure-tone acoustic trauma, we first measured gap detection performance in a series of three baseline trials (Fig. 1c, "G"). We next delivered pure-tone trauma (monaurally, 17 kHz, 115 dB SPL), and then measured gap detection afterward with a temporal resolution of $\sim$15 min (see Methods).

In animals that were not anesthetized during pure-tone trauma, the tinnitus index rose sharply after trauma (Fig. 2a) and remained elevated for hours. In contrast, when the same animals were briefly anesthetized with isoflurane during trauma (on a different day), there was little or no increase in the tinnitus index. When the tinnitus index did increase, it returned to baseline in less than an hour.

Across our sample of 10 animals, the maximum tinnitus index reached was five-fold higher when isoflurane was not used (Fig. 2c; 0.35 $\pm$ 0.31 vs. 0.07 $\pm$ 0.09, $p < 0.01$). Tinnitus duration, which we operationally defined as the amount of time the tinnitus index exceeded 0.05, was ten-fold greater when isoflurane was not used (Fig. 2d; 135 $\pm$ 164 min vs 13 $\pm$ 36 min, $p < 0.05$). Note that 0.05 here is an arbitrary threshold, but varying it over a wide range (0.001–0.25) did not change the result. A comparison of different time points after trauma also showed that tinnitus lasted much longer when isoflurane was not used during trauma. For animals that were anesthetized with isoflurane during trauma, 30% (3/10) showed tinnitus at some point within the first 100 min after trauma, but no animal showed tinnitus at any time point more than 100 min after trauma. For animals that were not anesthetized during trauma, 50% (5/10) of animals showed tinnitus at some point within the first 100 min after trauma. Between 100 and 200 min after trauma, 50% of animals showed tinnitus, and this percentage gradually decreased to 10% (1/10) by 500 min after trauma. Overall, for animals that were anesthetized with isoflurane during trauma, 30% (3/10) showed tinnitus at some point after trauma. For animals that were not anesthetized during trauma, 60% (6/10) showed tinnitus at some point after trauma. To allow a direct comparison between our results and previous studies, we also used a common measure of gap-detection based on the degree of inhibition of the startle response (percent gap-induced pre-pulse inhibition of acoustic startle, or %GPIAS, Turner et al., 2006; Yang et al., 2007). A reduction in %GPIAS is consistent with tinnitus. Fig. 2e shows that animals which were not anesthetized during trauma showed a reduction in %GPIAS that mirrored the tinnitus index increases in Fig. 2a. The maximum change in %GPIAS was $-42 \pm 39\%$ (Fig. 2g), which was significantly different from zero ($p < 0.01$). In contrast, when animals were anesthetized during trauma, there was no significant change in %GPIAS (Fig. 2f, g, maximum change was $-2 \pm 6\%$, $p > 0.3$). Taken together, these results indicate that isoflurane anesthesia during acoustic trauma largely blocked temporary tinnitus.

Interestingly, gap-detection deficits did not rise immediately after pure-tone trauma. The tinnitus index did not increase from baseline levels until 40–60 min after trauma, and did not reach peak values until 80–170 min after trauma. It is important to note here that the temporal precision of our measurements is only about 15 min (and specifically for the first measurement after trauma, averaged 30 min (range: 9–83 min), see Methods). Thus the delayed rise in tinnitus index after trauma appears to be real, but the initial dynamics of temporary tinnitus from onset to peak warrant a more detailed characterization with better temporal resolution.

We also found that the duration of tinnitus was longer for the first trauma exposure than for the second exposure $\sim$3 days later. This effect was driven mainly by animals that were not anesthetized during trauma, for which tinnitus lasted longer when it was the first exposure than when it was the second exposure ($p < 0.05$).

