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.

Keywords: 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), 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 on-going 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.

Methods

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 13183), 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 analyser; 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.

Acoustic reflex threshold measurement

Ipsilateral ARTs were measured using the GSI tympstar middle ear analyser 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 second) 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 minutes 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] X ear [plugged/control] X 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] X ear [plugged/control] X duration [4/7 days]) ANOVA. All data analysis was 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.

Figure 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 was 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, neither in the absolute ARTs nor 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. Figure 3 depicts the change of the ARTs over the earplug period: Fig. 3a shows the combined data from the 4- and the 7-day 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, characterisation 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 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 hours 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 modelling 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 focussed 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.

Acknowledgements

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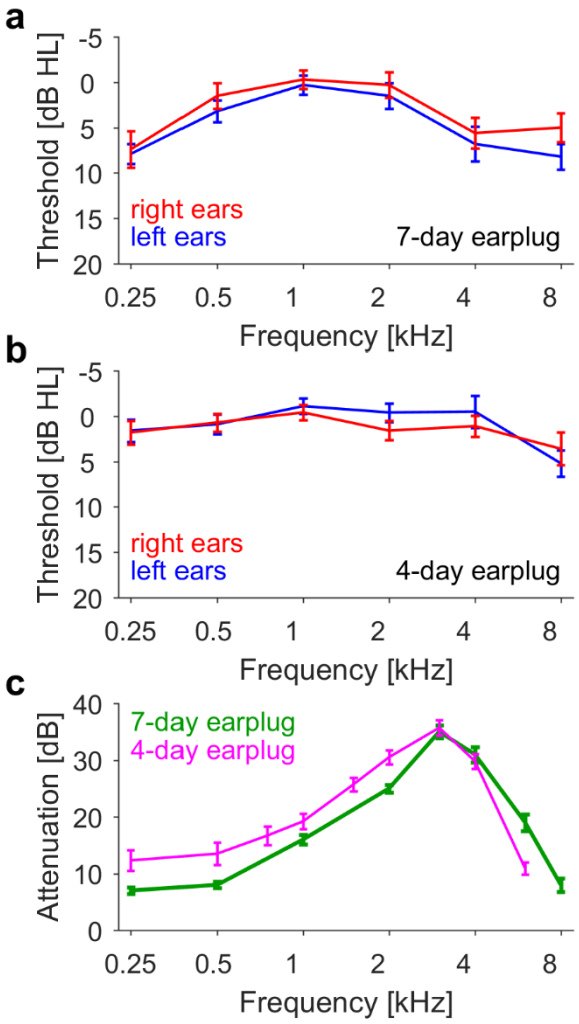

Figure 1: Audiograms and earplug attenuation. **a)** Mean audiograms of the left (blue line) and the right ears (red line) of the participants that wore an earplug for 7 days (n = 17). **b)** Mean audiograms of the left (blue line) and the right ears (red line) of the participants that wore an earplug 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.

