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Cortical Tonotopic Map Changes in Humans Are Larger in Hearing Loss Than in Additional 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

67   Tinnitus, a common and potentially devastating condition, is the presence of a 'phantom'

68   sound that often accompanies hearing loss. Hearing loss is known to induce plastic

69   changes in cortical and sub-cortical areas. Although plasticity is a valuable trait that

70   allows the human brain to rewire and recover from injury and sensory deprivation, it can

71   lead to tinnitus as an unwanted side effect. In this large fMRI study, we provide evidence

72   that tinnitus is related to a more conservative form of reorganization than in hearing loss

73   without tinnitus. This result contrasts with the previous notion that tinnitus is related to

74   excessive reorganization. As a consequence, treatments for tinnitus may need to enhance

75   the cortical plasticity, rather than reversing it.

76

77

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;

103   Eggermont, 2006), but few have directly investigated this relation. In previous

104   experimental work the observed tonotopic map plasticity was linked to hearing loss but

105   not to tinnitus (Weisz et al., 2005; Wienbruch et al., 2006; McMahon et al., 2016). In

106   humans, tonotopic map reorganization was reported in one MEG study on tinnitus. A

107   positive correlation was reported between the strength of the perceived tinnitus and the

108   extent of cortical reorganization (Muhlnickel et al., 1998). In contrast, other studies

109   reported no tonotopic plasticity related to tinnitus in humans (Langers et al., 2012) or

110   animals (Kotak et al., 2005; Yang et al., 2011). Instead, these animal studies identified

111   enhanced cortical excitation or reduced cortical inhibition in animals with binaural

112   hearing loss and behavioral signs of tinnitus. The release from inhibition in the hearing

113   loss affected area connects the tinnitus pitch with increased neuronal excitability (Yang

114   et al., 2011). In general, it is not well established that tonotopic map plasticity is a cortical

115   characteristic of tinnitus.

116

117   Animal-models of cortical tonotopic reorganization indicate that receptive fields of

118   neurons within the hearing loss affected area shift towards the intact receptors (Rajan

119   and Irvine, 1998; Eggermont and Komiya, 2000; Irvine et al., 2001; Muhlau et al., 2006).

120   This reorganization causes a downwards shift in the characteristic frequency of neurons,

121   in both temporary and lasting hearing loss (Irvine et al., 2000; Norena and Eggermont,

122   2005, 2006), thus altering the tonotopic map. In contrast, not all animal studies on hearing

123   loss found a downwards shift in tonotopic maps, but instead reported increased

124   excitability (Kotak et al., 2005) or decreased inhibition (Rajan, 1998) of the affected

125   frequency regions. In humans, one MEG study reported a shift of the cortical responsive

126   region towards the intact edge-frequency of the audiogram in hearing loss (Dietrich et al.,

127   2001). In summary, different correlates of tonotopic plasticity have been reported in

128   literature on hearing loss and tinnitus, and the translation of animal-models to human

129   imaging is sparse especially in tinnitus.

130

131   This large fMRI study examined the relation between hearing loss, tinnitus, and tonotopic

132   reorganization with loudness-matched sound stimuli in humans. Inclusion of participants

133   with high frequency hearing loss, both with and without tinnitus, allowed us to investigate

134   to what extent reorganization is a consequence of hearing loss, and whether any

135   reorganization is specifically related to tinnitus.

136

137   **Materials and methods**

138   The study was approved, in accordance with the principles of the declaration of Helsinki

139   (2013), by the medical ethical committee of the University Medical Center Groningen, the

140   Netherlands. Written informed consent was obtained and participants received

141   reimbursement for their participation.

142

143   Participants

144   A total of 113 participants, both male and female, were included in a larger MRI study. In

145   90 participants, three complete functional runs were obtained. This resulted in 35

146   participants with hearing loss and tinnitus, 17 participants with hearing loss without

147   tinnitus, and 38 healthy controls without hearing loss or tinnitus (Table 1). None of the

148   participants were using hearing aids to compensate their hearing loss, or ameliorate their

149   tinnitus. Pure tone audiometry was performed in a sound attenuating booth to determine

150   hearing thresholds for all participants at octave frequencies ranging from 0.125 to 8 kHz.

