NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION
RESEARCH ARTICLE
Hannah Brotherton et al. / Neuroscience xxx (xxxx) xxx–xxx

Earplug-induced changes in acoustic reflex thresholds suggest that increased subcortical neural gain may be necessary but not sufficient for the occurrence of tinnitus

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Abstract—The occurrence of tinnitus is associated with hearing loss and neuroplastic changes in the brain, but disentangling correlation and causation has remained difficult in both human and animal studies. Here we use earplugs to cause a period of monaural deprivation to induce a temporary, fully reversible tinnitus sensation, to test whether differences in subcortical changes in neural response gain, as reflected through changes in acoustic reflex thresholds (ARTs), could explain the occurrence of tinnitus.Forty-four subjects with normal hearing wore an earplug in one ear for either 4 ($n = 27$) or 7 days ($n = 17$). Thirty subjects reported tinnitus at the end of the deprivation period. ARTs were measured before the earplug period and immediately after taking the earplug out. At the end of the earplug period, ARTs in the plugged ear were decreased by 5.9 ± 1.1 dB in the tinnitus-positive group, and by 6.3 ± 1.1 dB in the tinnitus-negative group. In the control ear, ARTs were increased by 1.3 ± 0.8 dB in the tinnitus-positive group, and by 1.6 ± 2.0 dB in the tinnitus-negative group. There were no significant differences between the groups with 4 and 7 days of auditory deprivation.Our results suggest that either the subcortical neurophysiological changes underlying the ART reductions might not be related to the occurrence of tinnitus, or that they might be a necessary component of the generation of tinnitus, but with additional changes at a higher level of auditory processing required to give rise to tinnitus. *This article is part of a Special Issue entitled: [SI: Tinnitus Hyperacusis].*

Key words: tinnitus, auditory deprivation, acoustic reflex threshold, neural plasticity, earplug.

INTRODUCTION

The occurrence of tinnitus, a phantom auditory sensation, is correlated with cochlear damage, neuroplastic changes in the central auditory system, and changes in spontaneous neuronal activity (Roberts et al., 2010; Baguley et al., 2013; Schaette, 2013; Shore et al., 2016). However, the relative contributions of the different factors and their causal relations have remained largely unclear. Moreover, it has yet to be clarified which of the changes in the central auditory system might be necessary for the development of tinnitus, and which might be unrelated consequences of hearing loss.

In most patients, tinnitus is associated with audiometric hearing loss (Axelsson and Ringdahl, 1989; Pilgramm et al., 1999; Nicolas-Puel et al., 2002), and tinnitus pitch is generally matched to frequencies where hearing is impaired (Norena et al., 2002; König et al., 2006; Roberts et al., 2008; Sereda et al., 2011). However, tinnitus can also occur in subjects without audiometric hearing loss (Barnea et al., 1990; Sanchez et al., 2005), and it is currently an open question whether such subjects have sub-clinical cochlear damage (Schaette and McAlpine, 2011; Gu et al., 2012; Bramhall et al., 2018) or not (Gilles et al., 2016; Guest et al., 2017). Conversely, hearing loss does not always lead to tinnitus, as demonstrated by the fact that the prevalence of hearing loss is higher than the prevalence of tinnitus (Lockwood et al., 2002).

In humans, the presence of tinnitus has been linked to changes in the spontaneous neuronal activity in the central auditory system. Specifically, changes in spontaneous brain rhythms have been reported, with an increase in power in the delta frequency band and reduced power in the alpha frequency band (Weisz et al., 2005; Weisz et al., 2007; Adjamian et al., 2012). Modulation of the alpha/delta ratio was also observed during masking (Adjamian et al., 2012),

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https://doi.org/10.1016/j.neuroscience.2019.03.017

residual inhibition (Sedley et al., 2012; Sedley et al., 2015), and residual excitation (Sedley et al., 2012) of tinnitus, but these changes might be confined to a subset of participants (Sedley et al., 2012). Furthermore, significant increases in gamma band activity have been reported in cases of chronic tinnitus (Weisz et al., 2007; Lorenz et al., 2009) as well as for temporary tinnitus after noise exposure (Ortmann et al., 2011). However, other studies have not reported a consistent relation between gamma power and tinnitus (Adjamian et al., 2012; Sedley et al., 2012), or even an increase in gamma band activity during tinnitus suppression (Sedley et al., 2015). Potential difficulties in the interpretation of human neuroimaging results are underlined by recent reports that putative "tinnitus networks" in neuronal resting state activity could not be found when tinnitus and control subjects were carefully matched for hearing status (Davies et al., 2014), and that there might be no relation between EEG findings and psychometric or psychoacoustic properties of tinnitus (Pierzycki et al., 2016).

Animal studies have reported relations between behavioral measures that have been assumed to be indicative of tinnitus and a variety of changes in spontaneous neuronal activity after the induction of hearing loss, e.g. increased spontaneous firing rates (Brozoski et al., 2002; Kaltenbach et al., 2004; Bauer et al., 2008; Ahlf et al., 2012; Koehler and Shore, 2013), increases in spontaneous bursting activity (Bauer et al., 2008; Wu et al., 2016), and increases in neuronal synchrony (Engineer et al., 2011; Wu et al., 2016). These potential neural correlates have been observed all along the central auditory pathway, from the cochlear nucleus to the auditory cortex. Modeling studies suggest that the development of increased spontaneous firing rates could be caused by an increase in neuronal response gain after hearing loss (Schaette and Kempter, 2006; Parra and Pearlmutter, 2007; Schaette and Kempter, 2008, 2009; Chrostowski et al., 2011; Norena, 2011). Several studies have provided indications that subcortical changes in spontaneous neuronal activity might only occur in animals with behavioral evidence for tinnitus (Kaltenbach et al., 2004; Koehler and Shore, 2013; Wu et al., 2016), but other studies have found that increases in spontaneous neuronal activity might be a general consequence of hearing loss and not specific for tinnitus (Coomber et al., 2014), and that ablation of the dorsal cochlear nucleus, which has been proposed to play an important role in tinnitus generation, does not abolish the assumed behavioral signs of tinnitus (Brozoski and Bauer, 2005). Thus, a definite answer to the question of where the "tinnitus generator" is located, and which neuronal mechanisms underlie the development of the phantom sound, has not yet been found.