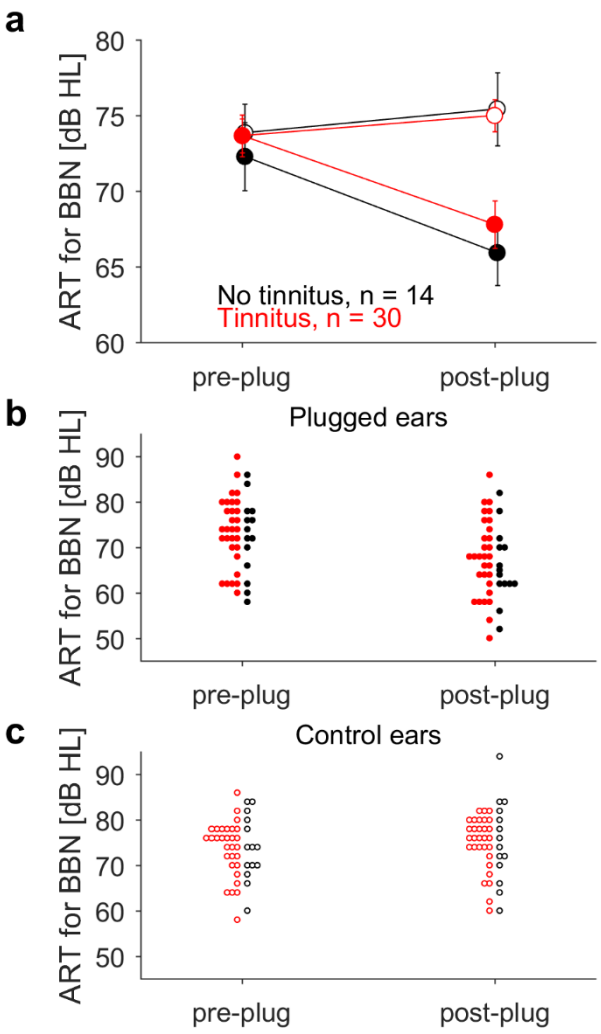

Figure 2: Acoustic reflex thresholds (ARTs) before and after unilateral auditory deprivation through an earplug. Participants experiencing tinnitus (n = 30) at the end of the earplug period are shown in red, those without tinnitus in black (n = 14). ARTs for the plugged ear are denoted by filled circles, 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.

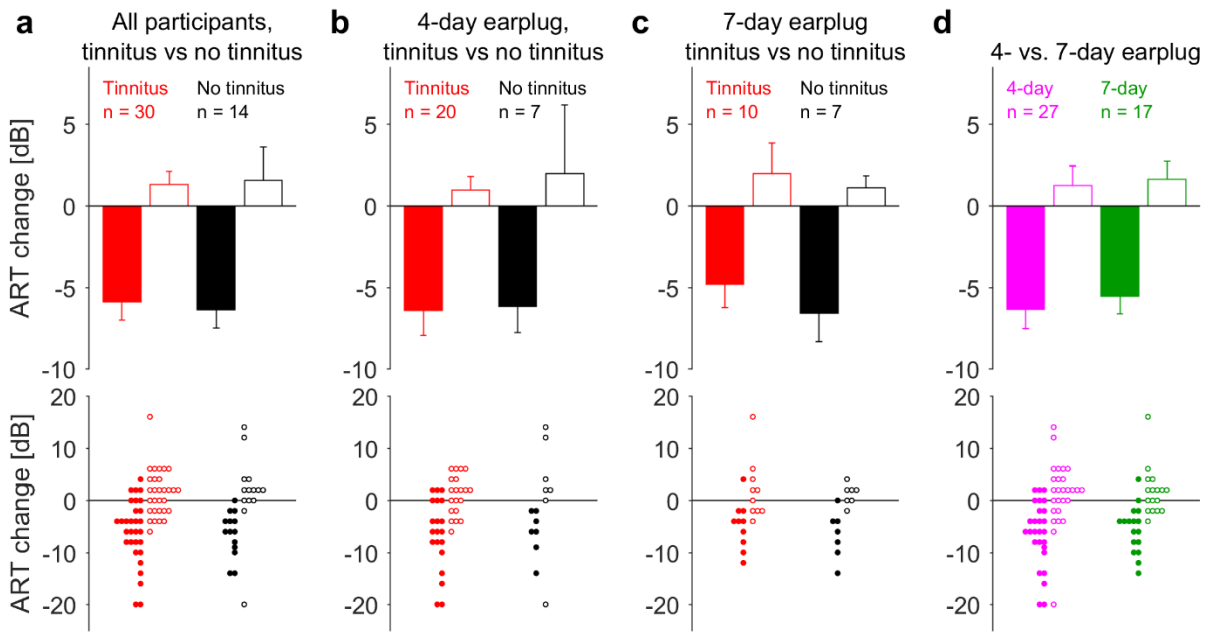

Figure 3: ART change from baseline after the earplug period. Top panels show mean ART changes, data from plugged ears is shown with filled bars, data from control ears with open bars. Bottom panels show individual participants, data from plugged ears is shown with filled circles, data from control ears 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.

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

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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.

Keywords: 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), 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 on-going 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.

Methods

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 13183), 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 analyser; 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.

