Structural Changes in Tinnitus Patients and Their Relationship to Hearing-Loss

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Abstract

Subjective tinnitus is the perception of sound without an external source. Tinnitus is highly associated with hearing-loss, but theories that attempt to explain tinnitus rarely consider hearing-loss as a cofounding variable. Leaver et al. (2011) propose that a gating mechanism is unable to properly inhibit the unwanted tinnitus signal. The key areas of this theory, amygdala and ventromedial prefrontal cortex, usually show less grey matter (GM) than controls. Perhaps a lack of GM prevents the gating system to correctly inhibit the tinnitus signal. Theories on tinnitus involve a mismatch in the functional reorganisation in several brain areas, especially the auditory cortex (Jastreboff, 1990). Structural studies often assume that the functional reorganisation is accompanied by structural reorganisation. Volumetric studies on tinnitus often find differences in the auditory, limbic, and frontal areas between tinnitus patients and controls.

This study compared anatomical MRI-scans of 23 tinnitus patients with 4 individuals with hearing-loss and 6 healthy controls. Tinnitus and control subjects have on average more grey matter in the left superior temporal cortex than hearing-impaired individuals. Region-of-Interest analysis showed that controls had more grey matter in the left parahippocampal cortex compared to tinnitus patients. Tinnitus patients had more grey matter than the hearing-loss group in auditory, limbic, and frontal areas. The findings suggest that hearing-loss group had the strongest decrease in GM volume in the limbic system. The tinnitus group is somewhere between the hearing-loss group and the controls.

The findings suggest that the limbic system is involved in the tinnitus percept, but it seems that the gating mechanism is not situated here. It might be that this gate is situated in the frontal cortex, but the frontal findings seem to be related to tinnitus distress. Structural reorganisation is possibly the strongest in hearing-impaired individuals, and only partially happening in tinnitus patients, suggesting that reorganisation may not have happened properly. It is emphasised that the sample group size and hearing-levels are not ideal, and tinnitus distress should be taken into account in further studies.

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Introduction

Tinnitus is the sensation of sound without an external source. When speaking about tinnitus, we typically mean subjective tinnitus, which can only be perceived by the sufferer. Usually, subjective tinnitus is accompanied by peripheral hearing loss (Eggermont & Roberts, 2004; Hoffman & Reed, 2004; Nicolas-Puel et al., 2006), although tinnitus with mild or even no hearing loss has been reported as well (Levine, 1999; Stouffer & Tyler, 1990). As the chance to acquire hearing loss increases with age, it may not come as a surprise that the prevalence of tinnitus also increases with age (Eggermont, 2012; Lockwood, Salvi, & Burkard, 2002). It remains unclear, however, whether tinnitus is a stand-alone pathology as a result of the hearing-loss or has other causes. One of reasons that makes finding the aetiology of tinnitus difficult is this connection between hearing-loss and tinnitus. As the likelihood to develop tinnitus, deafness, or both increases with age, it is difficult to dismantle their relationship.

Connection Hearing Loss and Tinnitus: Tonotopy and the Reorganisation Hypothesis

Early ideas on the cause of tinnitus involve exclusively the inner ear: the damaged nerve endings of the inner hair cells would deploy continuous stimulation in absence of a stimulus. This model is somewhat outdated (Mühlau et al., 2006). For example, nerve damage is usually associated with numbness. Thus damage to the hair cells would result in deafness and hearing-loss instead of tinnitus. Also, tinnitus remains present when the cochlea is destroyed or removed (Zacharek, Kaltenbach, Mathog, & Zhang, 2002). More recent models involve both the peripheral and central nervous system. Theories usually involve peripheral damage to the cilia, reorganisation of central auditory pathways, and changes in the parts of the limbic system that perform evaluation of the emotional content of sensory experiences (Eggermont, 2012; Jastreboff, 1990; Langers & de Kleine, 2016; Leaver et al., 2011; Mühlau et al., 2006). The idea of damage in the peripheral nervous system that triggers a mechanism in the brain is also seen in literature on phantom pain; tinnitus is sometimes compared to that (Flor, Birbaumer, & Turk, 1990; Muhlnickel, Elbert, Taub, & Flor, 1998; Rauschecker, 1999).

In the inner ear, the cochlea follows a tonotopic organisation. This means that the hair cells at the base of the cochlea are tuned to high frequency sounds, and hair cells that are situated more towards the apex are sensitive to lower frequency sounds. This tonotopic organisation is preserved in the auditory cortex (Brodmann area 41/42) (Pantev et al., 1995; Romani, Williamson, & Kaufman, 1975). Neurons at one end of the auditory cortex respond best to low frequencies; neurons at the other end respond best to high frequencies. As explained earlier, most theories on tinnitus involve a form of reorganisation of the auditory pathway and tonotopic

organisation. Several functional studies found changes related to tinnitus at the level of the auditory cortex (Arnold, Bartenstein, Oestreicher, Römer, & Schwaiger, 1996; Giraud et al., 1999; Lockwood et al., 1998; Mirz et al., 1999; Muhlnickel et al., 1998; Rauschecker, 1999), the thalamus (Lanting, De Kleine, & Van Dijk, 2009), and the inferior colliculus (Melcher, Sigalovsky, Guinan, & Levine, 2000).

A popular idea is that tinnitus is the result of 'neuroplasticity gone wrong' (Eggermont, 2012). In hearing-impaired individuals, the auditory input is diminished. This leads to less strengthening of connections between neurons in the auditory pathway. In turn, this could lead to changes in the tonotopic map. According to Hebbian theory (Hebb, 2005), synaptic connections are built based on the frequency of interactions between neurons. In tinnitus patients, the mechanism that strengthens or weakens connections is not working properly, due to a lack of inhibition of irrelevant activity. This irrelevant activity is processed and perceived as the tinnitus signal. This would mean that neurons in de auditory cortex that were initially sensitive to, for example, high frequency tones, also start to react to a lower frequency (Eggermont, 2007). Or these neurons react to random spontaneous activity (Schaette & Kempter, 2006), which also leads to the perception of tinnitus (Chrostowski, Yang, Wilson, Bruce, & Becker, 2011; Dominguez, Becker, Bruce, & Read, 2006). This mechanism is not limited to the auditory cortex, but various areas in the auditory system. Moreover, one study found that signs of tinnitus disappeared when the tonotopic representation was restored, which suggests reorganization is responsible for the tinnitus percept (Engineer et al., 2011). Nevertheless, other studies have not found functional differences in tonotopic maps of tinnitus patients without hearing-loss (Langers, de Kleine, & van Dijk, 2012).

Leaver and colleagues (2011) formulated a hypothesis that connects functional and structural changes in tinnitus patients. They propose a model of tinnitus that involves limbic and auditory interactions in the brain (Figure 1). Their model includes areas that are usually found in both functional and structural studies, such as the auditory cortex, the ventromedial prefrontal cortex (vmPFC), the nucleus accumbens (NAc), and medial geniculate nucleus (MGN, thalamus). In healthy individuals, the vmPFC and NAc control the auditory input that will be processed. A few studies found that these areas have less grey matter in tinnitus patients, and argue that at least one of these areas is unable to inhibit the tinnitus signal (Leaver et al., 2011; Leaver et al., 2012; Mühlau et al., 2006; Rauschecker, Leaver, & Mühlau, 2010). Leaver and colleagues argue that structural changes in the auditory cortex are a result of the inefficient control of auditory signal in lower level areas, because peripheral damage in the somatosensory system results in cortical map changes but causes even more reorganization at the thalamic level

(Ergenzinger, Glasier, Hahm, & Pons, 1998; Rauschecker, 1998). They associate structural findings in the auditory cortex with the characteristics of the perceived tinnitus signal, but other studies oppose this (Langers et al., 2012). Interestingly, a VBM and EEG comparison study by Vanneste, Van de Heyning, and De Ridder (2015) found no correlations between functional and anatomical changes in tinnitus patients. They attribute these findings to hearing-loss instead of tinnitus. However, they did not compare tinnitus patients to normal-hearing controls. This poses a problem, because the areas that are usually found in studies on tinnitus, such as the (left) auditory cortex and the right temporal lobe, have also been found in studies on hearing-impairment (Eckert, Cute, Vaden Jr, Kuchinsky, & Dubno, 2012; Lin et al., 2014; Vanneste et al., 2015).