In animal models of tinnitus, some of the discrepancies might be due to the use of different species or noise exposure paradigms. However, it is conceivable that the different behavioral tests used to detect the presence of tinnitus could have led to differences in the results, and there is currently an ongoing debate whether behavioral tests for detecting tinnitus in animals do reflect tinnitus or other consequences of experimentally induced hearing loss (Eggermont, 2013; Fournier and Hebert, 2013). In human studies, the heterogeneity of hearing loss makes it difficult to match tinnitus and control groups closely, which presents a potential confound. Moreover, the heterogeneity of tinnitus itself might introduce another source of variability. Finally, it is conceivable that neuroplastic changes might be a necessary pre-requisite for the development of tinnitus, like, for example, increased neuronal response gain in subcortical auditory structures, but that additional changes at higher processing stages, like failure of thalamic gating (Rauschecker et al., 2010) or altered evaluation of subcortical neuronal activity patterns (Sedley et al., 2016), might be required to explain conscious perception of tinnitus. Any of the confounds mentioned above would greatly increase the difficulty of teasing these factors apart.

One way of investigating the mechanisms underlying tinnitus generation, while avoiding some of these pitfalls, might be to study temporary tinnitus, which can be induced in human subjects through auditory deprivation by means of an earplug. We have recently demonstrated that wearing an earplug in one ear for several days reliably and fully reversibly induces the perception of tinnitus in the majority of subjects, and the descriptions of the tinnitus sounds were similar to those used by tinnitus patients to describe their auditory phantom (Schaette et al., 2012). Using the earplug paradigm, where all subjects experience the same defined type, degree and duration of temporary hearing loss, enables the investigation of hearing-loss-induced neurophysiological changes within subjects, and the comparison between subjects with and without phantom sounds makes it possible to separate those related to tinnitus perception from those related to hearing loss. Earplug-induced auditory deprivation has already been shown to increase the perceived loudness of sounds (Formby et al., 2003; Munro et al., 2014) and to decrease the sound level required to elicit the acoustic reflex (acoustic reflex threshold, ART) in the plugged ear (Munro and Blount, 2009; Munro et al., 2014; Brotherton et al., 2016, 2017). Decreases in ART might be caused by an increase in neuronal response gain at the level of the brainstem, i.e. a physiological change that would also be a candidate mechanism for the generation of tinnitus (Schaette and Kempter, 2006, 2009; Norena, 2011).

Here we report on the relation between the occurrence of tinnitus and changes in the ART after auditory deprivation through wearing an earplug in one ear for several days. Forty-four young participants with normal-hearing wore an earplug in one ear continuously for either 4 or 7 days. ARTs were measured with broadband noise as eliciting stimulus before the earplug period and immediately after the earplug was taken out at the end of the earplug period. We hypothesized that if the occurrence of tinnitus can be explained by subcortical changes in neuronal gain, the ARTs of participants experiencing tinnitus would differ from those that did not hear phantom sounds.

EXPERIMENTAL PROCEDURES

We have pooled the data from two previous studies where ARTs were measured and participants were asked about phantom sounds. In the first study (Munro et al., 2014), 17 volunteers (age range 20–28 years, mean age 23.5 ±

0.44 years; 11 female) wore an earplug for 7 days. In the second study (Brotherton et al., 2017), 27 volunteers (21 female; mean age, 24.7 ± 1.3 years; range 19–50 years) wore an earplug in one ear for 4 days. Pooling the data was possible because changes in ART induced by monaural earplug usage reach a plateau after 2–4 days (Brotherton et al., 2016). Both studies were approved by the ethics committee of the University of Manchester (Refs 663/07P and 13,183), and all participants gave written informed consent.

For both studies, participants were required to have normal hearing, i.e. thresholds of <20 dB HL from 0.25 kHz to 8 kHz, and no asymmetry >10 dB between ears at any frequency. A short health questionnaire was used to screen for other conditions, and persons reporting chronic tinnitus or intermittent tinnitus at the beginning of the study were excluded. Normal middle ear function was ensured through tympanometry using a GSI TympStar middle ear analyzer; participants were required to have middle ear pressure between +50 and −50 daPa and middle ear compliance of 0.3 to 1.6 cm^3.

Pure-tone audiometry

Pure tone audiometry was performed with an Aurical clinical audiometer and TDH-39 supra-aural headphones. Hearing threshold levels were measured for each ear separately at 0.25, 0.5, 1, 2, 4 and 8 kHz, using procedures recommended by the British Society for Audiology. The mean hearing thresholds are shown in Fig. 1a, b.

Sound-attenuating earplugs and measures of tinnitus

The participants were fitted monaurally (22 left ear, 22 right ear) with a reusable Mack's silicone putty ear plug (McKeon Products, United States) and instructed to wear it continuously for 4 or 7 days, except for daily ablutions. Sound attenuation of the earplug, i.e., the difference in ear canal sound level with and without the earplug in situ, was measured using a clinical probe tube microphone system and a broadband signal (pink noise) of 75 (Munro et al., 2014) or 65 dB SPL (Brotherton et al., 2017). The measures were made three times on each listener after the participant removed and refitted the earplug into each ear, to confirm that participants fitted the earplug with a maximum attenuation difference of 3 dB at 1 kHz and 2 kHz when fitting it themselves. The average attenuation levels are shown in Fig. 1c.