Acoustic reflex threshold measurement

Ipsilateral ARTs were measured using the GSI tympstar middle ear analyser 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 second) 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 minutes 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] X ear [plugged/control] X 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] X ear [plugged/control] X duration [4/7 days]) ANOVA. All data analysis was performed using Matlab (The MathWorks Inc., Natick, Massachusetts).

Results

At the end of the earplug period, 30 participants reported experiencing ~~stable, constant~~ 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.

Figure 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 was 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, neither in the absolute ARTs nor 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. Figure 3 depicts the change of the ARTs over the earplug period: Fig. 3a shows the combined data from the 4- and the 7-day 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, characterisation 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 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 hours 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 modelling studies suggest that ~~such~~ 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 focussed 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.

Acknowledgements

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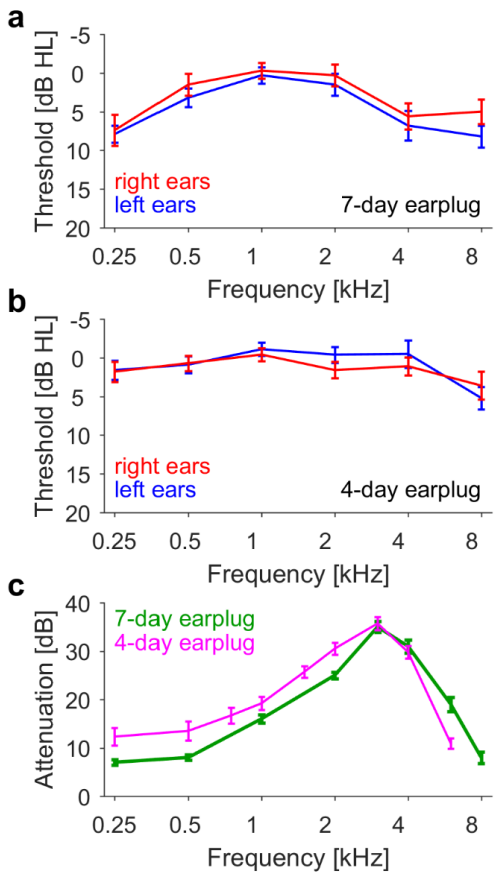

Figure 1: Audiograms and earplug attenuation. **a)** Mean audiograms of the left (blue line) and the right ears (red line) of the participants that wore an earplug for 7 days (n = 17). **b)** Mean audiograms of the left (blue line) and the right ears (red line) of the participants that wore an earplug 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~~y~~, n = 17). All error bars are ± s.e.m.

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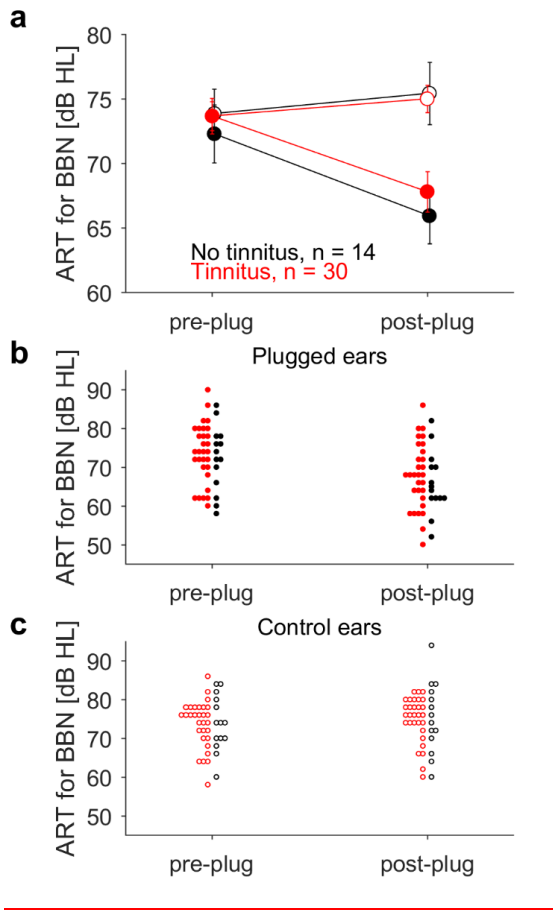

Figure 2: Acoustic reflex thresholds (ARTs) before and after unilateral auditory deprivation through an earplug. Participants experiencing tinnitus (n = 30) at the end of the earplug period are shown in red, those without tinnitus in black (n = 14). ARTs for the plugged ear are denoted by filled circles, 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.

