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ORIGINAL PAPER

The Importance of Aging in Gray Matter Changes Within Tinnitus Patients Shown in Cortical Thickness, Surface Area and Volume

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Abstract Aging and sensorineural hearing loss are known to be involved in the development of chronic tinnitus. This study explores the structural changes of gray matter using surface base methods and focuses more specifically on changes in cortical thickness in 127 tinnitus patients. The linear relationships between cortical thickness and behavioral measures including aging, tinnitus loudness, tinnitus duration, tinnitus distress, and hearing loss were analyzed. Three dimensional T1-weighted MR images were acquired and cortical gray matter volumes were segmented using FreeSurfer on Talairach space. The results showed that cortical thickness and volume are negatively correlated to age in widespread regions of frontal cortices, and positively to bilateral entorhinal cortex and left rostral anterior cingulate cortex. The cortical thickness changes related to hearing loss overlap with those related to normal aging. The gray matter volumes of bilateral amygdalae, hippocampi, nuclei accumbens, and thalami are all significantly negatively correlated to age. Tinnitus-related distress level and subjective loudness were negatively correlated only to the thalamic volume. The results suggest that the primary factor of long-term structural changes in chronic tinnitus patients is age and age related hearing loss, rather than hearing loss per se. Tinnitus related factors such as subjective tinnitus loudness, tinnitus duration, and the level of chronic tinnitus related distress were not correlated to important morphometric changes in this study.

Keywords Tinnitus · Aging · Gray matter · Prefrontal cortex · Limbic region

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Introduction

Tinnitus is considered an auditory phantom perception, and can be represented as a noise, a tone, or a complex sound percept. It has been attributed to maladaptive neuroplastic changes resulting from hearing loss, which can be due to trauma, noise exposure, or aging (Eggermont and Roberts 2004; Kreuzer et al. 2014). The degree of hearing loss is positively correlated to the prevalence of tinnitus (Chung et al. 1984), and aging is a major factor in hearing loss (i.e. presbycusis) (Huang and Tang 2010).

Tinnitus as a clinical entity is attracting more attention (Elgoyhen et al. 2015; Langguth et al. 2013) because of its increasing prevalence both in the young and elderly population. In the young population this has linked to increased exposure of loud music (Gilles et al. 2012; Muhr and Rosenhall 2010) as well as war related noise trauma (Helfer 2011). In the elderly population the prevalence of tinnitus may be increasing because the prevalence of chronic tinnitus is correlated to age (Shargorodsky et al. 2010) and therefore can be attributed to the lengthened average life expectancy, but also to a raised interest in quality of life, an increased awareness of the symptom, as well as higher health expectations (Nondahl et al. 2012).

The pathogenesis of tinnitus has been related to both functional (De Ridder et al. 2011; Mirz et al. 2000a; Schlee et al. 2009; Smits et al. 2007; van der Loo et al. 2011; Vanneste et al. 2011, 2010) and structural brain changes

(Husain et al. 2011; Landgrebe et al. 2009; Leaver et al. 2011; Mahoney et al. 2011; Muhlau et al. 2006). Previous research has provided evidence that both auditory (Eggermont and Roberts 2004; Kaltenbach and Afman 2000; Salvi et al. 2000) and non-auditory (De Ridder et al. 2011; Mirz et al. 2000a; Schlee et al. 2009; Smits et al. 2007; van der Loo et al. 2011; Vanneste et al. 2011, 2010) brain regions are associated with tinnitus. However, the obtained results are not straightforward since different studies show varying and different brain structures involved in tinnitus. Initially, structural gray matter changes were obtained in the auditory cortex, the thalamus, and the subcallosal frontal cortex in tinnitus patients (Muhlau et al. 2006). However, a follow up study performed by a different research group, analyzing a very similar study population, could not replicate these results (Landgrebe et al. 2009). Gray matter changes have also been shown in the right inferior colliculus and the left hippocampus for tinnitus patients relative to healthy controls (Boyen et al. 2012; Landgrebe et al. 2009). Interestingly, more recent studies suggest that gray matter changes in tinnitus patients are actually attributed to the tinnitus associated hearing loss rather than the tinnitus per se (Aldhafeeri et al. 2012; Husain et al. 2011; Vanneste et al. 2015). Furthermore, gray matter decreases are noted in individuals with hearing loss without tinnitus in the anterior cingulate and superior medial frontal gyri relative to patients with tinnitus and hearing loss (Husain et al. 2011). This was further explored and morphometric changes in the subcallosal brain regions were shown to negatively correlate with supra-clinical hearing frequencies (>8 kHz), but not with tinnitus (Melcher et al. 2012), therefore questioning the relationship between tinnitus and the structural changes in the subcallosal area previously attributed to tinnitus (Mühlau et al. 2006). In addition, a recent study detected structural differences in gray matter within auditory, hippocampal, thalamic, and cerebellar areas which were related to hearing loss, but not tinnitus, in tinnitus patients (Vanneste et al. 2015).