At the end of the first earplug fitting session, participants were given an "earplug logbook" to record earplug usage (expected to be continuous except for removal for cleaning). They were also told that there might be a possibility of experiencing phantom sounds during earplugs usage, and they were asked to take a note about their occurrence in the logbook. We deliberately did not mention "tinnitus" in all explanations and only talked about phantom auditory sensations or phantom sounds to avoid biasing the subjects by using the strongly suggestive term "tinnitus", which carries a negative connotation for many people.

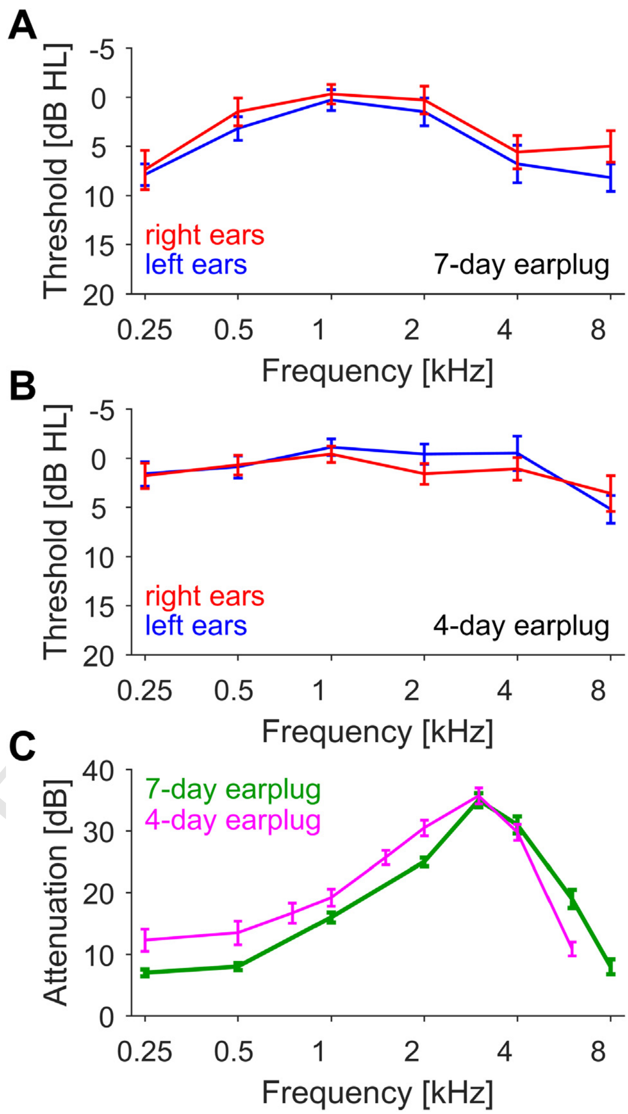

Fig. 1. Audiograms and earplug attenuation. (A) Mean audiograms of the left (blue line) and the right ears (red line) of the participants that wore an earplug for 7 days (n = 17). (B) Mean audiograms of the left (blue line) and the right ears (red line) of the participants that wore an earplug for 4 days (n = 27). (C) Mean earplug attenuation values of the unilateral earplugs in the 4-day (magenta, n = 27) and the 7-day group (green, n = 17). All error bars are ± s.e.m.

Acoustic reflex threshold measurement

Ipsilateral ARTs were measured using the GSI tympstar middle ear analyzer with a 226-Hz probe tone. Ipsilateral measurements involved presenting the eliciting stimulus and measuring the reflex in the same ear. The stimulus used to elicit a reflex was a broadband noise (BBN). The stimulus was of fixed duration (1 s) and presented at an initial level of 60 dB HL. The sound level was increased in 5-dB steps until the reflex was detected (reduction in compliance of >0.02 cm^3). Increasing the stimulus by a further 5 dB confirmed the reflex growth. The stimulus was decreased by 10 dB and increased in 2-dB steps to determine the ART. The stimulus was presented two additional times at the apparent ART to confirm repeatability and then increased by a further 2 dB to confirm reflex growth. If a change in compliance was not seen at the maximum stimulus eliciting level of 95 dB HL, 5 dB was added onto the maximum value as done in previous ART studies (Munro and Blount, 2009). In each case, ART measurements were completed within 30 min after removal of the earplug. For ART measurements, the tester was blinded to which ear had been plugged. Consequently, in half of the participants the previously plugged ear was therefore measured before the control ear.

Data analysis and statistical tests

The data were inspected before analysis to confirm that it was appropriate to use parametric statistics. Statistical analysis of the raw ART data was carried out using a three-factor (tinnitus [yes/no] × ear [plugged/control] × deprivation [pre/post]) repeated-measures analysis of variance (ANOVA). To assess whether different durations of earplug usage had an effect on the change in ART, we performed a three-factor (tinnitus [yes/no] × ear [plugged/control] × duration [4/7 days]) ANOVA. All data analyses were performed using Matlab (The MathWorks Inc., Natick, Massachusetts).

RESULTS

At the end of the earplug period, 30 participants reported experiencing tinnitus sounds at the time of ART measurement. These were classed as "tinnitus-positive" for all further analyses. Those who did not report tinnitus (n = 14) on the final day of the earplug period were classed as "tinnitus-negative". In the 7-day group, an additional four participants reported hearing tinnitus at some point during the earplug period, but the phantom sound disappeared before day 7, and they were thus classified as "tinnitus-negative" in our analyses of ARTs. In the 4-day group, this information was not collected. In both groups, the descriptions of the tinnitus sounds (see Tables 1 and 2) were similar to those typically given by tinnitus patients.