Because one ear was securely plugged with silicone elastomer during trauma, we expected animals to maintain normal hearing in that ear, and therefore that they should be able to hear the narrow-band background noise used in the gap-detection task (Turner et al., 2006). To confirm this, we used a separate noise-detection task, which did not have continuous background noise (Fig. 3). This task used a pre-pulse that consisted of 6 kHz, $^1/_3$ octave narrow-band noise, i.e., the same spectral characteristics as the continuous background noise in the gap-detection task. The pre-pulse level was at or below the level of the background noise used in the gap-detection task. We reasoned that if such a pre-pulse caused significant inhibition of the startle response, the animal was

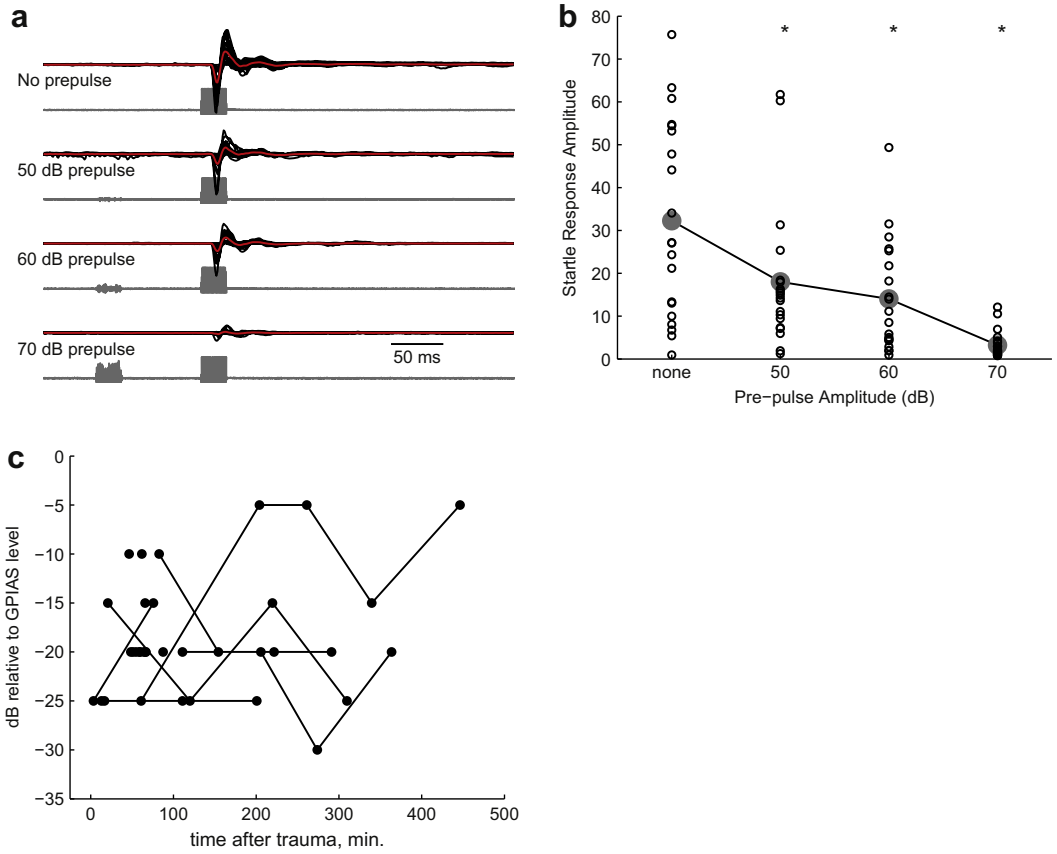

Fig. 3. Narrow band noise detection thresholds. a) Example of startle responses of an animal to a white noise burst without any background noise. In the top panel, the white noise burst is presented in isolation. In the lower panels, the burst is preceded by a narrow-band prepulse, with the prepulse level indicated at left (prepulse bandwidth was $^1/_3$ octave and center frequency was 6 kHz). Note that the startle response was progressively reduced as the prepulse level was increased. Startle response amplitude is in arbitrary units. b) Peak startle response amplitudes for the raw data shown in (a). Black circles: 20 individual trials; grey filled circles: mean across trials; *indicates that the prepulse caused a significant ($p < 0.05$) decrease in peak startle amplitude. We used the lowest prepulse level that significantly reduced startle as an estimate (upper bound) of detection threshold (50 dB in this example). c) Time course of detection threshold, expressed as dB relative to the background noise level used in the gap detection task. Detection threshold averaged -19 ± 6 dB and never exceeded -5 dB, indicating that animals could always hear the background noise used in the gap detection task.

able to detect the 6 kHz, $^1/_3$ octave narrow-band background noise, suggesting that hearing loss cannot account for the gap-detection deficits. Fig. 3a–b shows an example from one animal. The startle response was progressively reduced as the pre-pulse level was increased. In this example, pre-pulse inhibition was significant ($p < 0.05$) even when the pre-pulse was as low as 50 dB. This demonstrates that the animal could detect 50 dB narrow-band noise, indicating that the background noise used in the gap-detection task (which was 75 dB for this animal) was at least 25 dB above threshold. In other words, an upper bound on the noise-detection threshold in this example was −25 dB relative to the background noise level.