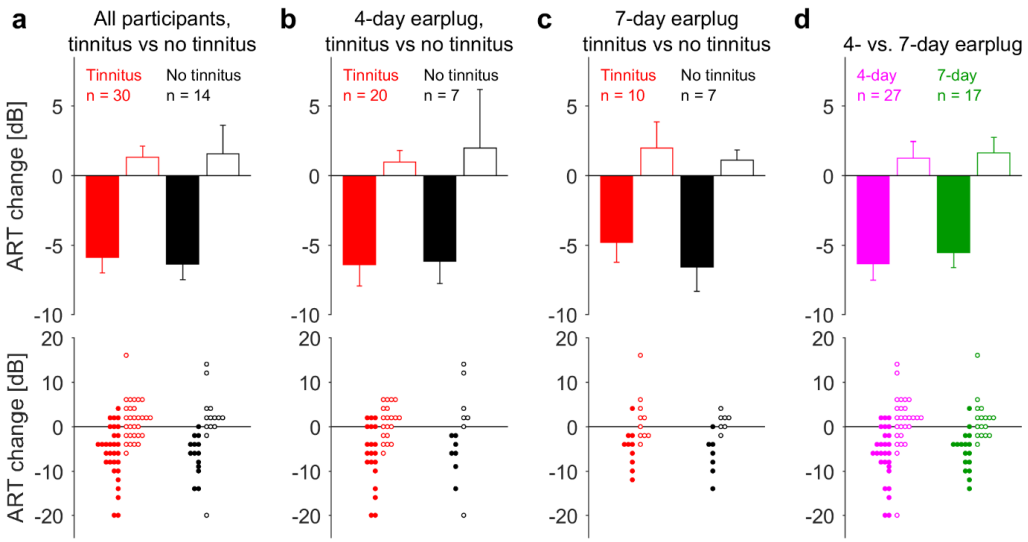

Figure 3: ART change from baseline after the earplug period. Top panels show mean ART changes, ~~D~~data from plugged ears is shown with filled bars, data from control ears with open bars. Bottom panels show individual participants, data from plugged ears is shown with filled circles, data from control ears with open circles. For the comparisons of tinnitus (red) versus no tinnitus (black), ~~P~~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.

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
~~A~~1	Y	Y	Tone
~~A~~2	Y	Y	None given
~~A3 4~~	Y	Y	Ringing
~~A4 6~~	Y	Y	None given
~~A5 7~~	Y	Y	None given
~~A6 8~~	Y	Y	Ringing
~~A7 9~~	Y	N	trains and whistles
~~A8 10~~	Y	N	soft humming
~~A9 11~~	N	N	
~~A10 4~~	N	N	
~~A11 7~~	Y	Y	high-pitched beep
~~A12 9~~	N	N	
~~A13 20~~	Y	N	humming, ringing, crackling
~~A14 21~~	Y	N	Ringing
~~A15 22~~	Y	Y	high-pitched tone
~~A16 23~~	Y	Y	Ringing
~~A17 24~~	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
18B3	N		
19B4	N		
20B5	Y	Tapping noise	Plugged ear only
21B6	N		
22B7	Y	Whistling	Plugged ear only
23B8	Y	Ringing	Plugged ear only
24B9	Y	White noise	Plugged ear only
25B10	Y	Ringing	Plugged ear only
26B11	N		
27B12	Y	Hissing	Plugged ear only
28B13	Y	Hissing	Plugged ear only
29B15	Y	pounding/drilling	In the head
30B16	Y	Ringing	Plugged ear only
31B17	Y	Buzzing/humming	Plugged ear only
32B18	N		
33B19	Y	Ringing and beating	Plugged ear only
34B20	Y	Hissing, Whistling, Beating	Plugged ear only
35B21	Y	Ringing	Plugged ear only
36B22	Y	Whistling, ringing and beating	Plugged ear only
37B23	Y	Ringing and beating	Plugged ear only
38B24	Y	Ringing	Plugged ear only
39B27	N		
40B28	Y	Ringing	Both ears, louder in plugged ear
41B29	N		
42B31	Y	Ringing	Both ears, louder in plugged ear
43B33	Y	Ringing and beating	Plugged ear only
44B34	Y	Ringing and beating	Plugged ear only

