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# Cortical Tonotopic Map Changes in Humans are Larger in Hearing Loss than in additional Tinnitus

*Short title*

Tonotopic Map Changes in Hearing Loss and Tinnitus

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**Abstract**

Neural plasticity due to hearing loss results in tonotopic map changes. Several studies have suggested a relation between hearing-loss-induced tonotopic reorganization and tinnitus. This large functional magnetic resonance imaging (fMRI) study on humans intended to clarify the relations between hearing loss, tinnitus and tonotopic reorganization. To determine the differential effect of hearing loss and tinnitus, both male and female participants with bilateral high frequency hearing loss, with and without tinnitus, and a control group were included. In a total of 90 participants, bilateral cortical responses to sound stimulation were measured with loudness matched pure-tone stimuli (0.25 - 8 kHz). In the bilateral auditory cortices, the high frequency sound-evoked activation level was higher in both hearing-impaired participant groups, compared to the control group. This was most prominent in the hearing loss group without tinnitus. Similarly, the tonotopic maps for the hearing loss without tinnitus group were significantly different from the controls, whereas the maps of those with tinnitus were not. These results show that higher response amplitudes and map reorganization are a characteristic of hearing loss, not of tinnitus. Both tonotopic maps and response amplitudes of tinnitus participants appear intermediate to the controls and hearing loss without tinnitus group. This observation suggests a connection between tinnitus and an incomplete form of central compensation to hearing loss, rather than excessive adaptation. One implication of this may be that treatments for tinnitus shift their focus towards enhancing the cortical plasticity on track, instead of reversing it.

Keywords: plasticity, auditory cortex, hearing loss, tinnitus, tonotopy

**Significance Statement**

Tinnitus, a common and potentially devastating condition, is the presence of a 'phantom' sound that often accompanies hearing loss. Hearing loss is known to induce plastic changes in cortical and sub-cortical areas. Although plasticity is a valuable trait that allows the human brain to rewire and recover from injury and sensory deprivation, it can lead to tinnitus as an unwanted side effect. In this large fMRI study, we provide evidence that tinnitus is related to a more conservative form of reorganization than in hearing loss without tinnitus. This result contrasts with the previous notion that tinnitus is related to excessive reorganization. As a consequence, treatments for tinnitus may need to enhance the cortical plasticity, rather than reversing it.

## Introduction

Peripheral damage causes plasticity to occur in the area of the central nervous system that corresponds to the loss of function. In the auditory domain hearing loss instigates plasticity that results in changes in tonotopic maps, spontaneous activity, and neural synchronicity (Robertson and Irvine, 1989; Eggermont and Roberts, 2004). Tonotopic maps are a striking feature of the mammalian auditory cortex and underlie the representation of complex sounds such as speech. This spatial separation of frequencies originates in the inner ear, where high frequencies are processed in the base of the cochlea and low frequencies in the apex. This separation is maintained from the cochlea to the auditory cortex (Brugge and Merzenich, 1973; Rauschecker et al., 1995). The tonotopic maps can be disrupted by hearing loss, the most prevalent sensory deficit in the elderly population.

The presence of clinical hearing loss increases the chances of developing tinnitus, the perception of sound in the absence of an external source. To this date the specific pathophysiology involved in tinnitus remains elusive. However, the tinnitus pitch is often constrained to the frequency regions affected by hearing loss (Schecklmann et al., 2012; Shekhawat et al., 2014; Sereda et al., 2015; Keppler et al., 2017), or to the border of the intact hearing region (Moore et al., 2010). These findings suggest that hearing loss and tinnitus are intricately related. Excessive or conservative tonotopic reorganization may differentiate between hearing loss with and without tinnitus.

Several papers have suggested a relation between hearing loss-induced tonotopic reorganization and tinnitus (Robertson and Irvine, 1989; Muhlnickel et al., 1998; Rauschecker, 1999; Eggermont and Roberts, 2004; Norena and Eggermont, 2005;

Eggermont, 2006), but few have directly investigated this relation. In previous experimental work the observed tonotopic map plasticity was linked to hearing loss but not to tinnitus (Weisz et al., 2005; Wienbruch et al., 2006; McMahon et al., 2016). In humans, tonotopic map reorganization was reported in one MEG study on tinnitus. A positive correlation was reported between the strength of the perceived tinnitus and the extent of cortical reorganization (Muhlnickel et al., 1998). In contrast, other studies reported no tonotopic plasticity related to tinnitus in humans (Langers et al., 2012) or animals (Kotak et al., 2005; Yang et al., 2011). Instead, these animal studies identified enhanced cortical excitation or reduced cortical inhibition in animals with binaural hearing loss and behavioral signs of tinnitus. The release from inhibition in the hearing loss affected area connects the tinnitus pitch with increased neuronal excitability (Yang et al., 2011). In general, it is not well established that tonotopic map plasticity is a cortical characteristic of tinnitus.