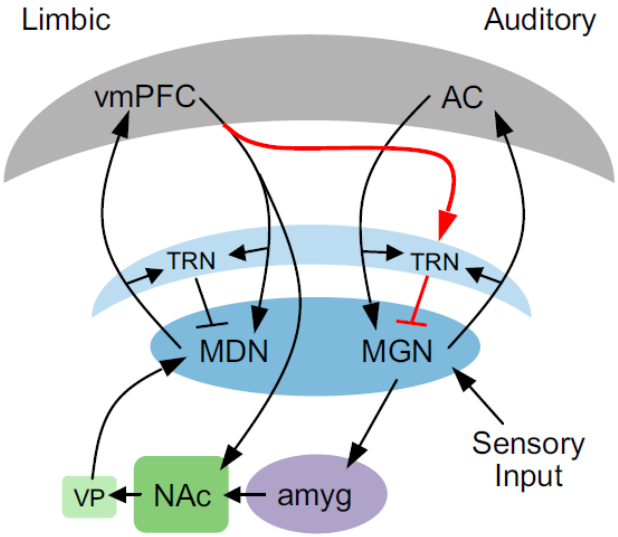

Figure 1: ***Schematic of interactions in tinnitus****. In healthy individuals, the limbic system may identify a sensory signal as irrelevant (e.g., transient tinnitus) and inhibit the unwanted signal at the medial geniculate nucleus (MGN) via the ventromedial prefrontal cortex (vmPFC) to the auditory thalamic reticular nucleus (TRN, red pathway). Thus, the spread of the unwanted signal is reduced in both circuits. In chronic tinnitus, inefficient vmPFC output prevents inhibition of the tinnitus signal, resulting in continued activity and constant perceptual presence of the tinnitus signal. Cortical structures are noted in gray, thalamus is noted in blue, basal ganglia in green, and amygdala in lavender. Abbreviations: medial dorsal nucleus (MDN), ventral pallidum (VP), amygdala (amyg), auditory cortex (AC). Retrieved from Leaver et al. (2011).*

Structural Differences between Normal-Hearing Individuals, Individuals with Hearing-Loss, and Tinnitus Patients

Reviews of the literature on functional and structural changes in the brain that are associated with tinnitus have been created by Eggermont (2012) and Langers & De Kleine (2016). The usual method to analyse grey matter volume is called voxel-based morphometry (VBM). An overview of studies that used VBM to investigate structural changes in tinnitus patients can be found in Table 1. Only studies with controls are included.

The temporal lobe, and in particular the auditory cortex, is an area of interest in tinnitus research. Schneider and others (2009) and Aldhafeeri and others (2012) found an association between tinnitus and structural changes in the auditory cortex. They found smaller grey matter volume in tinnitus patients compared to controls. In patients with unilateral tinnitus, the changes were observed on the ipsilateral side. Patients with bilateral tinnitus, on the other hand, showed reduced mHG volumes in both hemispheres. Boyen, Langers, De Kleine, and Van Dijk (2013) had contrary results: an increase in grey matter volume in the primary auditory cortex. Other studies found no differences in cortical thickness in the primary and secondary auditory cortex (Mühlau et al., 2006).

Furthermore, as the limbic system controls the auditory signal, these areas are often also regarded in tinnitus studies. Mühlau and others (2006) found changes situated at the thalamic level of the auditory system. The posterior thalamus including the medial geniculate nucleus (MGN) of tinnitus patients showed an increase in grey-matter concentration. They note that earlier studies on adult sensory plasticity found interactions between the cortex and the thalamus (Ergenzinger et al., 1998; Rauschecker, 1998; Suga & Ma, 2003). The authors found an additional decrease in grey matter in the subcallosal region including the nucleus accumbens (NAc). The subcallosal area is associated with negative emotions (Blood, Zatorre, Bermudez, & Evans, 1999) and aversive sounds (Zald & Pardo, 2002). Later studies could not replicate these findings (Landgrebe et al., 2009; Melcher, Knudson, & Levine, 2013). Instead, Landgrebe and others (2009) found a decrease in grey matter in the right inferior colliculus (IC) and in the left hippocampus.

In the frontal region, grey matter reductions have been found in the prefrontal cortex, in particular in the ventromedial prefrontal cortex (vmPFC) and in the dorsomedial prefrontal cortex (dmPFC) (Aldhafeeri et al., 2012; Husain et al., 2011; Leaver et al., 2011; Leaver et al., 2012; Melcher et al., 2013). Besides these regions, Melcher and colleagues also found

differences between tinnitus patients and controls in the ventral posterior cingulate cortex (cPCC). Finally, Vanneste and colleagues (2015) found convincing structural changes in the cerebellum.

Table 1 Grey matter findings in tinnitus using group contrasts. Underlined brain areas are findings using Region of Interest analysis. Otherwise, findings are the result of voxel-by-voxel whole brain analyses.
Abbreviations: PFC = prefrontal cortex, IC = inferior colliculi, SFG = superior frontal gyrus, MFG = medial frontal gyrus, AntC = anterior cingulate, STG = superior temporal gyrus, SMG = supramarginal gyrus), BA = Brodmann area, GM = grey matter.

Authors	Sample	Finding
Mühlau et al. (2006)	28 patients versus 28 controls; matched for age and gender	Subcallosal area (ventromedial PFC) ↓, thalamus (MGN) ↑
Schneider et al. (2009)	61 patients versus 45 controls; age, gender, and hearing as covariates	Heschl's gyrus ↓
Landgrebe et al. (2009)	28 patients versus 28 controls; matched for age and gender	Right IC ↓, left hippocampus ↓
Leaver et al. (2011)	11 patients versus 11 controls; hearing and age as covariates	Ventromedial PFC ↓
Husain et al. (2011)	8 patients versus 7 controls with hearing-loss versus 11 controls; matched for age and gender	No tinnitus findings. Hearing loss: SFG ↓, MFG ↓, right AntC ↓, STG ↓
Leaver et al. (2012)	23 patients versus 21 controls; matched for age and gender	Ventromedial PFC ↓, dorsomedial PFC ↓ left SMG ↓
Diesch et al. (2012)	63 patients versus 42 controls	Corpus callosum ↑ in female ↓ in male patients
Aldhafeeri et al. (2012)	14 patients versus 14 controls; matched for age, gender, and hearing	Right frontal cortex ↓, cingulate gyrus ↓, right primary auditory cortex ↓, left temporal cortex ↓
Boyen et al. (2013)	31 patients versus 16 controls with hearing-loss versus 24 controls; matched for gender	Left primary auditory cortex (BA 41) ↑, right auditory association cortex (BA 22) ↑, inferior temporal area (BA 20) ↑, limbic cortex (BA 35 & 36) ↑ in tinnitus vs normal-hearing control, frontal areas (BA 8, 9, & 11) ↑ in tinnitus vs. normal-hearing control
Melcher et al. (2013)	24 tinnitus patients versus 24 controls, matched for age, gender, and hearing level	PFC ↓, probability of decreased GM with increasing hearing-loss

It is no surprise that in functional and structural studies on tinnitus emotion-related brain areas are found to be deviating from normal-hearing controls. Grey matter decreases in the left hippocampus (Landgrebe et al., 2009) are associated with the pathophysiology of depression (de Geus et al., 2007; Vasic, Walter, Höse, & Wolf, 2008) and insomnia (Riemann et al., 2007). Both conditions are complaints among tinnitus patients (Crönlein, Langguth, Geisler, & Hajak, 2007). Moreover, it has been suggested that grey matter volume reductions in the auditory cortex and hippocampus are correlated to the amount of tinnitus distress

(Schecklmann et al., 2013; Vanneste et al., 2015), but others argue that the neural systems that process tinnitus and distress are separated (Leaver et al., 2012). When investigating these emotion-related areas in tinnitus patients, one should be aware of the apparent correlation between tinnitus and negative emotions. Volumetric differences between tinnitus patients and controls may not be a result of the tinnitus, but a consequence of tinnitus distress (Schecklmann et al., 2013).