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Figure 1

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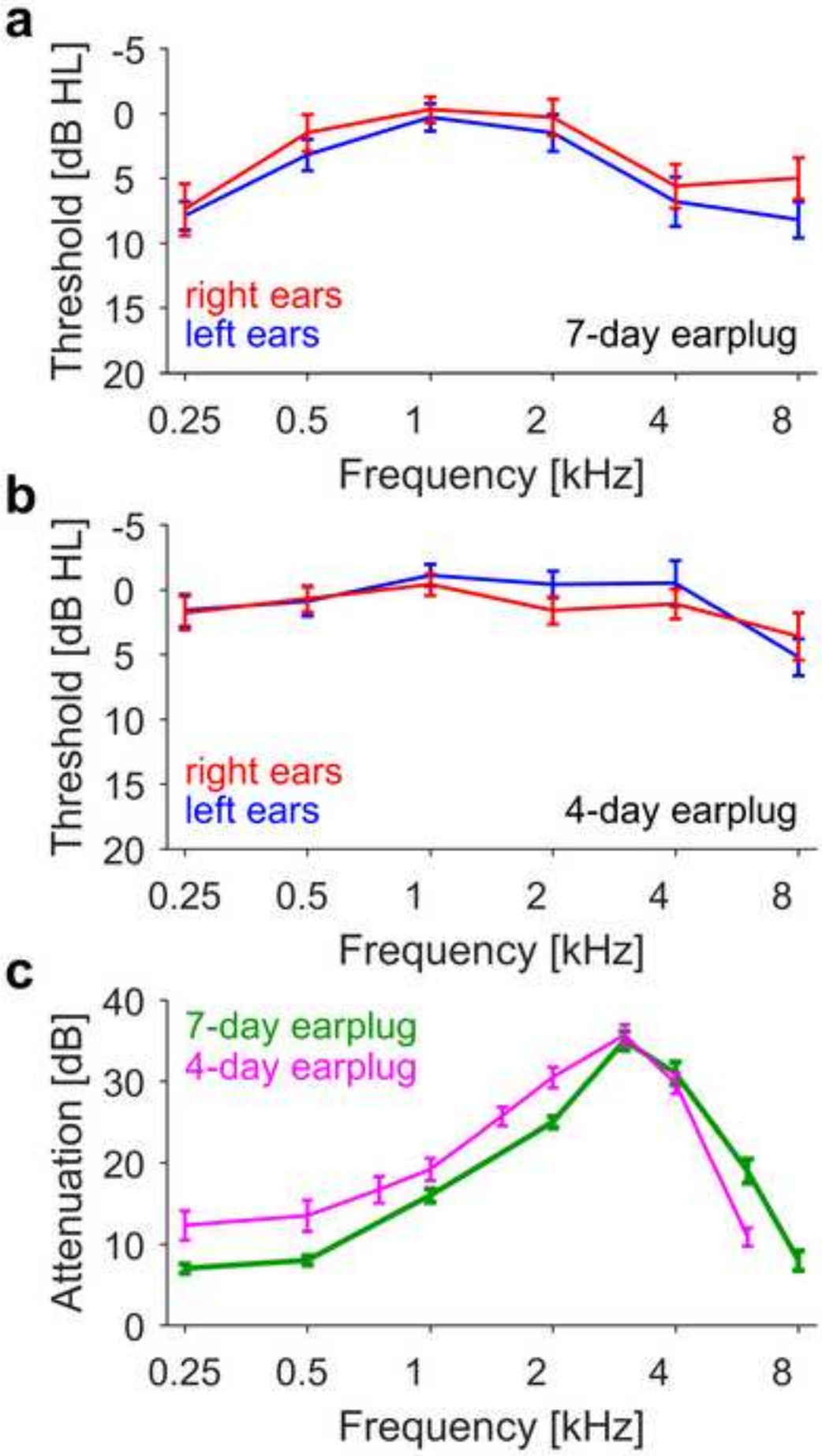