Animal-models of cortical tonotopic reorganization indicate that receptive fields of neurons within the hearing loss affected area shift towards the intact receptors (Rajan and Irvine, 1998; Eggermont and Komiya, 2000; Irvine et al., 2001; Muhlau et al., 2006). This reorganization causes a downwards shift in the characteristic frequency of neurons, in both temporary and lasting hearing loss (Irvine et al., 2000; Norena and Eggermont, 2005, 2006), thus altering the tonotopic map. In contrast, not all animal studies on hearing loss found a downwards shift in tonotopic maps, but instead reported increased excitability (Kotak et al., 2005) or decreased inhibition (Rajan, 1998) of the affected frequency regions. In humans, one MEG study reported a shift of the cortical responsive region towards the intact edge-frequency of the audiogram in hearing loss (Dietrich et al., 2001). In summary, different correlates of tonotopic plasticity have been reported in

literature on hearing loss and tinnitus, and the translation of animal-models to human imaging is sparse especially in tinnitus.

This large fMRI study examined the relation between hearing loss, tinnitus, and tonotopic reorganization with loudness-matched sound stimuli in humans. Inclusion of participants with high frequency hearing loss, both with and without tinnitus, allowed us to investigate to what extent reorganization is a consequence of hearing loss, and whether any reorganization is specifically related to tinnitus.

## Materials and methods

The study was approved, in accordance with the principles of the declaration of Helsinki (2013), by the medical ethical committee of the University Medical Center Groningen, the Netherlands. Written informed consent was obtained and participants received reimbursement for their participation.

### Participants

A total of 113 participants, both male and female, were included in a larger MRI study. In 90 participants, three complete functional runs were obtained. This resulted in 35 participants with hearing loss and tinnitus, 17 participants with hearing loss without tinnitus, and 38 healthy controls without hearing loss or tinnitus (Table 1). None of the participants were using hearing aids to compensate their hearing loss, or ameliorate their tinnitus. Pure tone audiometry was performed in a sound attenuating booth to determine hearing thresholds for all participants at octave frequencies ranging from 0.125 to 8 kHz. Tinnitus pitch and loudness were estimated with a matching procedure. In addition, the participants completed the Tinnitus Handicap Inventory (McCombe et al., 2001), the

Tinnitus Reactions Questionnaire (Wilson et al., 1991), the Hyperacusis Questionnaire (Khalfa et al., 2002) and the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983).

Group differences were tested with a Chi-square test of independence for the variable sex, and a three-group ANOVA followed-up by independent pairwise t-tests for the variable age. The questionnaire scores were assessed by means of a Kruskal-Wallis test and followed up by a pairwise Mann-Whitney test.

## EXPERIMENTAL DESIGN

### Data acquisition

All MRI data was obtained with a 3.0 T Philips Intera MRI scanner (Best, the Netherlands), at the Neuro Imaging Center Groningen. The scanner was equipped with a SENSE 32-channel head coil. Both structural and functional images were obtained for each participant. The structural image was a whole brain T1 weighted image (voxel size 1mm x 1mm x 1mm). The functional images were acquired in a sparse imaging sequence (Hall et al., 1999), as single shot EPI: 47 slices; no gap; scan matrix 72 x 67; descending slice order; TR of 10 seconds, TE 22 ms, Flip Angle 90°. For each participant a total of three runs, of each 65 EPI volumes, were acquisitioned.