Fig. 2 shows the mean ARTs before and after deprivation. ARTs measured from the previously plugged ears were decreased compared to baseline (by 5.9 ± 1.1 dB in the tinnitus-positive group, and by 6.3 ± 1.1 dB in the tinnitus-negative group), and ARTs measured from the control ears showed a slight increase over the earplug period (by 1.3 ± 0.8 dB in the tinnitus-positive group, and by 1.6 ± 2.0 dB in the tinnitus-negative group). There were a highly significant effect of earplug-induced deprivation (pre- vs post-plugging) ($F(1,84) = 13.0$, $p = 0.00052$), and a highly significant interaction between deprivation and ear (plugged/control) ($F(1,84) = 34.4$, $p < 0.0001$), but no significant effect of tinnitus ($F(1,84) = 0.18$, $p = 0.677$). Thus, there were no significant differences between tinnitus-positive and tinnitus-negative participants, either in the absolute ARTs or in the degree of ART change over the earplug period.

As we used two different lengths of auditory deprivation, we also analyzed whether the different durations of earplugging might have had an influence on the change in ARTs. Fig. 3 depicts the change of the ARTs over the earplug period: Fig. 3a shows the combined data from the 4- and the 7-day

Table 1. Occurrence and description of tinnitus in the 7-day earplug group. Please note that not all participants of the 7-day study gave description of their phantom sounds, as we did not conduct a detailed interview in this study.

Participant number	Tinnitus during earplug period	Tinnitus on day 7	Tinnitus description
1	Y	Y	Tone
2	Y	Y	None given
3	Y	Y	Ringing
4	Y	Y	None given
5	Y	Y	None given
6	Y	Y	Ringing
7	Y	N	trains and whistles
8	Y	N	soft humming
9	N	N	
10	N	N	
11	Y	Y	high-pitched beep
12	N	N	
13	Y	N	humming, ringing, crackling
14	Y	N	Ringing
15	Y	Y	high-pitched tone
16	Y	Y	Ringing
17	Y	Y	None given

Table 2. Occurrence, description and location of tinnitus in the 4-day earplug group.

Participant number	Tinnitus on day 4	Tinnitus description	Tinnitus Location
18	N		
19	N		
20	Y	Tapping noise	Plugged ear only
21	N		
22	Y	Whistling	Plugged ear only
23	Y	Ringing	Plugged ear only
24	Y	White noise	Plugged ear only
25	Y	Ringing	Plugged ear only
26	N		
27	Y	Hissing	Plugged ear only
28	Y	Hissing	Plugged ear only
29	Y	pounding/drilling	In the head
30	Y	Ringing	Plugged ear only
31	Y	Buzzing/humming	Plugged ear only
32	N		
33	Y	Ringing and beating	Plugged ear only
34	Y	Hissing, Whistling, Beating	Plugged ear only
35	Y	Ringing	Plugged ear only
36	Y	Whistling, ringing and beating	Plugged ear only
37	Y	Ringing and beating	Plugged ear only
38	Y	Ringing	Plugged ear only
39	N		
40	Y	Ringing	Both ears, louder in plugged ear
41	N		
42	Y	Ringing	Both ears, louder in plugged ear
43	Y	Ringing and beating	Plugged ear only
44	Y	Ringing and beating	Plugged ear only

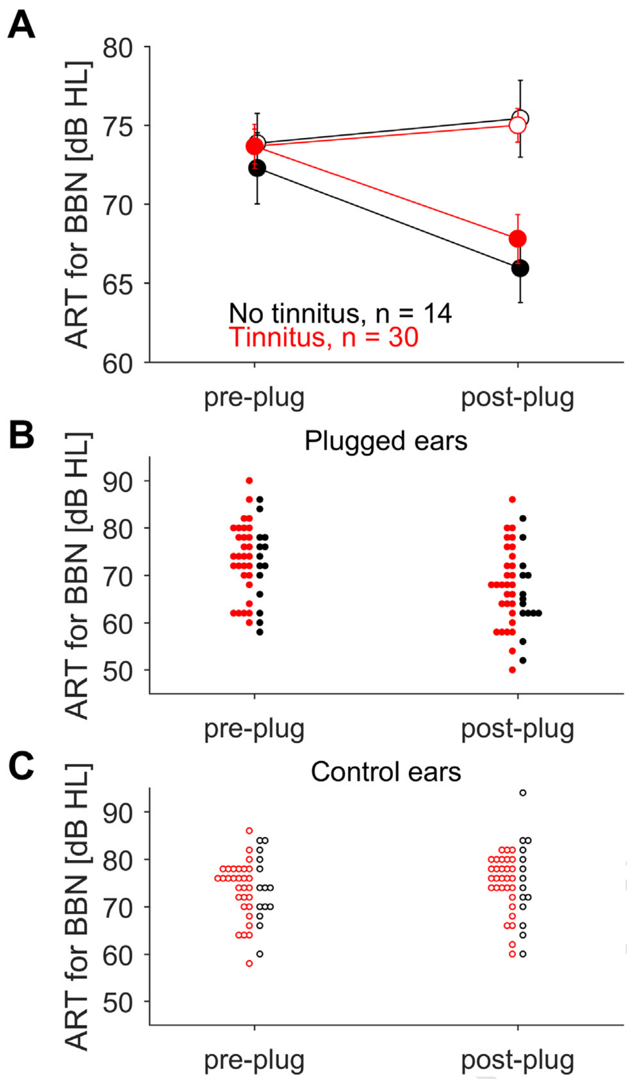

Fig. 2. Acoustic reflex thresholds (ARTs) before and after unilateral auditory deprivation through an earplug. Participants experiencing tinnitus (n = 30) at the end of the earplug period are shown in red, those without tinnitus in black (n = 14). ARTs for the plugged ear are denoted by filled circles, those for the open ears by open circles. Panel (A) shows mean ARTs before and after earplugging, panel (B) individual participants' ARTs for the plugged ears, and panel (C) individual participants' ARTs for the open control ears. There were no significant differences between participants with and without tinnitus. All error bars are ± s.e.m.

group, with participants divided into a tinnitus and a no-tinnitus group. Fig. 3b shows the same tinnitus-grouping for the 4-day earplug group, and Fig. 3c for the 7-day earplug group. Finally, Fig. 3d compares all participants of the 4- and the 7-day group, regardless of tinnitus. There were no differences in the magnitude of ART change between the group with 4 days and the group with 7 days of earplug-induced unilateral auditory deprivation, and no effect of tinnitus perception (three-factor ANOVA, no effect of earplug duration or tinnitus, $F(1,84) = 0.26$, $p = 0.61$ and $F(1,84) = 0.03$, $p = 0.86$, respectively).