As chronic tinnitus is more likely to arise in the elderly (Baguley, 2002; Eggermont & Roberts, 2004), and age is associated with (sub)cortical grey matter volume decrements (Bartzokis et al., 2001; Good et al., 2002; Raz et al., 2005; Zimmerman et al., 2006), it is important to control for age when investigating structural differences. Also, a fair amount of preceding studies did not control for hearing-loss (Landgrebe et al., 2009; Leaver et al., 2011; Melcher et al., 2013; Mühlau et al., 2006; Schneider et al., 2009). More recent studies did compare individuals with hearing-loss, tinnitus, and normal hearing controls (Boyen et al., 2013; Husain et al., 2011). There are researchers who argue that tinnitus-related findings are in fact hearing-loss related findings (Vanneste et al., 2015).

Literature on anatomical differences between tinnitus patients, patients with hearing-loss, and normal hearing individuals is relatively sparse and inconsistent. This study aims to contribute to the literature on structural differences in tinnitus patients, individuals with hearing loss, and normal hearing individuals. Differences in grey matter volume may reveal vulnerability factors for tinnitus or indicate neuroplasticity.

Expectations

Studies usually find functional differences between normal-hearing individuals and tinnitus patients in the auditory cortex (Eggermont, 2012; Langers & de Kleine, 2016), but structural differences are often also found outside of these areas. Studies on structural differences are inconsistent, but changes in cortical thickness are mainly found in three areas: the auditory cortex, the limbic system, and frontal regions. More specifically, the areas with the highest likelihood to show decreases between tinnitus patients and controls are the ventral medial prefrontal cortex, the nucleus accumbens, the cingulate cortex, the cerebellum, the left hippocampus, and the primary auditory area. Boyen and colleagues (2013) found mostly increases in these areas in tinnitus patients. It is possible that structural changes are found in both individuals with hearing loss and with tinnitus (Boyen et al., 2013; Husain et al., 2011; Melcher et al., 2013; Vanneste et al., 2015). Hearing-loss-related decreases in GM are expected in the in frontal gyri, the anterior cingulate, the superior temporal gyrus, and the

prefrontal cortex. An increase can be expected in the limbic cortex. It is important to consider that the relation between tinnitus and the brain could be asymmetric (Melcher et al., 2000; Mühlau et al., 2006; Schneider et al., 2009).

Materials and Methods

Participants

All participants were informed about the purpose of the study before giving their written consent. The study had been approved by the local Medical Ethics Committee of the University Medical Center Groningen. In total, 37 participants were tested. One participant showed neurological abnormalities and was excluded from analysis. The remaining 36 participants (13 female, mean age 59,1 [SD 8,8], 33 right-handed) were assigned to one of three groups: the tinnitus and hearing-loss group (Ti + HL), the hearing-loss only group (HL), and controls (Co). See Table 2 for the characteristics of the participants per group. Tinnitus patients suffered from chronic subjective tinnitus. They were recruited from the University Medical Centre Groningen Ear, Nose, and Throat policlinic and from Stichting HoorMij, a Dutch foundation for hearing-impaired individuals. Besides tinnitus, the patients had no psychiatric or neurological disorders. Participants did not use any hearing aids at the time of inclusion.

A standard tone audiometric test was performed on all participants, using eight different octave frequencies (0,25, 0,5, 1, 2, 3, 4, 6 and 8 kHz). Sometimes 0,125 kHz was also included. See Figure 2 for results. Controls had an average hearing threshold below 20 dB at 500-2000 Hz. If a participant did not meet this requirement, the participant was put in the hearing-loss group. Hearing impaired participants were put in a steep sloping or a gradual sloping groups. Participants qualified for steep slope hearing-loss if the difference between the average hearing threshold at 1 and 2 kHz and the average threshold at 4 and 8 kHz was more than 30 dB SPL. Participant who did not show such a discrepancy were classified as gradual hearing-loss participants. Participants had less than 30 dB difference on all frequencies between both ears.

Table 2: Subjects' characteristics

	T + HL (n = 26)	HL (n = 4)	Control (n = 6)
Age			
Mean (SD)	59,7 (7,9)	63,5 (3,9)	53,8 (13,0)
Range	41 - 71	58 – 67	28 – 63
Sex (female)	8	2	3
Handedness (right)	24	4	5
Average overall hearing threshold in dB SPL			
Left ear (SD)	37,6 (33)	16,6 (7,8)	10,7 (7,5)
Right ear (SD)	35,8 (26,3)	20,4 (10,1)	13,3 (13,1)

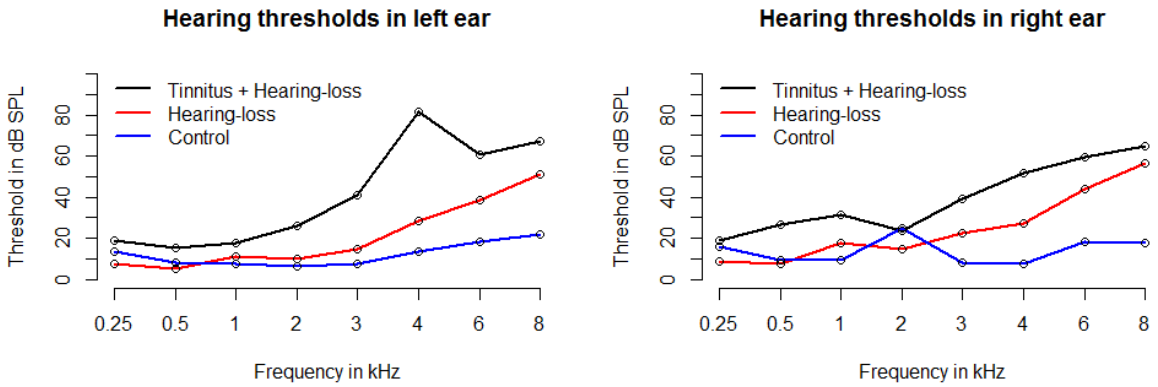

Figure 2: Mean audiograms for the tinnitus + hearing-loss group (black), hearing-loss group (red), and control group (blue)

Data Acquisition

MRI-scans have been performed using a Philips 3T scanner of the Neuroimaging Center (NiC) of the University Medical Center Groningen in Groningen which was equipped with a SENSE transmit/receive head coil. Multiple scans were executed, such as DTI, resting-state, fMRI (while performing a simple task), and an anatomical scan. The anatomical scan was a 3-dimensional high-resolution T1-weighted echo scan (160 slices; acquisition duration 614 s; repetition time (TR) 10,4 ms; echo time (TE) 0 ms; field of view 256 x 160 x 224 mm; matrix 256 x 229). Voxels were resliced to 1,5 x 1,5 x 1,5 mm, which is the result of a consideration of multiple factors. The relative small sample groups, together with chance of finding much

reorganisation on the thalamic level on the one hand, and less reorganisation on the cortical level on the other, are the reason that this voxel size was chosen (Ergenzinger et al., 1998; Rauschecker, 1998). This size is not unusual in VBM studies on tinnitus (Langers, 2014). Participants wore headphones during the scan. The scans were screened by a radiologist.