### Sound stimuli

During the fMRI experiments, loudness matched auditory stimuli were presented. Prior to the MRI session, participants performed a binaural loudness matching task in which the stimulus tones at 0.25, 0.5, 2, 4, and 8 kHz were all matched in perceived loudness to a 1-kHz tone at 40 dB SPL. This compensates for loudness distortion present in

sensorineural hearing loss (Moore and Glasberg, 2004). In addition, studies indicate that sound-evoked cortical activation correlates better with loudness rather than the level of sound stimuli (Hall et al., 2001; Langers et al., 2007). A two alternative-forced-choice, 1-up-1-down loudness matching procedure was used to approximate equal loudness sensation over all frequencies. An interleaved staircase method was applied, with a maximum of 15 trials per frequency, 7 reversals, and a step size of [10,5,5,3,3,1] dB SPL. This method yielded an equal loudness contour for each participant.

Procedure MRI

The individually loudness-matched auditory stimuli were presented during the relatively silent scanner intervals in the sparse sampling protocol. The auditory stimuli were 245 ms in length and were repeated at a 4-Hz repetition rate. Every volume acquisition consisted of 7.5 seconds of sound stimulation with one frequency, followed by 2 seconds of scanning. In addition to the sound stimuli, there was a silence condition. Stimulus conditions were presented binaurally in a quasi-random order via an MR Confon Sound System (Baumgart et al., 1998). Sound levels in the MRI were calibrated with a B&K 4134 microphone, inserted in the ear of a KEMAR dummy.

To control for effects of attention, participants were instructed to perform a visual valence task similar to the task used by Langers and van Dijk (2012). Participants were instructed that the sound stimuli were irrelevant and asked to concentrate on the visual task.

STATISTICAL ANALYSIS

Data Preprocessing

The fMRI data analysis was performed in Matlab (version 2018a), and with the aid of SPM12 (Statistical Parametric Mapping). Functional images were pre-processed, realigned, and co-registered to the anatomical image, then normalized to fit a standard brain (MNI), and resliced to a voxel-width of 2 mm. With the use of a Gaussian filter, the images were smoothed with a Gaussian kernel with full width-half maximum of 5mm. During preprocessing, a logarithmic transformation was applied to the fMRI volumes, to convert output to units of percentage signal change (Langers and van Dijk, 2012).

A second level analysis was performed to assess the response to sound, voxel-by-voxel, on group level, by means of an F-test on the 6 coefficients of the sound-frequency related regressors. A minimum cluster size of k > 1000 was used to exclude smaller activation clusters of no interest to tonotopic mapping. The remaining activation clusters were used to construct a Region-of-Interest (ROI) for further analyses (n = 5141 voxels).

Group comparisons

Group differences in median activation levels and corresponding Bayes Factors were calculated for each frequency. Differences in activation patterns between the groups were obtained by calculating the Euclidean distance per frequency, based on the mean signal change in all voxels:

$$d_{ab} = \sqrt{(\sum_i^n (x_{ai} - x_{bi})^2)},$$

where $a$ and $b$ refer to the two groups being compared, and the sum is taken over all n=5141 voxels in the cortical regions of interest. This distance was computed for each stimulus frequency. It is a measure of the difference in activation patterns between the

groups *a* and *b*. The voxels were assigned to the different frequencies according to their peak activation responsiveness. Permutation testing was performed to assess statistical significance of the group differences.

Principal Component Analysis

In order to obtain a robust measure for tonotopic map changes, a principal component analysis was performed by means of singular value decomposition, without centering (similar to Langers et al. (2012a)). The participant matrices (5141 × 6) were concatenated to form an aggregate matrix A of 462690 × 6 (90 participants × 5141 voxels × 6 frequencies). The principal components ($X_i$) were extracted from this matrix A. Frequency-wise analyses were performed on the aggregate matrix A, expressing percentage signal change instead of principal component loadings. The advantage of performing PCA on one concatenated matrix containing data of all participants is that all PCA derived component maps are based on the same principal components and can therefore be compared across participants (Langers et al., 2014).

Assessment of the statistical significance of these principal component scores was done by calculating, for each pair-wise group comparison, the Mahalanobis distance to quantify the magnitude of separation between the principal component clusters of the different groups. The method described here was coined by Goodpaster and Kennedy (Goodpaster and Kennedy, 2011), The Mahalanobis distance definition used was: $D_M(PC1, PC2) = \sqrt{d'\, C_W^{-1}\, d}$, based on the median voxel response per participant. With d expressed as the difference vector between the centroids of two groups according to $d = [C_{PC12} - C_{PC11}, C_{PC22} - C_{PC21}]$ , and $C_W^{-1}$as the pooled variance covariance matrix between two groups. To test if the cluster separation was significant between groups, a Hotelling's $T^2$