DISCUSSION

We have investigated whether there is a relation between the occurrence of tinnitus and changes in the ART after unilateral auditory deprivation through wearing an earplug. Out of 44 participants who wore an earplug continuously, 30 reported experiencing tinnitus at the end of the earplug period. ART measurements with BBN as the eliciting stimulus showed a significant decrease of ARTs measured from the previously plugged ear at the end of the earplug period, but no significant differences between participants with and without tinnitus. Therefore, the changes in subcortical neural response properties underlying the earplug-induced changes in ART are either not related to the occurrence of tinnitus, or they contribute to the occurrence of tinnitus, with a second mechanism determining whether a conscious percept emerges or not.

In this study, we have pooled the data from two investigations that used different durations of earplug usage. We had previously shown that changes in ARTs induced by monaural auditory deprivation through an earplug reach a plateau after 2–4 days of earplug usage (Brotherton et al., 2016). This was confirmed in our current study, as there was no difference in the change in ART from baseline between the 4-day- and the 7-day-earplug group (Fig. 3). The magnitude of changes in the ART observed in the present study was comparable to those seen in other investigations (Munro and Blount, 2009; Brotherton et al., 2016).

To describe the sounds that they experienced, our participants used descriptors that closely resemble those given by tinnitus patients (Tables 1 and 2). Moreover, characterization of the tinnitus sounds using a modified version of the tinnitus spectrum measurement method (Norena et al., 2002) in our previous study (Schaette et al., 2012) yielded "tinnitus spectra" that peaked in the region of the earplug-induced hearing loss, similar to results obtained from tinnitus patients (Norena et al., 2002; König et al., 2006; Roberts et al., 2008). It is thus plausible to assume that the earplug-induced temporary tinnitus and chronic tinnitus experienced by tinnitus patients are closely related phenomena. Our results thus offer potential insights into the mechanisms of tinnitus.

To investigate physiological changes in response to earplug-induced auditory deprivation, we measured changes in the ART, using BBN as an eliciting stimulus, which provides a quick test for changes across a wide range of frequencies. However, many participants described "narrowband" tinnitus sensations like whistling or ringing (Tables 1 and 2), suggesting that plasticity may have been limited to a relatively narrow range of frequency channels in the central auditory system, which might be probed in a more specific way with ART measurements using pure tone stimuli. A limiting factor, however, is that at the high sound intensities required to elicit the acoustic reflex, cochlear excitation patterns are very broad and even a pure tone will excite a large stretch of the basilar membrane (Diehl and Schaette, 2015), and therefore demonstrating a frequency-specific effect in ART measurements might be difficult at best.

As we assessed earplug-induced physiological changes in the central auditory system by measuring changes in the

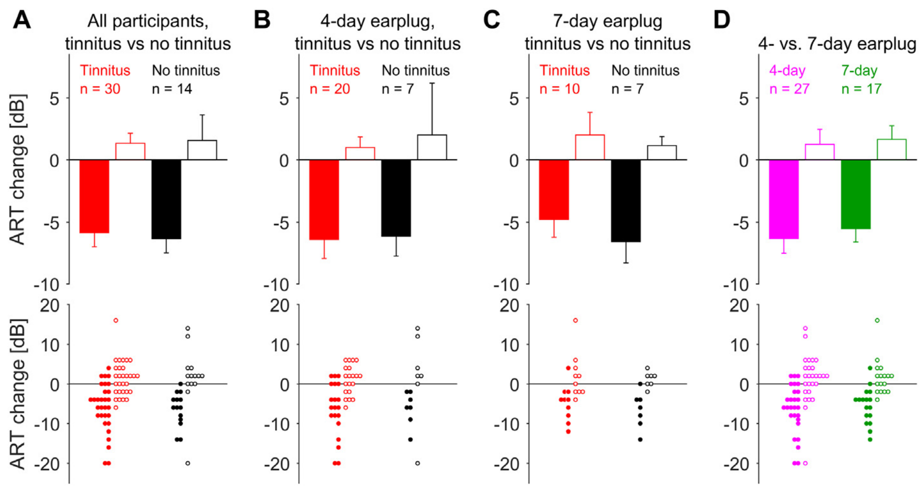

Fig. 3. ART change from baseline after the earplug period. Top panels show mean ART changes, data from plugged ears are shown with filled bars, data from control ears with open bars. Bottom panels show individual participants, data from plugged ears are shown with filled circles, data from control ears with open circles. For the comparisons of tinnitus (red) versus no tinnitus (black), panel (A) shows data from all participants (both earplug durations combined), panel (B) only from those with a 4-day earplug period, and panel (C) from participants with a 7-day earplug period. (D) Comparison of ART changes (participants with and without tinnitus combined) for 4-day (magenta) vs. 7-day (green) earplug duration. All error bars denote are ± s.e.m. Neither the occurrence of tinnitus nor the length of the earplug period had a significant effect on the ART change.

threshold of the acoustic reflex, we only probed a small part of the auditory brainstem: The pathway of the acoustic reflex arc involves the ipsilateral auditory nerve, ventral cochlear nucleus and superior olivary complex. From the superior olivary complex there are projections to the ipsilateral stapedius muscle through the ipsilateral facial nerve nucleus, and to the contralateral stapedius muscle through the contralateral facial nerve nucleus (Lee et al., 2006). Therefore, the decreases in the ipsilateral ART following unilateral earplug use suggest changes in neuronal processing, for example an increase in neuronal response gain (Brotherton et al., 2015), in either the ventral cochlear nucleus or the superior olivary complex. Animal studies have shown an increase in excitatory and a decrease in inhibitory synaptic neurotransmission in the ipsilateral ventral and dorsal cochlear nucleus after 24 h of unilateral earplugging (Whiting et al., 2009). Similarly, increases in neuronal response amplitudes have been observed in the VCN after noise-induced hearing loss (Cai et al., 2009). On the other hand, the amplitude of ABR wave III, which is thought to originate from the VCN (Melcher et al., 1996), was not significantly changed after 4 days of monaural earplugging (Brotherton et al., 2017), demonstrating the need for more research to pinpoint the mechanisms underlying the deprivation-induced changes in ARTs.