Data Processing

Voxel-based morphometry (VBM) (Ashburner & Friston, 2000; Ashburner, 2015, March 12; Wright et al., 1995) was performed using SPM12 software package (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK) running in MATLAB 2016a (MATLAB 2016a, The MathWorks, Inc., Natick, Massachusetts, United States). See Figure 3 for an overview of the pre-processing. Images were first manually aligned using the 'Display' option in SPM if they were skewed (Figure 3A). Images were segmented into grey matter, white matter, and cerebrospinal fluid using the default tissue probability templates from SPM12 (Figure 3B). The resulting images are GM probability maps which comprise voxels with values between 0 and 1 reflecting the likelihood of grey matter at a particular voxel (Melcher et al., 2013). The DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra, Ashburner, 2007) toolbox was used to create grey matter templates of the obtained grey matter images (Figure 3C). The images were spatially normalised into the Montreal Neurological Institute (MNI) stereotaxic space (Figure 3D). This is the data that will be used in the statistical analysis. The size of the reorganised areas can differ (Ergenzinger et al., 1998; Rauschecker, 1998). It is entirely possible to find both relatively large and small structural changes. Thus, 8 mm Gaussian FWHM smoothing was applied as a middle ground. Moreover, this setting has also been used in other VBM studies on tinnitus (Boyen et al., 2013; Husain et al., 2011; Landgrebe et al., 2009; Melcher et al., 2013; Mühlau et al., 2006; Schecklmann et al., 2013; Vanneste et al., 2015). Modulation was applied, to correct for deformation as a result of stretching and compressing the obtained images to the MNI standard during normalization. Modulation involves scaling by the amount of contraction, so that the total amount of grey matter in the modulated GM remains the same as it would be in the original images (Structural Brain Mapping Group, n.d.). This means that only the amount of grey matter will be taken into consideration during statistical analysis. This method usually leads to more findings than using unmodulated images when investigating volumetric differences in GM (see for example Boyen et al., 2013).

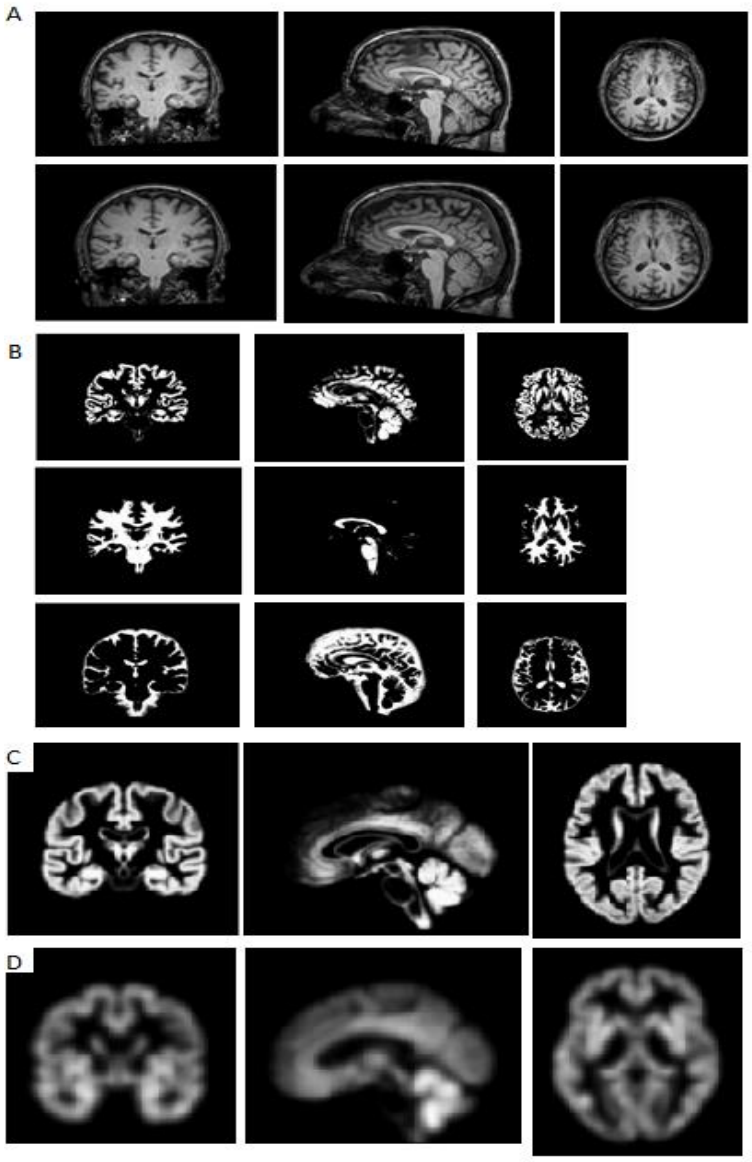

Figure 3: Data pre-processing. A: Reorientation of the raw data of one participant (top row). Here, the image was adjusted over the z-axis (bottom row), which is particularly visible in the transverse view. B: Segmentation of reoriented image of example participant into grey matter (top row), white matter (middle row), and cerebrospinal fluid (bottom row). C: Final grey matter template as produced by DARTEL over all participants. D: Normalised and smoothed result of pre-processing of example participant. This image is used during statistical analysis.
MNI coordinates x = 0, y = 0, z = 0 for all images

Statistical Analysis

Whole-brain analysis.

For the whole brain analysis (WBA), an Analysis of Covariance (ANCOVA) was performed with group membership as main effect and age and sex as covariates without interactions. The groups were split in to tinnitus patients with hearing-loss (Ti+HL), individuals with only hearing-loss (HL), and healthy controls (Co).

During statistical analysis, images were corrected for total grey matter volume using proportional scaling. The voxels were thresholded at 0,20 (out of 100) probability of tissue classification prior to statistical analyses. A voxel contains a probability value indicating the chance that a voxel is grey matter or not. When this probability is less than 0,20, the voxel is excluded from the analysis. This procedure prevents spurious findings (Ashburner, 2015, March 12). Total brain volume (grey + white matter) was used to calculate the global values in order to estimate the global effects, during whole brain voxel-by-voxel analysis.

As a form of data exploration, confidence thresholds were set to $p > 0,001$, without any other form of correction. Later, these results would be used for region of interest analysis. When the exploration was finished, the whole brain analysis was performed using a Family-Wise Error (FWE) correction (confidence threshold $p < 0,05$) to adjust for multiple comparisons at both voxel- and cluster level. T-contrasts were used to compare the average GM of two groups with the GM of the remaining group. This is a way to discover similarities between imbalanced experimental groups. Next, two sample t-tests were performed using the ANCOVA main effects as an inclusive mask in order to evaluate GM differences separately between the three subject groups. Again, age and gender were entered as covariates. A confidence threshold of $p < 0.05$ FWE corrected was applied as well.

Region-of-interest (ROI) analysis.