Figure 2

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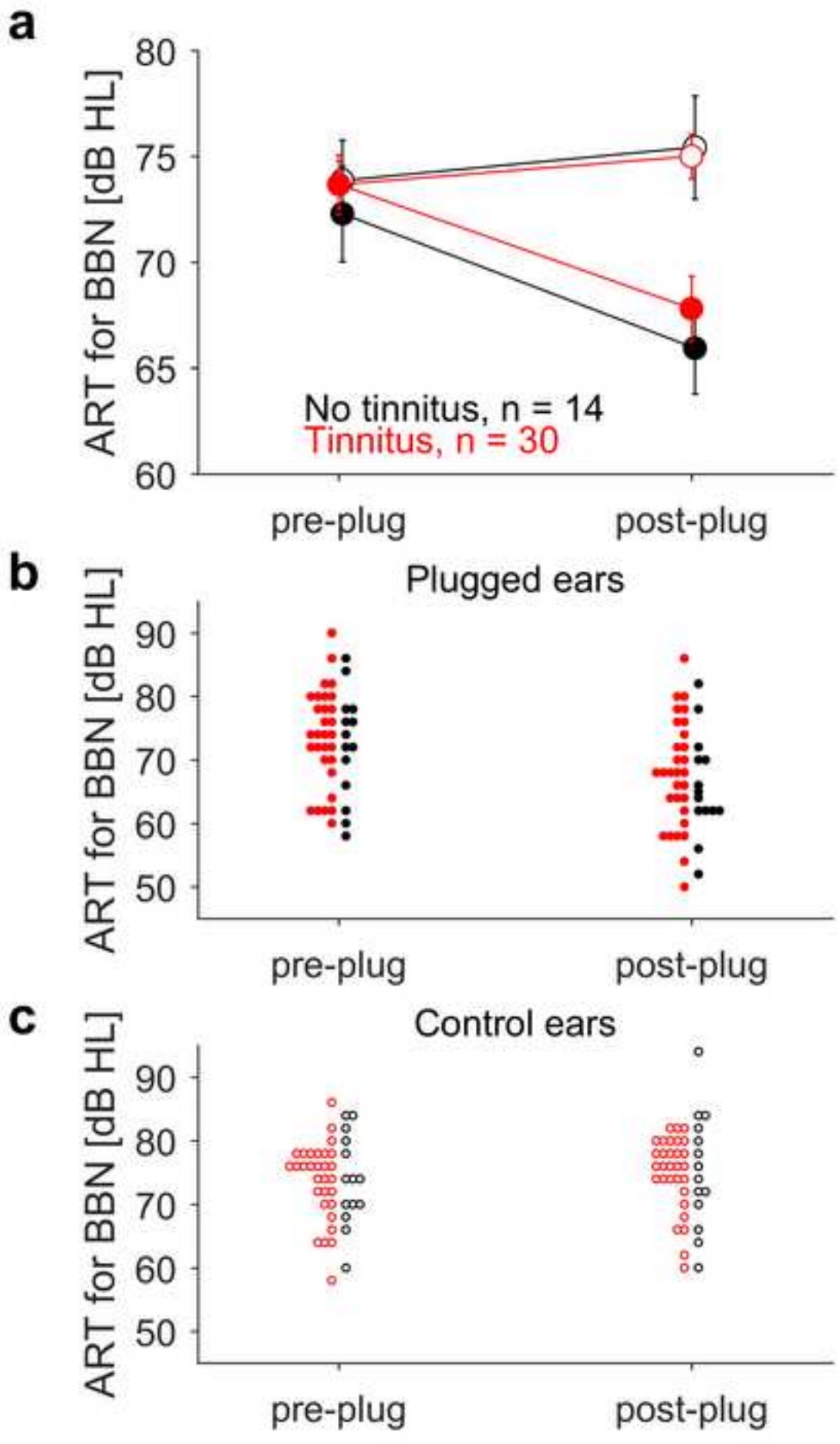

Figure 3

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