151   Tinnitus pitch and loudness were estimated with a matching procedure. In addition, the

152   participants completed the Tinnitus Handicap Inventory (McCombe et al., 2001), the

153 Tinnitus Reactions Questionnaire (Wilson et al., 1991), the Hyperacusis Questionnaire

154 (Khalfa et al., 2002) and the Hospital Anxiety and Depression Scale (Zigmond and Snaith,

155 1983).

156

157 Group differences were tested with a Chi-square test of independence for the variable sex,

158 and a three-group ANOVA followed-up by independent pairwise t-tests for the variable

159 age. The questionnaire scores were assessed by means of a Kruskal-Wallis test and

160 followed up by a pairwise Mann-Whitney test.

161

162 EXPERIMENTAL DESIGN

163 Data acquisition

164 All MRI data was obtained with a 3.0 T Philips Intera MRI scanner (Best, the Netherlands),

165 at the Neuro Imaging Center Groningen. The scanner was equipped with a SENSE 32-

166 channel head coil. Both structural and functional images were obtained for each

167 participant. The structural image was a whole brain T1 weighted image (voxel size 1mm

168 x 1mm x 1mm). The functional images were acquired in a sparse imaging sequence (Hall

169 et al., 1999), as single shot EPI: 47 slices; no gap; scan matrix 72 x 67; descending slice

170 order; TR of 10 seconds, TE 22 ms, Flip Angle 90°. For each participant a total of three

171 runs, of each 65 EPI volumes, were acquisitioned.

172

173 Sound stimuli

174 During the fMRI experiments, loudness matched auditory stimuli were presented. Prior

175 to the MRI session, participants performed a binaural loudness matching task in which

176 the stimulus tones at 0.25, 0.5, 2, 4, and 8 kHz were all matched in perceived loudness to

177 a 1-kHz tone at 40 dB SPL. This compensates for loudness distortion present in

178 sensorineural hearing loss (Moore and Glasberg, 2004). In addition, studies indicate that

179 sound-evoked cortical activation correlates better with loudness rather than the level of

180 sound stimuli (Hall et al., 2001; Langers et al., 2007). A two alternative-forced-choice, 1-

181 up-1-down loudness matching procedure was used to approximate equal loudness

182 sensation over all frequencies. An interleaved staircase method was applied, with a

183 maximum of 15 trials per frequency, 7 reversals, and a step size of [10,5,5,3,3,1] dB SPL.

184 This method yielded an equal loudness contour for each participant.

185

186 Procedure MRI

187 The individually loudness-matched auditory stimuli were presented during the relatively

188 silent scanner intervals in the sparse sampling protocol. The auditory stimuli were 245

189 ms in length and were repeated at a 4-Hz repetition rate. Every volume acquisition

190 consisted of 7.5 seconds of sound stimulation with one frequency, followed by 2 seconds

191 of scanning. In addition to the sound stimuli, there was a silence condition. Stimulus

192 conditions were presented binaurally in a quasi-random order via an MR Confon Sound

193 System (Baumgart et al., 1998). Sound levels in the MRI were calibrated with a B&K 4134

194 microphone, inserted in the ear of a KEMAR dummy.

195

196 To control for effects of attention, participants were instructed to perform a visual valence

197 task similar to the task used by Langers and van Dijk (2012). Participants were instructed

198 that the sound stimuli were irrelevant and asked to concentrate on the visual task.

199

200 STATISTICAL ANALYSIS

201 Data Preprocessing

202 The fMRI data analysis was performed in Matlab (version 2018a), and with the aid of

203 SPM12 (Statistical Parametric Mapping). Functional images were pre-processed,

204 realigned, and co-registered to the anatomical image, then normalized to fit a standard

205 brain (MNI), and resliced to a voxel-width of 2 mm. With the use of a Gaussian filter, the

206 images were smoothed with a Gaussian kernel with full width-half maximum of 5mm.