However, it is unclear whether these structural changes in tinnitus patients are associated with hearing loss per se, or whether the changes actually reflect over all aging, which in itself is also correlated to hearing loss, also known as presbycusis. Despite the interaction of aging and hearing loss (Huang and Tang 2010), aging generally induces changes in widespread brain areas that are not specifically related to age-related sensory deterioration (presbycusis, presbyopia) (Fjell et al. 2014; Salat et al. 2004). According to recent studies, the patients' age appears to be an important underlying factor in tinnitus (Brozoski et al. 2012; Lin et al. 2011; Pilgramm et al. 1999). It is known that cortical thinning in aging is constant and more invariable across individuals, with the largest changes seen in the frontal and temporal cortex, including (para)hippocampal regions, and in deeper structures such as the putamen, thalamus, and accumbens (Fjell et al. 2014).

Changes in cortical thickness and subcortical volume can be quantified with geometrical approaches applied to structural MR data. Volumetric brain reductions in healthy aging are likely only to a minor extent related to neuronal loss (Pakkenberg and Gundersen 1997), however the shrinkage is consistently going on in healthy aging brains, showing annual reductions in most brain areas (Fjell et al. 2009).

This study will explore the relationship between gray matter, hearing loss, and aging in chronic tinnitus patients. Gray matter integrity of the human brain will be measured using different geometric measures, applying surface-based analyses. Cortical thickness is based on parcellating the brain into a group of meshes (triangles) and their vertices, and measuring by averaging the shortest distances from white matter surface to gray matter surface and vice versa. This technique is known to be more robust than the widely used volumetry, as it is less sensitive to position errors and spatial variances (MacDonald et al. 2000) while also providing more precise measurements (Fischl and Dale 2000; Pereira et al. 2012). We have also measured the surface area by using the averaged size of all meshes that meet at each vertex on the white matter surface. The gray matter volume is calculated as the multiplication of the thickness and surface area (Dale et al. 1999; Fischl and Dale 2000; Fischl et al. 1999). Finally, the degree of curvature on the brain surface is measured from each tangent line on every vertex.

Previous research has already suggested that aging has a major impact on gray matter. In addition, it is known that hearing loss is associated with aging. However, it is less clear to what extent aging is contributing as a factor to structural changes in chronic tinnitus patients. We hypothesize that the gray matter integrity in tinnitus patients will depend on factors that are important in normal aging. Hence we try to disentangle the contribution of aging and hearing loss in tinnitus patients using different morphometric measures of gray matter.

Materials and Methods

Subjects

One-hundred and twenty-seven chronic tinnitus outpatients were recruited from the multidisciplinary Tinnitus Research Initiative (TRI) Clinic of the University Hospital of Antwerp, Belgium. Individuals with pulsatile tinnitus, Ménière's disease, otosclerosis, chronic headache, neurological disorders (e.g. brain tumors), and individuals being

treated for mental disorders were excluded in order to control for underlying confounding factors. In addition, patients with complex tinnitus (e.g. both a pure tone and narrow-band noise tinnitus) or with a broadband perception were not included in the study. According to the Declaration of Helsinki 2000, all subjects were informed about the purpose and the procedure of the study and gave a written consent. This study was approved by the local ethical committee of University of Antwerp Hospital. The written informed consent was collected from all participants. The demographic features and the clinical features of tinnitus and the average hearing loss level measured by the audiogram are shown in Table 1.