Computational modeling studies suggest that changes in synaptic strength, as have been observed in the VCN after earplugging (Whiting et al., 2009), could lead to an increase in neuronal gain sufficient to elevate the level of spontaneous neuronal activity in the cochlear nucleus (Schaette and Kempter, 2006, 2008; Schaette et al., 2012), which could underlie the perception of tinnitus (Schaette and Kempter, 2009; Norena, 2011). Recent animal and human studies have also implicated a role for the ventral cochlear nucleus in the generation of tinnitus (Gu et al., 2012; Coomber et al., 2014; Coomber et al., 2015). Therefore, an increase in neural gain in the cochlear nucleus could potentially underlie both a decrease in ARTs and the occurrence of tinnitus.

Animal studies have produced conflicting results about the relation between the occurrence of tinnitus and subcortical changes in spontaneous neuronal activity. Several studies have reported that increased spontaneous firing rates (Brozoski et al., 2002; Kaltenbach et al., 2004; Koehler and Shore, 2013), increased synchrony of spontaneous activity and increased spontaneous bursting (Wu et al., 2016) in the dorsal cochlear nucleus (the ventral division has not been investigated so far) correlated with assumed behavioral signs of tinnitus after noise exposure. However, other studies have indicated that increased spontaneous firing rates and bursting in the inferior colliculus could be related to hearing loss rather than tinnitus (Coomber et al., 2014; Ropp et al., 2014). Since noise-induced neuronal hyperactivity in the inferior colliculus is driven by the activity of neurons in the cochlear nucleus (Manzoor et al., 2012), the findings from the inferior colliculus also relate to the interpretation of cochlear nucleus results.

Two ways of reconciling conflicting results on the relation between changes in spontaneous neuronal activity and the occurrence of tinnitus, which also offers a framework for interpreting our results on the non-relation between changes in ARTs and the occurrence of tinnitus, are the gating hypothesis (Rauschecker et al., 2010) and the predictive coding hypothesis (Sedley et al., 2016). According to the gating hypothesis, tinnitus requires subcortical changes in neuronal activity patterns that constitute a tinnitus precursor, or a substrate for tinnitus. However, for conscious tinnitus perception to occur, an additional failure of a perceptual gating mechanism, e.g. at the level of the thalamus, is required; otherwise, the subcortical activity patterns that constitute the tinnitus precursor are simply filtered out since they do not provide relevant auditory information about the outside world. In the predictive coding hypothesis, hearing loss also alters subcortical patterns of spontaneous activity, but this tinnitus precursor is normally ignored as imprecise evidence against the prevailing percept of silence. Tinnitus perception then requires focused attention, and the phantom sound is only perpetuated when the default prediction is reset to expecting tinnitus. Following these hypotheses, hearing loss would always generate subcortical changes in neuronal response properties, which is consistent with our finding that both the tinnitus-positive and the tinnitus-negative group showed subcortical changes manifesting as significant decreases in

ARTs in the plugged ear, and also matches animal results that show hearing-loss-related changes in spontaneous neuronal activity without specificity for tinnitus (Coomber et al., 2014; Ropp et al., 2014). Conscious perception of tinnitus would then require additional changes at a higher level of the auditory pathway (Rauschecker et al., 2010; Leaver et al., 2011; Song et al., 2015a; Sedley et al., 2016), which were simply not assessed through our ART measurements. In a previous study, we have shown that changes in ARTs and changes in perceived loudness after earplugging show different patterns (Munro et al., 2014), suggesting that the earplug paradigm could enable studies of tinnitus-related changes in auditory processing, for example through neuroimaging before and after the earplug period. Moreover, since the tinnitus induced by the earplug was not perceived as bothersome by the participants, it would be possible to investigate just the neural correlates of the phantom sounds, without having to take into account the neural activity patterns related to tinnitus distress (Song et al., 2015b).

CONCLUSIONS

We have demonstrated that temporary tinnitus induced by auditory deprivation by means of an earplug might be used to assess tinnitus-related changes in the human auditory system. We have assessed subcortical changes in neural responses through ART measurements, and shown that changes in ARTs through auditory deprivation are not specific for tinnitus. Therefore, the neurophysiological changes underlying the decrease in ARTs might either not be related to the occurrence of tinnitus, or they might be a necessary component of the generation of a tinnitus precursor, but with additional changes at a higher level of auditory processing required to give rise to tinnitus.

ACKNOWLEDGMENTS

This study was supported by the NIHR Manchester Biomedical Research Centre. Q5

REFERENCES

Adjamian P, Sereda M, Zobay O, Hall DA, Palmer AR. (2012) Neuromagnetic indicators of tinnitus and tinnitus masking in patients with and without hearing loss. J Assoc Res Otolaryngol 13:715-731.

Ahlf S, Tziridis K, Korn S, Strohmeyer I, Schulze H. (2012) Predisposition for and prevention of subjective tinnitus development. PLoS One 7: e44519.

Axelsson A, Ringdahl A. (1989) Tinnitus — a study of its prevalence and characteristics. Br J Audiol 23:53-62.

Baguley D, McFerran D, Hall D. (2013) Tinnitus. Lancet 382:1600-1607.