207 During preprocessing, a logarithmic transformation was applied to the fMRI volumes, to

208 convert output to units of percentage signal change (Langers and van Dijk, 2012).

209

210 A second level analysis was performed to assess the response to sound, voxel-by-voxel,

211 on group level, by means of an F-test on the 6 coefficients of the sound-frequency related

212 regressors. A minimum cluster size of k > 1000 was used to exclude smaller activation

213 clusters of no interest to tonotopic mapping. The remaining activation clusters were used

214 to construct a Region-of-Interest (ROI) for further analyses (n = 5141 voxels).

215

216 Group comparisons

217 Group differences in median activation levels and corresponding Bayes Factors were

218 calculated for each frequency. Differences in activation patterns between the groups were

219 obtained by calculating the Euclidean distance per frequency, based on the mean signal

220 change in all voxels:

221

222 $d_{ab} = \sqrt{(\sum_{i}^{n}(x_{ai} - x_{bi})^2)}$,

223

224 where a and b refer to the two groups being compared, and the sum is taken over all

225 n=5141 voxels in the cortical regions of interest. This distance was computed for each

226 stimulus frequency. It is a measure of the difference in activation patterns between the

227   groups *a* and *b*. The voxels were assigned to the different frequencies according to their

228   peak activation responsiveness. Permutation testing was performed to assess statistical

229   significance of the group differences.

230

231   Principal Component Analysis

232   In order to obtain a robust measure for tonotopic map changes, a principal component

233   analysis was performed by means of singular value decomposition, without centering

234   (similar to Langers et al. (2012a)). The participant matrices (5141 × 6) were concatenated

235   to form an aggregate matrix A of 462690 × 6 (90 participants × 5141 voxels × 6

236   frequencies). The principal components (X_i) were extracted from this matrix A.

237   Frequency-wise analyses were performed on the aggregate matrix A, expressing

238   percentage signal change instead of principal component loadings. The advantage of

239   performing PCA on one concatenated matrix containing data of all participants is that all

240   PCA derived component maps are based on the same principal components and can

241   therefore be compared across participants (Langers et al., 2014).

242

243   Assessment of the statistical significance of these principal component scores was done

244   by calculating, for each pair-wise group comparison, the Mahalanobis distance to quantify

245   the magnitude of separation between the principal component clusters of the different

246   groups. The method described here was coined by Goodpaster and Kennedy (Goodpaster

247   and Kennedy, 2011), The Mahalanobis distance definition used was: $D_M(PC1, PC2) =$

248    $\sqrt{d'\,C_W^{-1}\,d}$, based on the median voxel response per participant. With d expressed as the

249   difference vector between the centroids of two groups according to $d = [C_{PC12} -$

250    $C_{PC11}, C_{PC22} - C_{PC21}]$, and C_W^{-1} as the pooled variance covariance matrix between two

251   groups. To test if the cluster separation was significant between groups, a Hotelling's T_2

252 statistic was calculated, according to the following equation: $T^2 = \frac{n1n2}{n1+n2} d' C_W^{-1} d$. The n

253 values indicate the sample sizes of the two groups. A larger T_2 statistic indicates a larger

254 distance between the PCA score centroids of the two groups. Next, an F-test was

255 performed and the F-value, the ratio of between group versus within group variance,

256 computed according to: $F(p, n1 + n2 - p - 1) = \frac{n1+n2-p-1}{p(n1+n2-2)} T^2$, with p being the

257 discriminator variables (the two PC's). The critical F-value was determined in a look-up

258 table, based on the numerator and denominator degrees of freedom at α = 0.05. This

259 critical F value determines if the variance between the centroids of two groups is

260 significant. Finally, a p-value was calculated for each group comparison to determine the

261 probability of this finding is small enough to reject the null-hypothesis, i.e. there are no

262 differences in PC scores between the groups.