Audiological and Behavioral Assessments

Patients were asked in person to report the side they perceive their tinnitus (unilateral or bilateral) and the duration of tinnitus as well the characteristic tone of their tinnitus (pure tone-like tinnitus or noise-like tinnitus). The patients were given tests by trained audiologists to measure the extent of hearing loss using a pure tone audiometry using the British Society of Audiology procedures at 0.125, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz (Audiology 2008). The hearing threshold, in dB HL (hearing level), was measured separately in both ears. The level of hearing loss for each individual was calculated at each frequency as the numerical average of the hearing thresholds in each ear.

All the subjects were tested for the frequency and the loudness level, in dB SL (sensation level), of their perceived tinnitus tones using the audiometric tinnitus matching analysis. For the unilateral tinnitus patients, the analysis was only performed on the ear opposite to the one where tinnitus is perceived. For the bilateral tinnitus patients, the matching analysis was performed on the ear with the less severe tinnitus symptoms, according to the subject's self-report. The pitch matching test of the tinnitus tone was performed by presenting either a 1 kHz pure tone, for pure tone tinnitus patients, or 1/3 octave-band noise centered at 1 kHz, for the narrow-band noise tinnitus patients. Both stimuli were presented to the ear with less severe tinnitus symptoms at a loudness of 10 dB above the patient's hearing threshold in that ear. The frequency of the stimulus was adjusted until the patient indicated that the presented sound most closely matches the pitch of the perceived tinnitus tone. The loudness of tinnitus tone was also matched to each individual patient using the same method. The tinnitus loudness (dB SL) was computed as the difference between the absolute tinnitus loudness and the hearing threshold (dB HL) at that frequency (Meeus et al. 2011, 2009).

The subjective loudness level of tinnitus was assessed by using a numeric rating scale (NRS) for loudness (LOUD; 'How loud is your tinnitus?': 0 = no tinnitus and 10 = as loud as imaginable), and the consequential distress level associated with tinnitus was verified with the verified Dutch translation of the tinnitus questionnaire (TQ) validated by Meeus et al. (2007). A broad spectrum of tinnitus-related psychological complaints was measured using 52 items on the questionnaire; including emotional and cognitive distress, intrusiveness, auditory perceptual difficulties, sleep disturbances, and somatic complaints (Hiller and Goebel 1992; McCombe et al. 2001) in tinnitus patients. Each item was scored on a three-point scale, ranging from 'true' (2 points), to 'partly true' (1 point), to 'not true' (0 points). The total score (from 0 to 84) was computed according to standard criteria published in the previous studies (Hiller and Goebel 1992; Hiller et al. 1994; Meeus et al. 2007).

Table 1 Demographic and clinical characteristics of the subjects (mean ± SD)

Variable (total n = 127)	Min	Max	Mean ± SD
Age	18	81	50.09 ± 14.40
Onset age	18	79	44.46 ± 14.44
Gender (male %)	N/A	N/A	70.08 %
TQ score	2	75	36.35 ± 16.68
LOUD (0-10)	1	10	5.34 ± 2.29
Duration (years)	0.13	45	5.69 ± 7.10
Laterality of tinnitus (unilateral %)	N/A	N/A	74.02 %
Type of tinnitus (pure tone %)	N/A	N/A	55.12 %
Averaged hearing loss (dB SL)	2.78	79.44	28.19 ± 17.14

Image Acquisition

Images were acquired using a 3.0 Tesla Siemens Trio scanner. A high-resolution scan (MPRAGE) was performed for each subject. The scans used a repetition time (TR) of 2300 ms, an echo time (TE) of 2.94 ms, an inversion time (TI) of 900 ms, and a flip angle of 9°. 160 sagittal slices were taken, using a matrix size of 256 × 256 mm^2, at a 1 × 1 × 1 mm^3 resolution.