We used multiple pre-pulse levels in the noise-detection task, and used the lowest pre-pulse level that significantly reduced startle as an estimate of noise-detection threshold. Because of the time constraints of the task, we used only 3 pre-pulse levels (in steps of 10 dB), and in most cases (as in Fig. 3b) even the lowest pre-pulse level (50–60 dB) produced significant inhibition. Therefore this estimate only provides an upper bound on noise-detection threshold. Nevertheless, this detection threshold was always below the background noise level used in the gap-detection task. We interleaved a sequence of gap-detection trials and noise-detection trials as shown in Fig. 1c (G: gap detection task, N: noise-detection task). Detection threshold for all animals, at every time point, was below the background noise level (Fig. 3c). Across animals, narrow-band noise detection thresholds (relative to the background noise level used in the gap-detection task) were -19 ± 6 dB before trauma and -19 ± 6 dB after trauma (not significant, $p > 0.8$). Likewise, the average estimated within-animal threshold shift after trauma was only 0.3 dB (not significant, $p > 0.8$), and was uncorrelated with tinnitus index ($p > 0.3$). This indicates that the animals could hear the narrow-band background noise in the gap-detection tasks, and that the acoustic trauma did not significantly affect detection thresholds for that narrow-band noise. Interestingly, in 9/10 animals, acoustic trauma produced a significant increase in the amplitude of the startle response to a white noise burst presented in isolation. Isoflurane also significantly reduced this startle response amplitude in 5/10 animals. Increased startle response amplitudes following a tinnitus inducer (such as acoustic trauma or salicylate) are consistent with previous studies, and have been suggested as a possible behavioral correlate of hyperacusis (Ison et al., 2007; Holt et al., 2010; Turner and Parrish, 2008; Sun et al., 2009).

4. Discussion

Here we adapted a behavioral method to measure temporary tinnitus following brief acoustic trauma, and used it to test whether anesthetizing rats with isoflurane during trauma had any effect on tinnitus. We found that tinnitus, as measured by gap-detection

deficits, was 5 times stronger and 10 times longer in duration when isoflurane was not used. This suggests that isoflurane largely prevents temporary noise-induced tinnitus.

It seems doubtful that our isoflurane result provides any direct clinically relevant insight. After all, individuals typically cannot be anesthetized during noise exposure, such as on the battlefield or on the factory floor. However, our results provide important methodological information for experimental studies of tinnitus, and may also shed some light on potential mechanisms of tinnitus. For experimental design of tinnitus studies, the use of anesthesia during noise exposure must be taken into careful consideration. While we used isoflurane because of the need for rapid recovery in our paradigm, other anesthetics such as pentobarbital have also been shown to reduce noise-induced hearing loss in mice (Chung et al., 2007) and might therefore show preventative effects on tinnitus similar to isoflurane. However, it is probably not necessary to conclude that anesthesia should never be used during noise exposure in tinnitus studies. Most experimental studies of chronic tinnitus typically use much longer and/or more intense trauma, such as 1–2 h exposure, and use of narrow or broadband noise instead of pure tones (e.g., Kraus et al., 2010; Brozoski et al., 2011). Since these studies report chronic tinnitus even when animals are anesthetized during trauma with isoflurane, these exposures must be intense enough to overcome any protective effect of isoflurane.