263

264 **Results**

265 To assess differences in cortical responsiveness to sounds, sparse-sampled sound-evoked

266 cortical activation was obtained for 38 control participants, 17 participants with hearing

267 loss but without tinnitus, and 35 participants with hearing loss and tinnitus (Table 1). The

268 participant groups with hearing loss were well matched on hearing loss (Fig 1A). There

269 are no significant differences between the hearing loss groups at the included octave

270 frequencies, except at 500 Hz (Mann-Whitney test, p = 0.05). The control group differs

271 significantly from both hearing loss groups on all frequencies (p < 0.05). Accordingly, the

272 mean equal loudness contours of the stimuli indicate that both hearing loss groups

273 needed higher sound intensities to perceive equal loudness at 4 and 8 kHz compared to

274 the control group (Fig 1B).

275

276 The groups differ significantly in terms of sex distribution (p = 0.014), with a significantly

277 larger proportion of men in the tinnitus group. A significant difference in age (F 14,72, p

278 < 0.001) exists between the groups, which is due to the difference between the tinnitus

279 and control group (p< 0.001) and the hearing loss and control group (p < 0.001). There is

280 no significant difference in age (p = 0.529) between the groups with hearing loss, with or

281 without tinnitus. HADS subscales did not show significant group differences. HQ score

282 distributions differed significantly between the groups (p = 0.001). Post-hoc testing

283 showed that the hearing loss and control groups did not differ significantly (p = 0.133), in

284 contrast to the tinnitus and hearing loss (p < 0.001) and the tinnitus and control

285 comparisons (p = 0.007). In the hearing loss group with tinnitus, 5 participants had HQ

286 scores that could indicate a reduced tolerance to sound, the exclusion of these participants

287 did not alter any of the measures displayed and hence they were included in the analyses.

288

289 Sound-evoked activation

290 To determine the sound-evoked cortical activation, regions of interest (ROIs) were

291 constructed based on the overall significantly activated voxels in response to sound,

292 across all 90 participants (FWE < 0.05, cluster size k > 1000; Fig 2A). This was done by

293 weighing all 6 sound-stimulus regressors equally in an omnibus F-test. All subsequent

294 second-level analyses were performed on these 5141 voxels corresponding roughly to the

295 bilateral auditory cortices. For each stimulus frequency, the average signal change was

296 computed across all voxels in the ROI. The cortical response to 8 kHz is significantly larger

297 in the tinnitus (Mann-Whitney test, p = 0.025, Z = 2.25, BF_{10} = 1.82) and the hearing loss

298 (p = 0.003, Z =2.94, BF_{10} = 5.24) groups compared to the control group, and this response

299 is large in comparison to voxels with different preferred frequencies (Fig 2B).

300 Nevertheless, the Bayes Factors (BF_{10}) indicate that this effect is more robust for the

301   hearing loss group without tinnitus. A one-way ANOVA indicated that the differences in

302   percentage signal change between participants was not explained by age ($F_{(2,41)} = 1.167$,

303      $p = 0.341$), or sex differences ($F_{(2,1)} = 0.287$, $p = 0.599$), but confirmed the significant

304   differences for group ($F_{(2,2)} = 4.17$, $p = 0.026$).

305

306   Similarity in cortical activation patterns was investigated by means of a Euclidean

307   distance measure, calculated for all three group comparisons. A small Euclidean distance

308   between two groups implies that their cortical activation patterns are similar. The cortical

309   activations patterns of the group with tinnitus and the control group are most similar to

310   each other, except at 8 kHz (Fig 2C). At 8 kHz, the activation pattern of the hearing loss

311   group without tinnitus diverged strongly, and significantly ($p < 0.0028$), from the control

312   group. In the group with tinnitus a similar but non-significant shift was observed.