Barnea G, Attias J, Gold S, Shahar A. (1990) Tinnitus with normal hearing sensitivity: extended high-frequency audiometry and auditory-nerve brain-stem-evoked responses. Audiology 29:36-45.

Bauer CA, Turner JG, Caspary DM, Myers KS, Brozoski TJ. (2008) Tinnitus and inferior colliculus activity in chinchillas related to three distinct patterns of cochlear trauma. J Neurosci Res 86:2564-2578.

Bramhall NF, Konrad-Martin D, McMillan GP. (2018) Tinnitus and auditory perception after a history of noise exposure: relationship to auditory brainstem response measures. Ear Hear 39:881-894.

Brotherton H, Plack CJ, Maslin M, Schaette R, Munro KJ. (2015) Pump up the volume: could excessive neural gain explain tinnitus and hyperacusis? Audiol Neurootol 20:273-282.

Brotherton H, Plack CJ, Schaette R, Munro KJ. (2016) Time course and frequency specificity of sub-cortical plasticity in adults following acute unilateral deprivation. Hear Res 341:210-219.

Brotherton H, Plack CJ, Schaette R, Munro KJ. (2017) Using acoustic reflex threshold, auditory brainstem response and loudness judgments to investigate changes in neural gain following acute unilateral deprivation in normal hearing adults. Hear Res 345:88-95.

Brozoski TJ, Bauer CA. (2005) The effect of dorsal cochlear nucleus ablation on tinnitus in rats. Hear Res 206:227-236.

Brozoski TJ, Bauer CA, Caspary DM. (2002) Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. J Neurosci 22:2383-2390.

Cai S, Ma WL, Young ED. (2009) Encoding intensity in ventral cochlear nucleus following acoustic trauma: implications for loudness recruitment. J Assoc Res Otolaryngol 10:5-22.

Chrostowski M, Yang L, Wilson HR, Bruce IC, Becker S. (2011) Can homeostatic plasticity in deafferented primary auditory cortex lead to travelling waves of excitation? J Comput Neurosci 30:279-299.

Coomber B, Berger JI, Kowalkowski VL, Shackleton TM, Palmer AR, Wallace MN. (2014) Neural changes accompanying tinnitus following unilateral acoustic trauma in the Guinea pig. Eur J Neurosci 40:2427-2441.

Coomber B, Kowalkowski VL, Berger JI, Palmer AR, Wallace MN. (2015) Modulating central gain in tinnitus: changes in nitric oxide synthase in the ventral cochlear nucleus. Front Neurol 6:53.

Davies J, Gander PE, Andrews M, Hall DA. (2014) Auditory network connectivity in tinnitus patients: a resting-state fMRI study. Int J Audiol 53:192-198.

Diehl PU, Schaette R. (2015) Abnormal auditory gain in Hyperacusis: investigation with a computational model. Front Neurol 6:157.

Eggermont JJ. (2013) Hearing loss, hyperacusis, or tinnitus: what is modeled in animal research? Hear Res 295:140-149.

Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake JA, Sudanagunta SP, Borland MS, Kilgard MP. (2011) Reversing pathological neural activity using targeted plasticity. Nature 470:101-104.

Formby C, Sherlock LP, Gold SL. (2003) Adaptive plasticity of loudness induced by chronic attenuation and enhancement of the acoustic background. J Acoust Soc Am 114:55-58.

Fournier P, Hebert S. (2013) Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? Hear Res 295:16-23.

Gilles A, Schlee W, Rabau S, Wouters K, Fransen E, Van de Heyning P. (2016) Decreased speech-in-noise understanding in young adults with tinnitus. Front Neurosci 10:288.

Gu JW, Herrmann BS, Levine RA, Melcher JR. (2012) Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. J Assoc Res Otolaryngol . Q6

Guest H, Munro KJ, Prendergast G, Howe S, Plack CJ. (2017) Tinnitus with a normal audiogram: relation to noise exposure but no evidence for cochlear synaptopathy. Hear Res 344:265-274.

Kaltenbach JA, Zacharek MA, Zhang J, Frederick S. (2004) Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. Neurosci Lett 355:121-125.

Koehler SD, Shore SE. (2013) Stimulus timing-dependent plasticity in dorsal cochlear nucleus is altered in tinnitus. J Neurosci 33:19647-19656.

König O, Schaette R, Kempter R, Gross M. (2006) Course of hearing loss and occurrence of tinnitus. Hear Res 221:59-64.

Leaver AM, Renier L, Chevillet MA, Morgan S, Kim HJ, Rauschecker JP. (2011) Dysregulation of limbic and auditory networks in tinnitus. Neuron 69:33-43.

Lee DJ, de Venecia RK, Guinan JJ, Brown MC. (2006) Central auditory pathways mediating the rat middle ear muscle reflexes. Anat Rec Part A 288A:358-369.

Lockwood AH, Salvi RJ, Burkard RF. (2002) Tinnitus. N Engl J Med 347:904-910.

Lorenz I, Muller N, Schlee W, Hartmann T, Weisz N. (2009) Loss of alpha power is related to increased gamma synchronization—a marker of reduced inhibition in tinnitus? Neurosci Lett 453:225-228.

Manzoor N, Licari FG, Klapchar M, Elkin R, Gao Y, Kaltenbach JA. (2012) Noise-induced hyperactivity in the inferior colliculus: its relationship with hyperactivity in the dorsal cochlear nucleus. J Neurophysiol 108:976-988.

Melcher JR, Guinan JJ, Knudson IM, Kiang NY. (1996) Generators of the brainstem auditory evoked potential in cat. II. Correlating lesion sites with waveform changes. Hear Res 93:28-51.

Munro KJ, Blount J. (2009) Adaptive plasticity in brainstem of adult listeners following earplug-induced deprivation. J Acoust Soc Am 126:568-571.