statistic was calculated, according to the following equation: $T^2 = \frac{n1n2}{n1+n2}\, d'\, C_W^{-1}\, d$. The n values indicate the sample sizes of the two groups. A larger $T_2$ statistic indicates a larger distance between the PCA score centroids of the two groups. Next, an F-test was performed and the F-value, the ratio of between group versus within group variance, computed according to: $F(p, n1 + n2 - p - 1) = \frac{n1+n2-p-1}{p(n1+n2-2)} T^2$ , with p being the discriminator variables (the two PC's). The critical F-value was determined in a look-up table, based on the numerator and denominator degrees of freedom at $\alpha$ = 0.05. This critical F value determines if the variance between the centroids of two groups is significant. Finally, a p-value was calculated for each group comparison to determine the probability of this finding is small enough to reject the null-hypothesis, i.e. there are no differences in PC scores between the groups.

## Results

To assess differences in cortical responsiveness to sounds, sparse-sampled sound-evoked cortical activation was obtained for 38 control participants, 17 participants with hearing loss but without tinnitus, and 35 participants with hearing loss and tinnitus (Table 1). The participant groups with hearing loss were well matched on hearing loss (Fig 1A). There are no significant differences between the hearing loss groups at the included octave frequencies, except at 500 Hz (Mann-Whitney test, p = 0.05). The control group differs significantly from both hearing loss groups on all frequencies ($p < 0.05$). Accordingly, the mean equal loudness contours of the stimuli indicate that both hearing loss groups needed higher sound intensities to perceive equal loudness at 4 and 8 kHz compared to the control group (Fig 1B).

The groups differ significantly in terms of sex distribution ($p = 0.014$), with a significantly larger proportion of men in the tinnitus group. A significant difference in age ($F_{14,72}$, $p < 0.001$) exists between the groups, which is due to the difference between the tinnitus and control group ($p < 0.001$) and the hearing loss and control group ($p < 0.001$). There is no significant difference in age ($p = 0.529$) between the groups with hearing loss, with or without tinnitus. HADS subscales did not show significant group differences. HQ score distributions differed significantly between the groups ($p = 0.001$). Post-hoc testing showed that the hearing loss and control groups did not differ significantly ($p = 0.133$), in contrast to the tinnitus and hearing loss ($p < 0.001$) and the tinnitus and control comparisons ($p = 0.007$). In the hearing loss group with tinnitus, 5 participants had HQ scores that could indicate a reduced tolerance to sound, the exclusion of these participants did not alter any of the measures displayed and hence they were included in the analyses.

Sound-evoked activation

To determine the sound-evoked cortical activation, regions of interest (ROIs) were constructed based on the overall significantly activated voxels in response to sound, across all 90 participants (FWE < 0.05, cluster size $k > 1000$; Fig 2A). This was done by weighing all 6 sound-stimulus regressors equally in an omnibus F-test. All subsequent second-level analyses were performed on these 5141 voxels corresponding roughly to the bilateral auditory cortices. For each stimulus frequency, the average signal change was computed across all voxels in the ROI. The cortical response to 8 kHz is significantly larger in the tinnitus (Mann-Whitney test, $p = 0.025$, $Z = 2.25$, $BF_{10} = 1.82$) and the hearing loss ($p = 0.003$, $Z = 2.94$, $BF_{10} = 5.24$) groups compared to the control group, and this response is large in comparison to voxels with different preferred frequencies (Fig 2B). Nevertheless, the Bayes Factors ($BF_{10}$) indicate that this effect is more robust for the

hearing loss group without tinnitus. A one-way ANOVA indicated that the differences in percentage signal change between participants was not explained by age ($F(2,41) = 1.167$, $p = 0.341$), or sex differences ($F(2,1) = 0.287$, $p = 0.599$), but confirmed the significant differences for group ($F(2,2) = 4.17$, $p = 0.026$).

Similarity in cortical activation patterns was investigated by means of a Euclidean distance measure, calculated for all three group comparisons. A small Euclidean distance between two groups implies that their cortical activation patterns are similar. The cortical activations patterns of the group with tinnitus and the control group are most similar to each other, except at 8 kHz (Fig 2C). At 8 kHz, the activation pattern of the hearing loss group without tinnitus diverged strongly, and significantly ($p < 0.0028$), from the control group. In the group with tinnitus a similar but non-significant shift was observed.