313

314   Additional analyses were performed to investigate if the highest responsiveness levels at

315   8 kHz could be explained by the highest levels of stimulation. Due to the presence of high-

316   frequency hearing loss, both hearing loss groups with and without tinnitus were

317   stimulated at higher intensities in the high frequencies than the control group. For each

318   participant, the percentage signal change in response to 8 kHz stimulation was plotted

319   against the intensity of stimulation (Fig 2D). The highest stimulation levels occurred in

320   the tinnitus group, whereas the highest percentage signal change occurred in the hearing

321   loss group. The over-representation of high frequencies persists when only moderate

322   hearing losses (≤60 dB HL at 8 kHz) or mild stimuli levels (< +1SD control mean) are

323   considered. This suggests that the higher levels of activation are not the direct result from

324   higher levels of stimulation.

325

Principal component analysis

326

327 To obtain robust tonotopic response maps principal component analysis was used (PCA).

328 The first and second principal component's response profiles, over all voxels, were

329 obtained by an analysis that included all three participant groups (Fig 3A, B). We included

330 the first two principal components, with the first principal component explaining 73% of

331 the variance in the signal and the second component an additional 11%. The first principal

332 component reflects overall responsiveness to sound stimulation (Fig 3A), as a direct

333 comparison to the overall activation confirmed.

334

335 The tonotopic maps could be inferred from the cascaded response profile of the second

336 principal component, which shows a stage wise increase from negative loadings on low

337 frequencies to positive loadings on high frequencies (Fig 3B). The aggregate responses

338 were portioned into individual spatial response maps to compute the average group maps

339 (Fig 3C). This showed that the high frequencies are more dominant in the spatial

340 frequency group maps of both hearing loss groups, compared to the controls. This high

341 frequency dominance is strongest for the hearing loss group without tinnitus (Fig 3C).

342

343 Assessment of the differences in principle component scores of the first and the second

344 principle component was done by calculating the Mahalanobis distance, Hotelling's T_2, F-

345 statistics and p-values, see Table 2. These analyses showed that the principle component

346 scores, both for the first and the second principle components, of the hearing loss group

347 without tinnitus were significantly different from those of the control group, as indicated

348 by the critical F value and p value ($p = 0.012$) at a level of p for multiple comparisons

349 ($p=0.0167$). The difference between the principle component scores of the hearing loss

350 group with tinnitus and the control group nearly reached significance ($p=0.0175$),

351 whereas the hearing loss groups, with and without tinnitus, were not significantly

352 different from one another (p=0.5864).

353

354 **Discussion**

355 Our findings show that functional reorganization of the auditory cortex is less pronounced

356 in hearing loss with tinnitus than in hearing loss without tinnitus. Both the response

357 amplitudes and the tonotopic map characteristics in participants with tinnitus were

358 intermediate to those of normal hearing control participants and hearing loss participants

359 without tinnitus. Thus, the reorganization is a consequence of hearing loss and is more

360 conservative in hearing loss with tinnitus. In other words, the presence of tinnitus in

361 hearing loss appears not to relate to excessive cortical plasticity but rather to more

362 diminished adaptation than in hearing loss alone.

363

364 The increased response amplitudes in both hearing loss groups were present only at 8

365 kHz. At this frequency the hearing loss was largest, of the frequencies tested, for the

366 majority of our hearing loss participants (75%). This is typical for (age-related) high-

367 frequency sensorineural hearing loss (Gates and Mills, 2005). It is worth noting that the

368 stimuli in our experiments were loudness matched across frequency for each participant

369 individually. This loudness matching ensured that all stimuli were audible and perceived

370 as equally loud, regardless of raised hearing thresholds. Consequently, the stimulus

371 intensity levels at higher sound frequencies were increased in the hearing loss groups,

372 with and without tinnitus, compared to the normal hearing participants (Fig 1). In the

373 tinnitus group, this effect was not related to the tinnitus frequency. Even though most

374 tinnitus participants had high frequency tinnitus (see Table 1), the tinnitus pitch was not

375 significantly correlated with the frequency eliciting the highest percentage signal change

376   (R = -.217, p = 0.276). The lack of significant correlation suggests that the increased

377   responsiveness at 8 kHz is not related to the tinnitus itself but rather to the accompanying

378   hearing loss. This is in line with the finding that this increase in responsiveness is present

379   in both the hearing loss group with and without tinnitus.