Munro KJ, Turtle C, Schaette R. (2014) Sub-cortical plasticity and modified loudness following short-term unilateral deprivation: evidence of multiple neural gain mechanisms within the auditory system. to appear in. J Acoust Soc Am .

Nicolas-Puel C, Faulconbridge RL, Guitton M, Puel JL, Mondain M, Uziel A. (2002) Characteristics of tinnitus and etiology of associated hearing loss: a study of 123 patients. Int Tinnitus J 8:37-44.

Norena AJ. (2011) An integrative model of tinnitus based on a central gain controlling neural sensitivity. Neurosci Biobehav Rev 35:1089-1109.

Norena A, Micheyl C, Chery-Croze S, Collet L. (2002) Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. Audiol Neurootol 7:358-369.

Ortmann M, Muller N, Schlee W, Weisz N. (2011) Rapid increases of gamma power in the auditory cortex following noise trauma in humans. Eur J Neurosci 33:568-575.

Parra LC, Pearlmutter BA. (2007) Illusory percepts from auditory adaptation. J Acoust Soc Am 121:1632-1641.

Pierzycki RH, McNamara AJ, Hoare DJ, Hall DA. (2016) Whole scalp resting state EEG of oscillatory brain activity shows no parametric relationship with psychoacoustic and psychosocial assessment of tinnitus: a repeated measures study. Hear Res 331:101-108.

Pilgramm M, Rychlick R, Lebisch H, Siedentrop H, Goebel G, Kirchhoff D. (1999) Tinnitus in the Federal Republic of Germany: a representative epidemological study. In: & Hazell JWP, editor. Sixth international tinnitus seminar. London: The Tinnitus and Hyperacusis Centre.

Rauschecker JP, Leaver AM, Muhlau M. (2010) Tuning out the noise: limbic-auditory interactions in tinnitus. Neuron 66:819-826.

Roberts LE, Moffat G, Baumann M, Ward LM, Bosnyak DJ. (2008) Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift. J Assoc Res Otolaryngol 9:417-435.

Roberts LE, Eggermont JJ, Caspary DM, Shore SE, Melcher JR, Kaltenbach JA. (2010) Ringing ears: the neuroscience of tinnitus. J Neurosci 30:14972-14979.

Ropp TJ, Tiedemann KL, Young ED, May BJ. (2014) Effects of unilateral acoustic trauma on tinnitus-related spontaneous activity in the inferior colliculus. J Assoc Res Otolaryngol 15:1007-1022.

Sanchez TG, Medeiros IR, Levy CP, Ramalho Jda R, Bento RF. (2005) Tinnitus in normally hearing patients: clinical aspects and repercussions. Braz J Otorhinolaryngol 71:427-431.

Schaette R. (2013) Tinnitus in men, mice (as well as other rodents), and machines. Hear Res , https://doi.org/10.1016/j.heares.2013.1012.1004.

Schaette R, Kempter R. (2006) Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. Eur J Neurosci 23:3124-3138.

Schaette R, Kempter R. (2008) Development of hyperactivity after hearing loss in a computational model of the dorsal cochlear nucleus depends on neuron response type. Hear Res 240:57-72.

Schaette R, Kempter R. (2009) Predicting tinnitus pitch from patients' audiograms with a computational model for the development of neuronal hyperactivity. J Neurophysiol 101:3042-3052.

Schaette R, McAlpine D. (2011) Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. J Neurosci 31:13452-13457.

Schaette R, Turtle C, Munro KJ. (2012) Reversible induction of phantom auditory sensations through simulated unilateral hearing loss. PLoS One , https://doi.org/10.1371/journal.pone.0035238.

Sedley W, Teki S, Kumar S, Barnes GR, Bamiou DE, Griffiths TD. (2012) Single-subject oscillatory gamma responses in tinnitus. Brain 135:3089-3100.

Sedley W, Gander PE, Kumar S, Oya H, Kovach CK, Nourski KV, Kawasaki H, Howard MA, Griffiths TD. (2015) Intracranial mapping of a cortical tinnitus system using residual inhibition. Curr Biol 25:1208-1214.

Sedley W, Friston KJ, Gander PE, Kumar S, Griffiths TD. (2016) An integrative tinnitus model based on sensory precision. Trends Neurosci 39:799-812.

Sereda M, Hall DA, Bosnyak DJ, Edmondson-Jones M, Roberts LE, Adjamian P, Palmer AR. (2011) Re-examining the relationship between audiometric profile and tinnitus pitch. Int J Audiol 50:303-312.

Shore SE, Roberts LE, Langguth B. (2016) Maladaptive plasticity in tinnitus—triggers, mechanisms and treatment. Nat Rev Neurol 12:150-160.

Song JJ, Vanneste S, De Ridder D. (2015) Dysfunctional noise cancelling of the rostral anterior cingulate cortex in tinnitus patients. PLoS One 10e0123538.

Song JJ, Vanneste S, Schlee W, Van de Heyning P, De Ridder D. (2015) Onset-related differences in neural substrates of tinnitus-related distress: the anterior cingulate cortex in late-onset tinnitus, and the frontal cortex in early-onset tinnitus. Brain Struct Funct 220:571-584.

Weisz N, Moratti S, Meinzer M, Dohrmann K, Elbert T. (2005) Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography. PLoS Med 2:e153.

Weisz N, Muller S, Schlee W, Dohrmann K, Hartmann T, Elbert T. (2007) The neural code of auditory phantom perception. J Neurosci 27:1479-1484.

Whiting B, Moiseff A, Rubio ME. (2009) Cochlear nucleus neurons redistribute synaptic AMPA and glycine receptors in response to monaural conductive hearing loss. Neuroscience 163:1264-1276.

Wu C, Martel DT, Shore SE. (2016) Increased synchrony and bursting of dorsal cochlear nucleus fusiform cells correlate with tinnitus. J Neurosci 36:2068-2073.

(Received 28 October 2018, Accepted 7 March 2019)
(Available online xxxx)