Additional analyses were performed to investigate if the highest responsiveness levels at 8 kHz could be explained by the highest levels of stimulation. Due to the presence of high-frequency hearing loss, both hearing loss groups with and without tinnitus were stimulated at higher intensities in the high frequencies than the control group. For each participant, the percentage signal change in response to 8 kHz stimulation was plotted against the intensity of stimulation (Fig 2D). The highest stimulation levels occurred in the tinnitus group, whereas the highest percentage signal change occurred in the hearing loss group. The over-representation of high frequencies persists when only moderate hearing losses (≤60 dB HL at 8 kHz) or mild stimuli levels (< +1SD control mean) are considered. This suggests that the higher levels of activation are not the direct result from higher levels of stimulation.

**Principal component analysis**

To obtain robust tonotopic response maps principal component analysis was used (PCA). The first and second principal component's response profiles, over all voxels, were obtained by an analysis that included all three participant groups (Fig 3A, B). We included the first two principal components, with the first principal component explaining 73% of the variance in the signal and the second component an additional 11%. The first principal component reflects overall responsiveness to sound stimulation (Fig 3A), as a direct comparison to the overall activation confirmed.

The tonotopic maps could be inferred from the cascaded response profile of the second principal component, which shows a stage wise increase from negative loadings on low frequencies to positive loadings on high frequencies (Fig 3B). The aggregate responses were portioned into individual spatial response maps to compute the average group maps (Fig 3C). This showed that the high frequencies are more dominant in the spatial frequency group maps of both hearing loss groups, compared to the controls. This high frequency dominance is strongest for the hearing loss group without tinnitus (Fig 3C).

Assessment of the differences in principle component scores of the first and the second principle component was done by calculating the Mahalanobis distance, Hotelling's $T_2$, F-statistics and p-values, see Table 2. These analyses showed that the principle component scores, both for the first and the second principle components, of the hearing loss group without tinnitus were significantly different from those of the control group, as indicated by the critical F value and p value ($p = 0.012$) at a level of p for multiple comparisons ($p=0.0167$). The difference between the principle component scores of the hearing loss group with tinnitus and the control group nearly reached significance ($p=0.0175$),

whereas the hearing loss groups, with and without tinnitus, were not significantly different from one another (p=0.5864).

## Discussion

Our findings show that functional reorganization of the auditory cortex is less pronounced in hearing loss with tinnitus than in hearing loss without tinnitus. Both the response amplitudes and the tonotopic map characteristics in participants with tinnitus were intermediate to those of normal hearing control participants and hearing loss participants without tinnitus. Thus, the reorganization is a consequence of hearing loss and is more conservative in hearing loss with tinnitus. In other words, the presence of tinnitus in hearing loss appears not to relate to excessive cortical plasticity but rather to more diminished adaptation than in hearing loss alone.

The increased response amplitudes in both hearing loss groups were present only at 8 kHz. At this frequency the hearing loss was largest, of the frequencies tested, for the majority of our hearing loss participants (75%). This is typical for (age-related) high-frequency sensorineural hearing loss (Gates and Mills, 2005). It is worth noting that the stimuli in our experiments were loudness matched across frequency for each participant individually. This loudness matching ensured that all stimuli were audible and perceived as equally loud, regardless of raised hearing thresholds. Consequently, the stimulus intensity levels at higher sound frequencies were increased in the hearing loss groups, with and without tinnitus, compared to the normal hearing participants (Fig 1). In the tinnitus group, this effect was not related to the tinnitus frequency. Even though most tinnitus participants had high frequency tinnitus (see Table 1), the tinnitus pitch was not significantly correlated with the frequency eliciting the highest percentage signal change

(R = -.217, p = 0.276). The lack of significant correlation suggests that the increased responsiveness at 8 kHz is not related to the tinnitus itself but rather to the accompanying hearing loss. This is in line with the finding that this increase in responsiveness is present in both the hearing loss group with and without tinnitus.

Generally, the stimulus levels were similar in the two hearing loss groups, although in some instances the intensities were larger in the hearing loss group with tinnitus (Fig 2C; data points at 80-110 dB SPL). Hence, it is quite remarkable that the cortical responses were largest in the hearing loss group without tinnitus, despite that the stimulus intensities did not surpass those of the hearing loss group with tinnitus. Similarly, the largest differences in the tonotopic map were found when contrasting the hearing loss group without tinnitus to the normal hearing participants. Conversely, the tonotopic map of the hearing loss participants with tinnitus was more similar to those of normal hearing participants (Fig 2 and 3). Since these differences cannot simply be accounted for by the differences in stimulus intensities, it may reflect different degrees of (re)organization of the auditory system for participants with hearing loss and tinnitus compared to those without tinnitus.