380

381   Generally, the stimulus levels were similar in the two hearing loss groups, although in

382   some instances the intensities were larger in the hearing loss group with tinnitus (Fig 2C;

383   data points at 80-110 dB SPL). Hence, it is quite remarkable that the cortical responses

384   were largest in the hearing loss group without tinnitus, despite that the stimulus

385   intensities did not surpass those of the hearing loss group with tinnitus. Similarly, the

386   largest differences in the tonotopic map were found when contrasting the hearing loss

387   group without tinnitus to the normal hearing participants. Conversely, the tonotopic map

388   of the hearing loss participants with tinnitus was more similar to those of normal hearing

389   participants (Fig 2 and 3). Since these differences cannot simply be accounted for by the

390   differences in stimulus intensities, it may reflect different degrees of (re)organization of

391   the auditory system for participants with hearing loss and tinnitus compared to those

392   without tinnitus.

393

394   The majority of tinnitus related fMRI studies included participants with normal hearing

395   thresholds or mild hearing losses. The results across these studies are variable. Gu et al.

396   reported elevated auditory cortex activation in tinnitus participants with normal hearing

397   (Gu et al., 2010). Unfortunately, their hyperacusis controlled design resulted in rather

398   small participant groups (n = 7 with tinnitus, n = 5 without tinnitus). In a similar fMRI

399   study by Langers et al., cortical response amplitudes were similar between normal

400   hearing participants with and without tinnitus, expect for a small region in the lateral

16

401 portion of left Heschl's gyrus (Langers et al., 2012). Similarly, Lanting et al. reported no

402 differences in cortical response amplitudes in relation to unilateral tinnitus and mild to

403 moderate hearing loss (Lanting et al., 2008). In contrast, Hofmeier et al. showed a

404 pronounced reduction of the cortical responses in tinnitus participants with mild hearing

405 loss in a study that excluded hyperacusis (Hofmeier et al., 2018).

406

407 The present study included participants with moderate to profound high-frequency

408 hearing loss. In both hearing loss groups, with and without tinnitus, an increased

409 responsiveness to 8-kHz stimulation was observed in comparison to the normal hearing

410 control group. These findings are in line with Ghazaleh et al., whom reported no tinnitus-

411 related differences in tonotopic map characteristics in participants with unilateral

412 hearing loss and tinnitus (Ghazaleh et al., 2017). Boyen et al. also found no differences in

413 cortical responses between hearing loss with and without tinnitus (Boyen et al., 2014).

414 Even though the hearing loss in the Hofmeier study was very mild, up to 40 dB per

415 frequency, the results are very similar to that of the current study. There is no obvious

416 explanation for the variability across these studies, however, the studies with larger

417 participant groups (Lanting et al., 2008; Langers et al., 2012; Hofmeier et al., 2018)

418 suggest that response amplitudes are either similar of reduced in tinnitus.

419

420 The reduced sound-evoked cortical amplitudes in hearing loss with tinnitus (Fig 2 B;

421 (Hofmeier et al., 2018)), in comparison to hearing loss without tinnitus, have been

422 interpreted as a failure to increase response gain (Knipper et al., 2013; Hofmeier et al.,

423 2018). This failure to increase response gain in the presence of heightened spontaneous

424 activity presumably results in tinnitus. The cortical inability in tinnitus to adapt

425 sufficiently to hearing loss finds a rational in reduced levels of Arc, a cytoskeletal protein

426   involved in long-term synaptic plasticity (Nikolaienko et al., 2018), as reported in the

427   auditory cortex of tinnitus animals (Tan et al., 2007; Rüttiger et al., 2013). Whereas,

428   generally, Arc is mobilized after inducing hearing loss (Kapolowicz and Thompson, 2016),

429   the expression of Arc is significantly reduced in animals that develop tinnitus (Rüttiger et

430   al., 2013). These findings support the notion that at a cortical level tinnitus, in the

431   presence of hearing loss, is associated with insufficient adaptation to hearing loss.