What is the mechanism by which isoflurane anesthesia prevents tinnitus? Isoflurane has multiple mechanisms of action: suppressing synaptic excitation, enhancing synaptic inhibition, and decreasing excitability (Hemmings et al., 2005), as well as reducing the production of reactive oxygen species that can lead to tissue damage (Nakagawara et al., 1986). Isoflurane could thereby provide a neuroprotective effect, preventing damage to hair cells (as shown by Kim et al., 2005) or central auditory neurons. Since the neuroprotective effect of isoflurane reduces noise-induced hearing loss (Kim et al., 2005; Chung et al., 2007), this mechanism could directly explain the prevention of temporary tinnitus that we observed. Although we demonstrated that hearing loss at 6 kHz is unlikely to account for the gap-detection deficits that we used to measure tinnitus, it is still likely that the temporary tinnitus was caused by noise-induced hearing loss at higher frequencies. Pure-tone trauma, such as we used here, has been shown to produce a temporary threshold shift that is restricted to a range of frequencies (1–2 octaves) centered half an octave above the trauma frequency frequency (Davis et al., 1950; Liberman and Kiang, 1978; Lonsbury-Martin and Meikle, 1978; Scholl and Wehr, 2008). For the 17 kHz tone that we used, the temporary threshold shift is therefore expected to be centered at 24 kHz, which we confirmed in a previous study (Scholl and Wehr, 2008), although we did not test for hearing loss at 24 kHz here. Our results are consistent with a model in which tinnitus results from hyperexcitability in central auditory neurons tuned to frequencies well below the region of hearing loss. These findings are also consistent with reports of increased spontaneous and evoked activity observed in cortical neurons with characteristic frequencies below the trauma frequency (Norena et al., 2003; Scholl and Wehr, 2008), and with behavioral evidence of tonal tinnitus below the trauma frequency in rats (Turner et al., 2006). Thus isoflurane could prevent low-frequency tinnitus by preventing high-frequency hearing loss.

Another possibility is that isoflurane may suppress activity in brain regions necessary for induction of tinnitus. For example, pre-trauma lesions of dorsal cochlear nucleus were recently shown to prevent induction of tinnitus by acoustic trauma (Brozoski et al., 2011). Isoflurane suppresses auditory brainstem response amplitudes (Stronks et al., 2010), suggesting that suppression of activity in dorsal cochlear nucleus neurons by isoflurane could be one mechanism by which isoflurane during trauma prevents tinnitus.

An important concern is whether a gap-detection deficit can be unambiguously attributed to a tinnitus percept, rather than hearing loss. To control for this possibility, our strategy was to interleave a noise-detection task. This demonstrated that animals could detect the same narrow-band noise used in the gap-detection task even at levels 19 dB below that used as background noise. Moreover, there was no change in noise detection thresholds after trauma, nor were changes in detection thresholds correlated with tinnitus severity. These results make sense because the 6 kHz narrow-band noise was well below the expected hearing loss produced by a 17 kHz tone, which should be centered at 24 kHz. The gap-detection deficits are therefore difficult to account for by hearing loss, providing support for the interpretation that they are caused by tonal tinnitus that fills in and reduces the saliency of the gap (Turner et al., 2006). Since our estimates of noise-detection thresholds are upper bounds, we know that the animal could detect the narrow-band noise used in the gap-detection task. Nevertheless, we would not have been able to detect small increases in noise-detection thresholds. If present, these could have reduced the perceived loudness of the background noise, which could reduce gap salience and could therefore have contributed to gap-detection deficits. This interpretation is not mutually exclusive with the presence of tinnitus. Another concern is that one study of temporary tinnitus in humans reported a tinnitus percept that appeared as early as 5 min after exposure (Chermak and Dengerink, 1987), whereas we did not observe an increase in gap detection deficits until 40–60 min after exposure. It is not clear whether this reflects a difference in the underlying tinnitus phenomena, or a methodological difference (i.e., perceptual report vs. measurements of gap-detection deficits). More generally, there will always be an inherent uncertainty in the interpretation of behavioral measures of a phantom sensation such as tinnitus.

Another potential concern is that the isoflurane anesthesia during the brief trauma presentation may have caused general arousal or performance deficits. However, animals were briefly anesthetized for earplug insertion in both cases, differing by only 3–4 min of exposure, and testing did not start until 30 $\pm$ 16 min later. Thus it seems unlikely that our results could be explained by general effect of isoflurane; rather, the main difference between the two groups was whether the brief period of isoflurane anesthesia overlapped with acoustic trauma or not.