For the ROI analysis, the modulated data was examined for various subdivisions of the brain. The choice of regions of analysis were based on a combination of previous literature (see Table 1) and outcomes of the uncorrected results of the whole-brain analysis (see Table 3). The ROIs based on the data exploration were made by entering the peak voxel of a cluster of more than 50 voxels in the IBASPM 116 collection of WFU_pickatlas (Maldjian, Laurienti, Burdette, & Kraft, 2003). So-called ‘masks’ were created by picking the resulting area. Settings were 3D and dilation 1. The mask was then applied to the contrast that previously showed significant differences in GM volume. In addition to ROIs based on the data exploration, more ROIs were created based on literature. These ROIs were the primary auditory cortex / superior temporal cortex (BA 41 & 42), Heschl’s gyrus, the thalamus, the ventromedial prefrontal cortex (BA 10, 11, 12, 14, 25, & 32), the hippocampus, anterior cingulate, supramarginal gyrus (BA 40), limbic cortex (BA 35 & 36, also called perirhinal cortex), frontal areas (BA 8, 9, & 11), and the corpus callosum. Hemispheres were considered separately, except for the corpus callosum. See Appendix I for all ROIs created for the analysis.

T-contrasts were used to compare the average GM of two groups with the GM of the remaining group. Two sample t-tests were performed using the ANCOVA main effects as an inclusive mask. Age and gender were entered as covariates. Then, the mask was added. A confidence threshold of $p < 0.05$ FWE corrected was applied to all contrasts to correct for multiple comparisons.

Results

Correlation with age and sex.

In order to find correlations with variables, a multiple regression was performed. This analysis showed that age slightly correlates with the amount of grey matter at the frontal cortex (T = 3,94; p < 0,001 no correction; peak voxel MNI coordinates ±18, 32, 60), but this did not hold when family wise error was applied. Nevertheless, other studies have found that age is strongly associated with the amount of GM (Bartzokis et al., 2001; Good et al., 2002; Raz et al., 2005; Sullivan, Marsh, Mathalon, Lim, & Pfefferbaum, 1995; Zimmerman et al., 2006). Gender had a stronger association with amount of grey matter in many areas when no correction was applied. When FWE was applied, one voxel survived in the temporal area (T = 5,93; p < 0,001 FWE < 0,05; peak voxel MNI coordinates ±38, -5, -18). Age and gender were used as covariates in further analyses.

Whole-brain Voxel-by-voxel Comparisons

Initially, an exploratory analysis using p>0,001 uncorrected showed differences in grey matter in many brain areas. See Table 3 for clusters with more than 50 voxels. No significant clusters of more than 50 voxels were found when contrasting HL + Co > Ti and HL > Ti. Only the Ti + Co > HL contrast showed significant differences in the amount of grey matter when a confidence threshold of p < 0.05 FWE was applied to correct for multiple comparisons. The averaged tinnitus and control subjects had more grey matter than patients with hearing-loss in the left superior temporal cortex, in an area that is also called the primary auditory cortex (BA 42, see Figure 4) (T = 6,02, peak voxel p = 0,025 FWE-corrected, k = 4, cluster p = 0,032, peak voxel MNI coordinates -62 -14 9).

Table 3: Voxel-by-voxel comparisons of GM amount obtained from the modulated images. A threshold of p < 0,001 uncorrected was applied as well as an extend threshold (k) of 50 adhering voxels. Brodmann area numbers are specified besides the general name if this was provided by wfu_Pickatlas.

Contrast	Area (Brodmann area)	Cluster size (k)	p-value cluster uncor.	T-value peak voxel	p-value peak voxel uncor.	Peak voxel (MNI coordinates)		
Ti > HL + Co	Middle cingulum left	270	0,065	5,05	< 0,001	-6	-12	36
	Superior temporal pole left	444	0,022	4,69	< 0,001	-45	-5	-20
	Superior temporal pole right	365	0,035	4,65	< 0,001	47	3	-18
	Lingual cortex left	80	0,296	4,47	< 0,001	-20	-45	-11
	Superior frontal cortex left	92	0,263	4,33	< 0,001	-15	-15	54
HL > Ti + Co	Cerebellum left	114	0,214	3,59	< 0,001	-47	-68	-24
	Cerebellum left	64	0,351	3,22	0,001	-32	-81	-27
Co > Ti + HL	Superior frontal cortex left	82	0,290	5,14	< 0,001	-21	15	45
	Lingual cortex left (18)	189	0,116	4,86	< 0,001	-8	-74	-5
	Parahippocampal cortex left	54	0,392	4,68	< 0,001	-17	0	-35
	Heschl's gyrus left (22)	140	0,171	4,60	< 0,001	-56	-11	8
	Middle frontal gyrus left (10)	90	0,268	4,57	< 0,001	-24	45	27
	Middle temporal cortex right	64	0,351	3,98	< 0,001	53	-62	9
Ti + Co > HL	Superior temporal cortex (42) left	412	0,027	6,02	< 0,001	-62	-14	9
	Middle frontal cortex	440	0,023	5,27	< 0,001	-21	45	26
	Lingual gyrus left (18)	635	0,008	5,60	< 0,001	-8	-74	-5
	Inferior temporal cortex left	94	0,258	5,13	< 0,001	-50	-38	-26
	Middle temporal cortex left	555	0,012	4,74	< 0,001	-44	0	-23
	Heschl's gyrus right (13)	224	0,089	4,59	< 0,001	41	-20	3
	Gyrus rectus right	58	0,0375	4,59	< 0,001	6	42	-23
	Lingual cortex right	141	0,170	4,42	< 0,001	12	-68	-5
	Lingual cortex left (19)	55	0,388	4,32	< 0,001	-18	-47	-11
	Superior frontal cortex right (10)	98	0,249	4,29	< 0,001	26	53	8
	Cerebellum left	55	0,388	4,22	< 0,001	-32	3	-35
	Superior temporal cortex right	86	0,279	4,11	< 0,001	54	-36	9
	Insula right	81	0,293	3,78	< 0,001	39	-14	15
Ti + HL > Co	Cerebellum left	50	0,411	3,71	< 0,001	-32	-84	-29
TI > Co	Superior temporal pole right	79	0,254	4,17	< 0,001	39	14	-21
Co > Ti	Parahippocampal cortex left	87	0,232	5,17	< 0,001	-17	0	-35
Ti > HL	Superior temporal gyrus (42) left	232	0,058	5,22	< 0,001	-63	-14	9
	Middle frontal cortex left	399	0,017	4,83	< 0,001	-21	47	27
	Superior temporal cortex left	615	0,004	4,79	< 0,001	-47	3	-12
	Gyrus rectus right	65	0,294	4,56	< 0,001	6	42	-21
	Heschl's gyrus right (13)	172	0,096	4,39	< 0,001	39	-21	5
	Lingual cortex left (19)	58	0,322	4,38	< 0,001	-18	-47	-11
	Middle orbital frontal cortex right	53	0,344	3,67	0,001	8	45	-9

HL > Co	Cerebellum left	56	0,018	12,88	< 0,001	-44	-69	-29
Co > HL	Lingual cortex left	143	0,001	25,97	< 0,001	-8	-77	-5
	Middle frontal gyrus left	56	0,018	16,72	< 0,001	-2	44	21

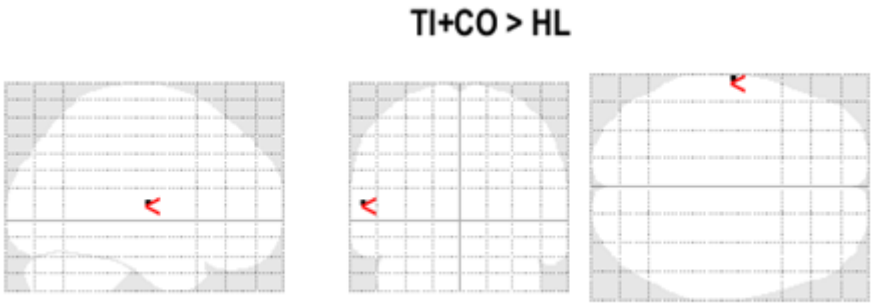

Figure 4: Ti + Co > HL contrast. The red arrow indicates the peak voxel. FWE threshold of p< 0,05 was applied. Coordinates of peak voxel: -62 -14 9. T = 6,02 p = 0,025.