432

433   The enhanced representation of high frequencies in hearing loss appears to contrast with

434   some animal models of tonotopic reorganization. Several animal studies reported the

435   absence of high frequency responsiveness in the auditory cortex, and over-representation

436   of low-frequencies in animals with induced high frequency hearing loss (Rajan and Irvine,

437   1998; Irvine et al., 2000; Norena and Eggermont, 2005). The differences between these

438   animal studies and our human data presumably relate to differences in techniques used

439   to assess cortical neural activity. The animal models were based on best- or characteristic

440   frequencies of cortical neurons, which are measured with near -threshold stimuli. This

441   method is especially informative of the spatial localization and extent of the cortical area

442   that preferentially responds to a certain frequency. In our study we measured BOLD-

443   responses at supra-threshold levels, the BOLD response is informative of the cortical area

444   that responds to sound stimulation as well as the intensity or amplitude of this response.

445   Therefore, these findings may not contrast each other but instead investigate a different

446   aspect of the cortical responses to sound.

447

448   Finally, although our results show group differences in the auditory cortex, it is not clear

449   whether these differences arise due to changes in the function of the cochlea or the brain.

450   Naturally, sensorineural hearing loss involves cochlear pathology. However, the

451 differences observed between the hearing-impaired participants with tinnitus and those

452 without tinnitus may be due to both cochlear and central differences. Recent evidence

453 suggests that tinnitus is associated with both reduced ribbon synapse density in the

454 cochlea (Rüttiger et al., 2013; Zhang et al., 2014), and reduced ARC expression in the

455 cortex (Rüttiger et al., 2013; Singer et al., 2013). With the measures of the present study,

456 i.e. pure tone audiometry and MRI, it is not possible to identify differences in cochlear

457 pathology between the hearing loss groups.

458

459 Limitations

460 In earlier studies by Profant et al. the authors described that with increasing age, stronger

461 sound evoked responses where observed in the auditory cortex (Profant et al., 2015;

462 Profant et al., 2014). To investigate if the observed group differences in the present study

463 were not caused by age differences, we plotted per group the age of participants against

464 their high frequency evoked cortical activation to observe any correlation. This

465 demonstrated that none of the groups showed any significant or near significant

466 correlation between age and high-frequency evoked cortical activation levels (THL R = -

467 .105, p = 0.547; HL R = .119, p = 0.650; CO R =0.246, p = 0.137). However, it must be noted

468 that our hearing loss group without tinnitus has fewer younger people compared to the

469 hearing loss group with tinnitus.

470

471 In conclusion, hearing loss was associated with higher levels of sound-evoked cortical

472 responsiveness and this increase was most pronounced in the group with hearing loss but

473 without tinnitus. Both in terms of response amplitudes and tonotopic map characteristics,

474 the participants with hearing loss and tinnitus appear intermediate to the controls and

475 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.

479

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677

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

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

703 group.

704

705 Fig 3. Characterization of tonotopic organization by principal component analysis (PCA).

706 (A) Frequency dependent response profile of the first and (B) second principal

707 component. (C) Spatial frequency group maps, based on the component strength of the

708 second principal component. Positive component scores indicate high frequency

709 responsiveness (i.e. more responsive to high than to low frequencies), whereas a negative

710 score indicates responsiveness to low frequencies. A Hotelling's T_2 statistic was

711 calculated to compare the principal component clusters and indicated a statistically

712 significant difference between the second principle component scores of the hearing loss

713 group without tinnitus compared those of the control group (p = 0.012).

714

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

716 fMRI study.

717

718 Table 2. Summary of pair-wise cluster separation of the first and second component given

719 by Mahalanobis distances, Hoteling's T_2 statistic, F0-statistics and p-values.