Acknowledgments

This research was supported by the Tinnitus Research Consortium.

References

Atherley, G.R., Hempstock, T.I., Noble, W.G., 1968. Study of tinnitus induced temporarily by noise. J. Acoust. Soc. Am. 44, 1503–1506.
Bauer, C.A., Brozoski, T.J., 2001. Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. J. Assoc. Res. Otolaryngol. 2, 54–64.
Bauer, C.A., Brozoski, T.J., Holder, T.M., Caspary, D.M., 2000. Effects of chronic salicylate on GABAergic activity in rat inferior colliculus. Hear. Res. 147, 175–182.
Bauer, C.A., Brozoski, T.J., Rojas, R., Boley, J., Wyder, M., 1999. Behavioral model of chronic tinnitus in rats. Otolaryngol. Head Neck Surg. 121, 457–462.
Brozoski, T.J., Wisner, K.W., Sybert, L.T., Bauer, C.A., 2011. Bilateral dorsal cochlear nucleus lesions prevent acoustic-trauma induced tinnitus in an animal model. J. Assoc. Res. Otolaryngol. 13 (1), 55–66.
Chermak, G.D., Dengerink, J.E., 1987. Characteristics of temporary noise-induced tinnitus in male and female subjects. Scand. Audiol. 16, 67–73.
Chung, J.W., Ahn, J.H., Kim, J.Y., Lee, H.J., Kang, H.H., Lee, Y.K., Kim, J.U., Koo, S.W., 2007. The effect of isoflurane, halothane and pentobarbital on noise-induced hearing loss in mice. Anesth. Analg. 104, 1404–1408 (table of contents).
Cooper Jr., J.C., 1994. Health and nutrition examination survey of 1971–75: part II. Tinnitus, subjective hearing loss, and well-being. J. Am. Acad. Audiol. 5, 37–43.

Davis, H., Morgan, C.T., Hawkins Jr., J.E., Galambos, R., Smith, F.W., 1950. Temporary deafness following exposure to loud tones and noise. Acta Otolaryngol. Suppl. 88, 1–56.

Eggermont, J.J., 2008. Role of auditory cortex in noise – and drug-induced tinnitus. Am. J. Audiol. 17, S162–S169.

Hemmings Jr., H.C., Akabas, M.H., Goldstein, P.A., Trudell, J.R., Orser, B.A., Harrison, N.L., 2005. Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol. Sci. 26, 503–510.

Holt, A.G., Bissig, D., Mirza, N., Rajah, G., Berkowitz, B., 2010. Evidence of key tinnitus-related brain regions documented by a unique combination of manganese-enhanced MRI and acoustic startle reflex testing. PLoS One 5, e14260.

Ison, J.R., Allen, P.D., O'Neill, W.E., 2007. Age-related hearing loss in C57BL/6J mice has both frequency-specific and non-frequency-specific components that produce a hyperacusis-like exaggeration of the acoustic startle reflex. J. Assoc. Res. Otolaryngol. 8, 539–550.

Jastreboff, P.J., Brennan, J.F., Coleman, J.K., Sasaki, C.T., 1988. Phantom auditory sensation in rats: an animal model for tinnitus. Behav. Neurosci. 102, 811–822.

Kim, J.U., Lee, H.J., Kang, H.H., Shin, J.W., Ku, S.W., Ahn, J.H., Kim, Y.J., Chung, J.W., 2005. Protective effect of isoflurane anesthesia on noise-induced hearing loss in mice. Laryngoscope 115, 1996–1999.

Kraus, K.S., Mitra, S., Jimenez, Z., Hinduja, S., Ding, D., Jiang, H., Gray, L., Lobarinas, E., Sun, W., Salvi, R.J., 2010. Noise trauma impairs neurogenesis in the rat hippocampus. Neuroscience 167, 1216–1226.

Liberman, M.C., Kiang, N.Y., 1978. Acoustic trauma in cats. Cochlear pathology and auditory-nerve activity. Acta Otolaryngol. Suppl. 358, 1–63.

Lobarinas, E., Sun, W., Cushing, R., Salvi, R., 2004. A novel behavioral paradigm for assessing tinnitus using schedule-induced polydipsia avoidance conditioning (SIP-AC). Hear. Res. 190, 109–114.