Region-of-Interest (ROI) Analysis

Several contrasts showed GM differences when a FWE-correction was applied (See Table 4). No differences in grey matter volume were found when using the contrasts HL > Ti + Co, Ti + HL > Co, Co + HL > Ti, Ti > Co, HL > Ti, HL > Co, an Co > HL. Contrary to the whole-brain analysis, the ROI-analysis showed differences between separate groups. Controls had more grey matter in the left parahippocampal cortex compared to tinnitus patients with hearing-loss (see Figure 5) (T=5,17, peak voxel p = 0,005, k = 40, cluster p = 0,015, peak voxel MNI coordinates -17 0 -35). Furthermore, tinnitus patients had more grey matter than individuals with hearing-loss in the temporal area (both Heschl's gyri, left superior temporal gyrus), frontal areas (left middle frontal cortex, right gyrus rectus), and the limbic cortex (BA 36).

Table 4: ROI comparisons of GM amount obtained from the modulated images. A threshold of p < 0,05 FWE-corrected was applied. Brodmann areas numbers are specified besides the general name if this was reported by wfu_Pickatlas.

Contrast	Area (Brodmann area)	Cluster size (k)	p-value cluster FWE-cor.	T-value peak voxel	p-value peak voxel FWE-cor.	Peak voxel (MNI coordinates)		
Ti > HL + Co	Middle cingulum left	76	0,009	5,05	0,007	-6	-12	39
	Superior temporal pole left	73	0,010	4,69	0,012	-45	-5	20
	Superior temporal pole right	100	0,007	4,65	0,014	47	3	-18
	Middle cingulum left	76	0,009	5,05	0,007	-6	-12	36
	Lingual cortex left	8	0,034	4,47	0,028	-20	-45	-11
Co > Ti + HL	Superior frontal cortex left	8	0,032	5,14	0,012	-21	15	45
	Lingual cortex left (18)	38	0,016	4,86	0,012	-8	-74	-5
	Parahippocampal cortex left	13	0,031	4,68	0,011	-17	0	-35
	Heschl's gyrus left (22)	109	0,010	4,60	0,005	-56	-11	8
	Superior frontal gyrus left	8	0,032	5,14	0,012	-21	15	45
	Middle frontal gyrus left (10)	3	0,040	4,57	0,043	-24	45	27
Ti + Co > HL	Superior temporal cortex (42) left	100	0,006	6,02	0,001	-62	-14	9
	Middle frontal cortex	106	0,004	5,27	0,003	-21	45	26
	Lingual gyrus left (18)	177	0,002	5,60	0,002	-8	-74	-5
	Lingual gyrus left (19)	3	0,041	4,32	0,039	-18	-47	-11
	Inferior temporal cortex left	17	0,024	5,13	0,009	-50	-38	-26
	Middle temporal cortex left (20)	2	0,042	4,53	0,034	-51	-6	-24
	Middle temporal cortex left	15	0,025	4,74	0,029	-44	0	-23
	Heschl's gyrus right (13)	89	0,012	4,59	0,006	41	-20	3
	Rectus right	28	0,024	4,59	0,010	6	42	-23
	Lingual cortex right	6	0,036	4,42	0,035	14	-68	-5
	Lingual cortex left (19)	3	0,041	4,32	0,039	-18	-47	-11
	Lingual cortex left (18)	177	0,002	5,60	0,002	-8	-74	-5
	Cerebellum left	2	0,042	4,99	0,014	-50	-39	-27
	Insula right	32	0,019	4,59	0,020	41	-20	3
Co > Ti	Parahippocampal cortex left	40	0,015	5,17	0,005	-17	0	-35
Ti > HL	Superior temporal gyrus (42) left	55	0,012	5,22	0,004	-63	-14	9
	Middle frontal cortex left	1	0,044	4,83	0,040	-21	47	27
	Gyrus rectus right	19	0,025	4,56	0,016	6	42	-21
	Heschl's gyrus right (13)	44	0,017	4,39	0,016	39	-21	5
	Heschl's gyrus right (6)	7	0,036	3,87	0,039	53	-6	5
	Limbic cortex left (BA 36)	11	0,031	4,63	0,012	-50	-39	-27
	Limbic cortex left (BA 36)	4	0,039	4,14	0,034	-20	-45	-11

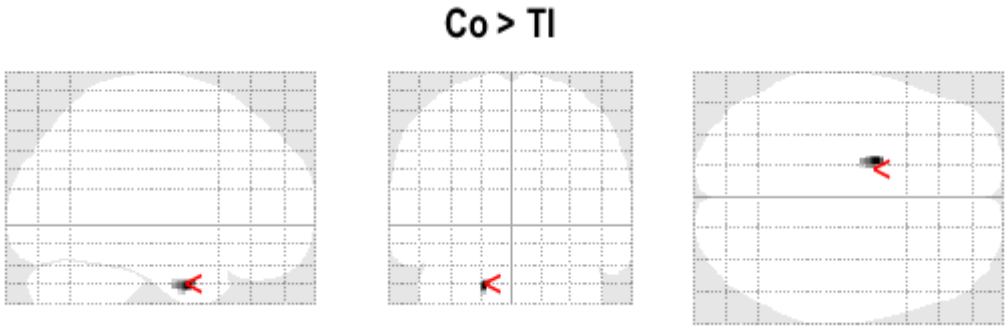

Figure 5: Co > Ti contrast with the left parahippocampal cortex as region of interest. The red arrow points to the peak voxel. FWE threshold of p< 0,05 was applied. Coordinates of peak voxel: -17 0 -35 T= 5,17 p = 0,005.

Discussion

This study attempted to make a distinction between the amount of grey matter that is associated with tinnitus, hearing-loss, and normal hearing. Only FWE-corrected findings will be discussed, as these are more reliable than uncorrected findings.

Differences were expected between tinnitus patients and the control groups in the ventral medial prefrontal cortex, the nucleus accumbens, the cingulate cortex, the cerebellum, the left hippocampus, and the primary auditory area. Hearing-loss related decreases were expected in the frontal gyri, the anterior cingulate, the superior temporal gyrus, and the prefrontal cortex. An increase was expected in the limbic cortex. A whole-brain analysis showed that, when averaged, the tinnitus and control subjects had more grey matter than hearing-impaired individuals in the superior temporal cortex (Figure 4), which is an area where differences were expected based on previous literature. On the other hand, the averaged hearing-loss and control subjects do not have more GM than tinnitus subjects. ROI analysis showed that control subjects have more grey matter in the left parahippocampal cortex than tinnitus subjects (Figure 5). Tinnitus subjects had more grey matter than the hearing-loss group in the temporal, frontal, and limbic cortex.