The majority of tinnitus related fMRI studies included participants with normal hearing thresholds or mild hearing losses. The results across these studies are variable. Gu et al. reported elevated auditory cortex activation in tinnitus participants with normal hearing (Gu et al., 2010). Unfortunately, their hyperacusis controlled design resulted in rather small participant groups (n = 7 with tinnitus, n = 5 without tinnitus). In a similar fMRI study by Langers et al., cortical response amplitudes were similar between normal hearing participants with and without tinnitus, expect for a small region in the lateral

portion of left Heschl's gyrus (Langers et al., 2012). Similarly, Lanting et al. reported no differences in cortical response amplitudes in relation to unilateral tinnitus and mild to moderate hearing loss (Lanting et al., 2008). In contrast, Hofmeier et al. showed a pronounced reduction of the cortical responses in tinnitus participants with mild hearing loss in a study that excluded hyperacusis (Hofmeier et al., 2018).

The present study included participants with moderate to profound high-frequency hearing loss. In both hearing loss groups, with and without tinnitus, an increased responsiveness to 8-kHz stimulation was observed in comparison to the normal hearing control group. These findings are in line with Ghazaleh et al., whom reported no tinnitus-related differences in tonotopic map characteristics in participants with unilateral hearing loss and tinnitus (Ghazaleh et al., 2017). Boyen et al. also found no differences in cortical responses between hearing loss with and without tinnitus (Boyen et al., 2014). Even though the hearing loss in the Hofmeier study was very mild, up to 40 dB per frequency, the results are very similar to that of the current study. There is no obvious explanation for the variability across these studies, however, the studies with larger participant groups (Lanting et al., 2008; Langers et al., 2012; Hofmeier et al., 2018) suggest that response amplitudes are either similar of reduced in tinnitus.

The reduced sound-evoked cortical amplitudes in hearing loss with tinnitus (Fig 2 B; (Hofmeier et al., 2018)), in comparison to hearing loss without tinnitus, have been interpreted as a failure to increase response gain (Knipper et al., 2013; Hofmeier et al., 2018). This failure to increase response gain in the presence of heightened spontaneous activity presumably results in tinnitus. The cortical inability in tinnitus to adapt sufficiently to hearing loss finds a rational in reduced levels of Arc, a cytoskeletal protein

involved in long-term synaptic plasticity (Nikolaienko et al., 2018), as reported in the auditory cortex of tinnitus animals (Tan et al., 2007; Rüttiger et al., 2013). Whereas, generally, Arc is mobilized after inducing hearing loss (Kapolowicz and Thompson, 2016), the expression of Arc is significantly reduced in animals that develop tinnitus (Rüttiger et al., 2013). These findings support the notion that at a cortical level tinnitus, in the presence of hearing loss, is associated with insufficient adaptation to hearing loss.

The enhanced representation of high frequencies in hearing loss appears to contrast with some animal models of tonotopic reorganization. Several animal studies reported the absence of high frequency responsiveness in the auditory cortex, and over-representation of low-frequencies in animals with induced high frequency hearing loss (Rajan and Irvine, 1998; Irvine et al., 2000; Norena and Eggermont, 2005). The differences between these animal studies and our human data presumably relate to differences in techniques used to assess cortical neural activity. The animal models were based on best- or characteristic frequencies of cortical neurons, which are measured with near -threshold stimuli. This method is especially informative of the spatial localization and extent of the cortical area that preferentially responds to a certain frequency. In our study we measured BOLD-responses at supra-threshold levels, the BOLD response is informative of the cortical area that responds to sound stimulation as well as the intensity or amplitude of this response. Therefore, these findings may not contrast each other but instead investigate a different aspect of the cortical responses to sound.