Loeb, M., Smith, R.P., 1967. Relation of induced tinnitus to physical characteristics of the inducing stimuli. J. Acoust. Soc. Am. 42, 453–455.

Lonsbury-Martin, B.L., Meikle, M.B., 1978. Neural correlates of auditory fatigue: frequency-dependent changes in activity of single cochlear nerve fibers. J. Neurophysiol. 41, 987–1006.

Milbrandt, J.C., Holder, T.M., Wilson, M.C., Salvi, R.J., Caspary, D.M., 2000. GAD levels and muscimol binding in rat inferior colliculus following acoustic trauma. Hear. Res. 147, 251–260.

Nakagawara, M., Takeshige, K., Takamatsu, J., Takahashi, S., Yoshitake, J., Minakami, S., 1986. Inhibition of superoxide production and Ca^{2+} mobilization in human neutrophils by halothane, enflurane, and isoflurane. Anesthesiology 64, 4–12.

Nondahl, D.M., Cruickshanks, K.J., Wiley, T.L., Klein, R., Klein, B.E., Tweed, T.S., 2002. Prevalence and 5-year incidence of tinnitus among older adults: the epidemiology of hearing loss study. J. Am. Acad. Audiol. 13, 323–331.

Norena, A.J., Moffat, G., Blanc, J.L., Pezard, L., Cazals, Y., 2010. Neural changes in the auditory cortex of awake guinea pigs after two tinnitus inducers: salicylate and acoustic trauma. Neuroscience 166, 1194–1209.

Norena, A.J., Tomita, M., Eggermont, J.J., 2003. Neural changes in cat auditory cortex after a transient pure-tone trauma. J. Neurophysiol. 90, 2387–2401.

Norena, A.J., Eggermont, J.J., 2005. Enriched acoustic environment after noise trauma reduces hearing loss and prevents cortical map reorganization. J. Neurosci. 25, 699–705.

Ralli, M., Lobarinas, E., Fetoni, A.R., Stolzberg, D., Paludetti, G., Salvi, R., 2010. Comparison of salicylate- and quinine-induced tinnitus in rats: development, time course, and evaluation of audiologic correlates. Otol. Neurotol. 31, 823–831.

Scholl, B., Wehr, M., 2008. Disruption of balanced cortical excitation and inhibition by acoustic trauma. J. Neurophysiol. 100, 646–656.

Shargorodsky, J., Curhan, G.C., Farwell, W.R., 2010. Prevalence and characteristics of tinnitus among US adults. Am. J. Med. 123, 711–718.

Stolzberg, D., Chen, G.D., Allman, B.L., Salvi, R.J., 2011. Salicylate-induced peripheral auditory changes and tonotopic reorganization of auditory cortex. Neuroscience 180, 157–164.

Stronks, H.C., Aarts, M.C., Klis, S.F., 2010. Effects of isoflurane on auditory evoked potentials in the cochlea and brainstem of guinea pigs. Hear. Res. 260, 20–29.

Sun, W., Lu, J., Stolzberg, D., Gray, L., Deng, A., Lobarinas, E., Salvi, R.J., 2009. Salicylate increases the gain of the central auditory system. Neuroscience 159, 325–334.

Turner, J.G., Brozoski, T.J., Bauer, C.A., Parrish, J.L., Myers, K., Hughes, L.F., Caspary, D.M., 2006. Gap detection deficits in rats with tinnitus: a potential novel screening tool. Behav. Neurosci. 120, 188–195.

Turner, J.G., Parrish, J., 2008. Gap detection methods for assessing salicylate-induced tinnitus and hyperacusis in rats. Am. J. Audiol. 17, S185–S192.

Vernon, J.A., Møller, A.R., 1995. Mechanisms of Tinnitus. Allyn and Bacon, Boston.

Yang, G., Lobarinas, E., Zhang, L., Turner, J., Stolzberg, D., Salvi, R., Sun, W., 2007. Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats. Hear. Res. 226, 244–253.

Zhang, X., Yang, P., Cao, Y., Qin, L., Sato, Y., 2011. Salicylate induced neural changes in the primary auditory cortex of awake cats. Neuroscience 172, 232–245.