Inefficient Gating System is not Situated in the Limbic Areas

Leaver and colleagues (2011) and Rauschecker and colleagues (2010) propose a malfunctioning gating system as the cause of the tinnitus signal. They support their hypothesis by pointing out areas showing an increase in functional activity while simultaneously displaying a decrease in grey matter volume. This gating area is properly inhibiting the tinnitus signal in healthy individuals with hearing-loss, but lacks the grey matter needed to inhibit the tinnitus signal in patients. The key areas are in the thalamus (MGN), the nucleus accumbens, or in the ventromedial prefrontal cortex. Irrelevant input from these areas ends up

in the auditory cortex, where long-term reorganization sets in to establish chronic tinnitus through Hebbian mechanisms. Leaver and colleagues founded their theory on older studies that did not include hearing-loss as a variable, while tinnitus is strongly linked to hearing-loss. This complicates finding the locus (or loci) of the disinhibition by a malfunctioning gate(s). This may explain why findings are so diverse (Table 1). Melcher et al. (2013), Husain et al. (2011), Vanneste et al. (2015), and Boyen et al. (2013) have all found differences that were solely associated to hearing-loss in the frontal and limbic areas of the brain, such as the frontal gyri, the superior temporal gyrus, the anterior cingulate, and the hypothalamus.

If the tinnitus signal is indeed a result of poor filtering in these gating areas, as Leaver and colleagues are posing, both control groups should have more grey matter in the thalamus and less grey matter in the frontal areas and limbic areas, compared to the tinnitus group. This study found no differences between groups in the thalamus, nucleus accumbens, or the ventromedial prefrontal cortex. This could mean that a malfunctioning gating system is not situated here, or that functional change may not lead to structural change (Vanneste et al., 2015). However, other frontal and limbic differences in GM volume have been identified. The finding that the left parahippocampal cortex is thinner in tinnitus subjects compared to normal-hearing controls has not been found in earlier studies, although Landgrebe and colleagues (2009) found a decrease in GM in the adhering hippocampus. They suggest that the hippocampus is directly related to the tinnitus pathophysiology. This cannot be concluded from the present study, as the HL > Ti contrast did not show any results. Boyen and colleagues (2013) found an increase in GM in the hearing-loss group compared to the tinnitus group in a cluster that stretches the parahippocampal gyrus and limbic cortex (BA 35 & 36). The present study, however, found a decrease in GM amount in the hearing-loss group compared to the tinnitus group in the limbic cortex. The data suggest that normal hearing controls have the most GM in the limbic system, then the tinnitus patients, and then the individuals with hearing-loss. This ‘Co > Ti > HL’ finding supports the hypotheses that the limbic system is at least involved in the pathophysiology of tinnitus, but cannot confirm a gating mechanism (Jastreboff, 1990). The findings do not suggest that a lack of grey matter in the limbic system is causing the tinnitus percept (Landgrebe et al., 2009; Leaver et al., 2011; Mühlau et al., 2006; Rauschecker et al., 2010), because the tinnitus group had no systematic decrease of GM in a particular limbic area compared to both control groups. The hypothesis that the (pre-)frontal cortex is involved as a gate, remains a possibility. Note that few voxels in the middle frontal cortex and rectus gyrus remained after FWE-correction, which makes the finding not very trustworthy.

The 'Reorganisation Gone Wrong' Hypothesis

The reorganisation of the cortex in the auditory system is a returning subject in tinnitus research. Evidence for functional reorganisation of the auditory cortex as a result of tinnitus is identified by some and not by others (for reviews see Eggermont, 2012; and Langers and De Kleine, 2016). Langers and De Kleine (2016) point to methodologic differences causing this disparity. Functional reorganisation is often found in the auditory system, the limbic system, the frontal lobe, and the cerebellum. Auditory-input is reduced in hearing-impaired individuals. As the auditory system is no longer activated as it used to be, neurons in the pathway may lower their activation threshold, and pick up irrelevant activation (Chrostowski et al., 2011; Dominguez et al., 2006). This is where the reorganisation goes wrong: irrelevant input is processed as sound. Leaver and colleagues (2011) assume that this irrelevant activation is sent to the auditory cortex as a result of disinhibition in the frontal and limbic areas. In the literature, it is often assumed that functional reorganisation is accompanied by structural reorganisation. The current literature do not explain how functional changes in tinnitus patients lead to structural changes. Structural findings have also been found in areas where functional findings have been observed (Table 1; Langers & De Kleine, 2016; Eggermont, 2012), but this is not always the case (Vanneste et al., 2015).

Again, many studies did not acknowledge hearing-impairment as a cofounding variable. This is concerning, because changes related to tinnitus could also be related to hearing-loss. For example, GM differences in the auditory cortex and the right temporal lobe have also been found in studies on hearing-impairment (Eckert et al., 2012; Lin et al., 2014; Vanneste et al., 2015). This study attempted to separate hearing-loss effects from tinnitus effects. In tinnitus patients, brain areas might still be used to process 'sound', even if it is not physically present. In hearing-impaired individuals, these areas might be used for something else. If functional reorganisation is indeed accompanied by structural organisation, hearing-impaired individuals should show differences in GM volume compared to tinnitus patients and controls. A decrease in grey matter volume would be expected in hearing-impaired individuals, as the auditory cortex is not used as much. Because our tinnitus group also suffered from hearing-loss, it can be assumed that parts of their auditory system may also be repurposed for something else. Tinnitus precepts greatly differ, suggesting that there are differences in the way the functional reorganisation might develop. Structural changes may therefore be not as severe as in individuals who are only hearing-impaired, but they might still

happen. This could be a reason why functional and structural findings differ much: they are diverse and may be rather small. Another option is that the tinnitus-percept is directly causing reorganisation in the auditory system (Jastreboff, 1990). After all, the patient group suffers from continuous tinnitus affecting their quality of life, while being hearing-impaired at the same time. This is in contrast to normal-hearing and hearing-impaired individuals, who do not experience one strong sound.

It was found that individuals with hearing-loss have less grey matter than the averaged tinnitus and control group in the left auditory cortex (WBA) and that individuals with tinnitus and hearing-loss have more grey matter in the left superior temporal gyrus and right Heschl's gyrus (ROI-analysis). These observations are in line with the first hypothesis: the hearing-loss group is different from the other groups. This suggests that the functional reorganisation due to hearing-loss in the auditory cortex was accompanied by structural changes. The tinnitus group and control group do not significantly differ from each other. Perhaps neurons in the auditory cortex of tinnitus patients remain more active after diminished input and might be indeed producing or processing the tinnitus percept.

Furthermore, individuals with tinnitus and hearing-loss have more grey matter in the limbic cortex and the gyrus rectus (ROI-analysis) than individual with only hearing-loss. We have already concluded that the hearing-loss group shows the strongest decrease in GM volume in the limbic system, then the tinnitus-group, and then the control group. It could be that structural reorganisation in the limbic systems was again most pronounced in the hearing-loss group. Compared to the controls, some reorganisation might have occured in the tinnitus group: enough to be found in a ROI-analysis, but not enough the be found when the whole brain is considered. The finding that the tinnitus group has more GM than the hearing-loss group in the gyrus rectus is difficult to place in context of the literature. Hearing-loss related decreases in GM in the prefrontal cortex have been observed (Husain et al., 2011; Melcher et al., 2013), as well as decreases in GM in hearing-impaired individuals (regardless of having tinnitus) compared to controls (Boyen et al., 2013). Hearing-related differences in the gyrus rectus have not yet been reported in the literature, but increases in GM volume in the gyrus rectus have been associated with depression (Ballmaier et al., 2004; Elderkin-Thompson, Hellemann, Pham, & Kumar, 2009), which is not uncommon in tinnitus patients (de Geus et al., 2007; Vasic et al., 2008).

Interestingly, no GM differences were found between the control group and the hearing-loss group. This is strange, because these differences have been found in earlier studies. This would provide further support for the idea that structural reorganisation is the

strongest in individuals with hearing-loss. Perhaps in this study the unequal group sizes are the cause of this. The literature often implies that differences are small, which signifies a need for large sample groups. The present study only had four participants with hearing-loss and six controls.