Finally, although our results show group differences in the auditory cortex, it is not clear whether these differences arise due to changes in the function of the cochlea or the brain. Naturally, sensorineural hearing loss involves cochlear pathology. However, the

differences observed between the hearing-impaired participants with tinnitus and those without tinnitus may be due to both cochlear and central differences. Recent evidence suggests that tinnitus is associated with both reduced ribbon synapse density in the cochlea (Rüttiger et al., 2013; Zhang et al., 2014), and reduced ARC expression in the cortex (Rüttiger et al., 2013; Singer et al., 2013). With the measures of the present study, i.e. pure tone audiometry and MRI, it is not possible to identify differences in cochlear pathology between the hearing loss groups.

Limitations

In earlier studies by Profant et al. the authors described that with increasing age, stronger sound evoked responses where observed in the auditory cortex (Profant et al., 2015; Profant et al., 2014). To investigate if the observed group differences in the present study were not caused by age differences, we plotted per group the age of participants against their high frequency evoked cortical activation to observe any correlation. This demonstrated that none of the groups showed any significant or near significant correlation between age and high-frequency evoked cortical activation levels (THL $R = -.105$, $p = 0.547$; HL $R = .119$, $p = 0.650$; CO $R = 0.246$, $p = 0.137$). However, it must be noted that our hearing loss group without tinnitus has fewer younger people compared to the hearing loss group with tinnitus.

In conclusion, hearing loss was associated with higher levels of sound-evoked cortical responsiveness and this increase was most pronounced in the group with hearing loss but without tinnitus. Both in terms of response amplitudes and tonotopic map characteristics, the participants with hearing loss and tinnitus appear intermediate to the controls and the hearing loss participants without tinnitus. This suggests that tinnitus is related to an

476 incomplete form of central compensation to hearing loss, rather than excessive
477 adaptation. As a consequence, treatments for tinnitus may need to enhance the cortical
478 plasticity, rather than reversing it.
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678 Fig 1. Hearing characteristics of participants. (A) Audiometric thresholds used in the MRI
679 scanning protocol are indicated here, with their corresponding SE. (B) During MRI
680 scanning, stimuli were presented at loudness levels equal to the 40-phon loudness curve.
681 All stimuli were thus matched in loudness to a 1-kHz pure tone at 40 dB SPL. The average
682 levels of the stimuli are depicted per group, for the six frequencies presented along with
683 their corresponding SE.
684
685 Fig 2. Sound-evoked activation levels. (A) Regions-of-interest based on overall activated
686 voxels (n = 5141) in response to sound, across all 90 participants. (B) Group level
687 responsiveness profile, based on percentage signal change in ROI voxels in response to
688 the six presented frequencies. A significant difference, at $p < 0.05$, in the responsiveness
689 levels is observed for both hearing loss groups, with and without tinnitus, compared to
690 the control group, in response to 8 kHz stimulation ($p = 0.02$ and $p = 0.003$). However,
691 significance remains when corrected for multiple comparisons (Bonferroni corrected
692 0.05/6=0.008), only for the hearing loss group without tinnitus. (C) Euclidian distance
693 between response profiles of participant groups, per frequency. The distance was
694 computed using the response amplitudes of all voxels as spatial response profile. A
695 smaller distance indicates more similar voxel responses on that frequency. The statistical
696 significance of the distances was determined by means of permutation testing (n =
697 50000). The distance between hearing loss without tinnitus and controls is significant for
698 8 kHz ($p < 0.0028$, Bonferroni corrected). (D) Mean percentage signal change per group
699 during 8 kHz stimulation. Per participant, the level of stimulation (in dB SPL) at 8 kHz is
700 plotted against the mean percentage signal change over all voxels in the region-of-
701 interest. Even though the absolute and mean highest percentage signal change occurred

in the hearing loss group, the highest levels of stimulation were applied in the tinnitus group.

Fig 3. Characterization of tonotopic organization by principal component analysis (PCA). (A) Frequency dependent response profile of the first and (B) second principal component. (C) Spatial frequency group maps, based on the component strength of the second principal component. Positive component scores indicate high frequency responsiveness (i.e. more responsive to high than to low frequencies), whereas a negative score indicates responsiveness to low frequencies. A Hotelling's $T_2$ statistic was calculated to compare the principal component clusters and indicated a statistically significant difference between the second principle component scores of the hearing loss group without tinnitus compared those of the control group ($p = 0.012$).

Table 1. Demographics and questionnaire scores of the three participants groups in this fMRI study.

Table 2. Summary of pair-wise cluster separation of the first and second component given by Mahalanobis distances, Hoteling's $T_2$ statistic, F0-statistics and p-values.