Technical considerations

There are a few important remarks to be made about the study. First, the participant group was severely imbalanced. Group sizes usually start at about 10 participants in each group (see Table 1). Small groups might result in spurious findings (type I errors) or results without enough statistical power (type II errors). Several researchers have stated that individual differences in the auditory cortex are possibly too fine or too variable for VBM to discover any volumetric differences (Mühlau et al., 2006; Schneider et al., 2009; Vanneste et al., 2015), but Melcher et al. (2013) reject this. They acknowledge the possibility that the sample of tinnitus patients used for their study and the studies by Mühlau et al. (2006) and Landgrebe et al. (2009) could be too different to find reliable grey matter decrements. This problem is also very possible for this study, because the sample groups were small.

Furthermore, although the mean age of the Ti + HL and the HL groups are not very different, the range over which this has been calculated is larger in the tinnitus group. The control group is relatively younger. Age-based GM differences have been found in the frontal and temporal lobules (Bartzokis et al., 2001; Sullivan et al., 1995). This could be the reason why the Ti + Co > HL contrast showed differences in GM volume, but analysis of the separate groups did not. The mean age of Ti and Co combined is about five years younger than the mean age of the HL group. However, the standard deviations overlap, so it remains the question whether the difference in volume is due to age or to reorganisation. The correlational analysis showed that gender is also an important factor, which is consistent with earlier findings (Good et al., 2002). The study by Good and others (2002) found significant volumetric sex differences in areas such as the central and temporal sulcus, the cingulate and Heschl's gyrus, frontal areas (more in females), the temporal lobe, the limbic cortex, and the cerebellum (more in males). These are areas that have also been found in this study. Thus, although adding age and sex as a covariate already helps to distinguish tinnitus effects from other cofounding factors, this analysis should be repeated when participant groups are more balanced in age and gender.

It is somewhat peculiar that on one hand the decrease in GM is situated in the left superior temporal cortex, and on the other hand in the right Heschl's gyrus. These areas are almost identical. This difference could be a result of the classification system used by wfu_Pickatlas.

The Family-Wise Error (FWE) correction provided by SPM to correct for multiple comparisons is based on a Bonferroni-correction (Bonferroni, 1936; Dunn, 1961). In a

Bonferroni-procedure, the p-value threshold α (in this case 0,05) is divided by the number of tests that are done on a data set. This corrects for false positives (type I errors). The correction tends to be too strict when applied to a large dataset, such as a whole-brain analysis that examines all voxels, because the resulting threshold after correction is very small. The corrected whole-brain analysis produced only a small cluster in the Ti + Co > HL contrast. This contrast may yield a larger cluster when a more appropriate correction is applied. Other clusters may survive too. An alternative is to control for the False Discovery Rate (FDR) instead, but this option is not provided by SPM. This problem is less present in the ROI-analyses cluster, as ROIs are smaller subsets of the data.

Conclusions and Further Directions

This study attempted to add to the literature of tinnitus-related grey matter changes and tried to disentangle the relationship between tinnitus and hearing-loss. Structural changes in the temporal lobe seem to be associated with hearing-loss, and might be a result of structural reorganisation due to diminished auditory input. The tinnitus group is not different from the control group, suggesting that the tinnitus percept may be processed as normal auditory input (Chrostowski et al., 2011; Dominguez et al., 2006; Jastreboff, 1990). Furthermore, findings in the limbic system suggest that normal-hearing individuals have the most grey matter here, then tinnitus patients, and then hearing-impaired individuals. This suggest that structural reorganisation is the strongest in hearing-impaired individuals, but also a present in tinnitus patients. No evidence was found for a malfunctioning gating system in the limbic system (Landgrebe et al., 2009; Mühlau et al., 2006), but the limbic system is probably involved in the pathophysiology of tinnitus. The frontal findings are difficult to interpret. The (pre)frontal area could be the malfunctioning part of gating mechanism (Leaver et al., 2011; Rauschecker et al., 2010) or, more likely, be a reflection of the negative emotions accompanying tinnitus (Ballmaier et al., 2004; de Geus et al., 2007; Elderkin-Thompson et al., 2009; Vasic et al., 2008).

As mentioned in the introduction, it is ambiguous whether findings in the hippocampus, auditory cortex, and now also the frontal cortex, can be attributed to the tinnitus signal or to tinnitus distress (Boyen et al., 2013; Landgrebe et al., 2009; Schecklmann et al., 2013; Vanneste et al., 2015). It is known that tinnitus patients have higher stress-levels that the healthy population (Crönlein et al., 2007). Emotion-related areas are mainly situated in the limbic system, where this study has found differences between tinnitus patients and controls. Note that Schecklmann and colleagues (2013) regard tinnitus duration equal to tinnitus

distress, and mention the frontal, temporal, and parietal areas, cingulate cortex, insula, (para-)hippocampal areas, and the amygdala as areas associated with tinnitus distress. This may suggest that the findings in this study are stress-related. However, the studies that support this claim do not make a clear distinction between tinnitus signal and tinnitus distress when presenting their findings. The present study has not done so either. An analysis within the tinnitus-group may shed a light on this. Additionally, (f)MRI-studies on tinnitus should include tinnitus distress as a variable in the future, in particular when studying the limbic system. This might even be possible for this study in a later stage. Participants filled out the Tinnitus Handicap Inventory (THI) (Newman, Jacobson, & Spitzer, 1996), the Tinnitus Questionnaire (TQ) (Goebel & Hiller, 1994; Meeus, Blaivie, & Van de Heyning, 2007), and the Tinnitus Reaction Questionnaire (TRQ) (Wilson, Henry, Bowen, & Haralambous, 1991). The scores can be analysed and related to the findings of this study. Also, in addition to an anatomical scan, an fMRI-scan was made during the scanning session. During this scan, participants were asked to indicate the valence of several images from the International Affective Picture System (IAPS) (Lang, Bradley, & Cuthbert, 1999) as either positive, negative, or neutral. Although this is a coarse measurement of general emotional state, the idea that tinnitus and negative emotions are connected could be further strengthened by investigating the outcome of this task. For example, tinnitus patients could rate images of neutral objects as negative a bit more often.

This study assumes that functional changes are accompanied by structural changes, although other studies have found otherwise (Vanneste et al., 2015). A combination of structural and functional imaging of tinnitus-patients may clarify this matter. Another option is to investigate brain networks related to tinnitus to investigate functional and structural changes together (De Ridder et al., 2014; Eggermont, 2012; Leaver et al., 2011). This makes it possible to literally connect structural and functional findings in the frontal, temporal, and limbic areas, while also detangling the relationship between tinnitus and hearing-loss in the brain.

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Appendix I

List of all Regions of Interest that were considered. Names were defined by WFU_Pickatlas IBASPM 116 in SPM. Hemispheres were analysed separately, expect the corpus callosum. BA = Brodmann area, L = left, R = right

Name	Hemisphere
Anterior Cingulate	L & R
BA 8 + 9 +11	L & R
BA 10 + 11 + 12 + 14 + 25 + 32	L & R
BA 18	L
BA 35	L
Ba 35 + 36	L & R
BA 36	L
BA 41	L & R
BA 41 + 42	L & R
Cerebellum (crus)	L & R
Corpus callosum	-
Heschl's gyrus	L & R
Hippocampus	L & R
Inferior temporal cortex	L
Insula	R
Lingual cortex	L & R
Middle temporal cortex	L & R
Middle cingulum cortex	L
Middle frontal cortex	L
Middle orbitofrontal cortex	R
Parahippocampal cortex	L
Rectus cortex	R
Superior temporal cortex	L
Superior frontal cortex	L & R

Superior temporal pole	L & R
Supramarginal gyrus	L & R
Thalamus	L & R

Afterword

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