Surface-Based Image Analysis

The cortical surfaces from the T1 MR images were reconstructed using the automated pipeline of FreeSurfer 5.3.0 (http://www.surfter.nmr.mgh.harvard.edu/) (Dale et al. 1999; Fischl and Dale 2000; Fischl et al. 1999). This process included motion correction by linear transformation, skull stripping, intensity normalization, segmentation of the gray matter from the whole brain, and transformation of the individual space into Talairach space, developed by Montreal Neurological Institute (Collins et al. 1994). This

was done by applying transformation parameters from the individual volumes to the average volume composed of previously aligned large datasets (Dale et al. 1999). The reconstructed surface file containing cortical curvature information was spatially filtered using a Gaussian kernel of 10 mm full-width half-maximum (FWHM) in order to reduce the noise level and enhance the statistical power.

To measure the cortical thickness in native space, the white and gray matter surfaces were inversely transformed into the native space. As described above, each pair of white and gray matter surface vertices is corresponded as the inner white matter mass is first corrected for topologies (e.g. removing interior holes and impossible connections), spatially smoothed to white–gray matter surface, and expanded to the outer surface. The surface of the outer gray matter is corrected by calculating the Euler's number. Cortical thickness was measured on each mesh of vertices by calculating the distance between the point on one surface and the closest corresponding point on the opposite surface, and then averaging the two values measured from each side to the other (Fischl and Dale 2000). The gray matter surface area was calculated on the surface of white matter, as the average of the size of the meshes that meet at each vertex. The tessellated white surface is inflated to match the cortical boundary, minimizing any distortions regarding the size of each mesh, hence the area calculated on the white surface will represent the estimation of gray matter surface area (Fischl et al. 1999; Winkler et al. 2012). Finally, the gray matter volume is calculated as a multiplication of the thickness and surface area.

The curvature of the gray matter was calculated on each individual using mean and Gaussian curvature measures. The principal curvature values represent the maximum and minimum bending of the surface and were originally measured from each tangent vectors on vertices; the mean curvature (1/mm) is defined as the arithmetic mean, and the Gaussian curvature ($1/mm^2$) is by the multiplication of the principal curvature values (Abbena et al. 2006). Each curvature measure was statistically analyzed on the individual smoothed surfaces. Significant correlations of tinnitus-related factors and the curvature measures were shown on the flattened population-average, landmark-and surface-based atlas (Van Essen 2005) using Caret 5.64 (Van Essen et al. 2001) (http://www.nitrc.org/projects/caret).

Statistical Analysis

Statistical analysis was performed in each hemisphere separately on the smoothed curvature volume in the standard Talairach space, using the QDEC application in FreeSurfer. The general linear model was used to regress the values at each vertex on the smoothed curvature with the predictors of average hearing loss, LOUD, TQ, and the duration in the whole sample population. The estimated intracranial volume of each subject was also taken into account as a nuisance covariate in the GLM. Therefore, all linear relationships of each tinnitus-related factors to the gray matter measures were acquired after the correction for the intracranial volume. Each linear relationship was measured by applying separate GLMs with a single predictor for the different gray matter indices. In addition, the linear relationship of the gray matter integrity to the age with the average hearing loss as the nuisance covariate and vice versa were acquired, accounting for presbycusis (Gates and Mills 2005). The tinnitus-related predictors were tested for the degree of bivariate correlation to each other and any significant collinearity that influences the results (Table 2). We also applied general linear model to find the significant group effects between different types of tinnitus. The comparison included (i) pure tone versus narrow-band noise tinnitus; (ii) unilateral versus bilateral tinnitus; (iii) left versus right ear within the unilateral tinnitus subjects ($n = 94$).

The results were corrected for the multiple comparison using the false-discovery rate (FDR) method (significant at corrected $p < .05$) (Hagler et al. 2006). Regions with statistical significance were selected based on the results of post hoc correction and areas with significantly different thickness were represented with the tenth logarithm of p values and the color code that shows the direction of the correlation.

The volumes of selected limbic structures were calculated based on the spatially normalized images of each individual in Talairach space (Fischl et al. 2002). The regions of interest were the bilateral amygdalae, hippocampi, nucleus accumbens and thalami. The average volume of each of the structures was correlated to the predictors of age, average hearing loss, LOUD, TQ, and the duration of tinnitus using Pearson's bivariate correlation and hierarchical regression analysis (IBM SPSS Statistics 22.0).

Results

Different statistical models were tested to assess the linear relationship of the tinnitus-related factors and the gray matter integrity. The whole brain analysis of cortical thickness, surface area and volume revealed that there is a statistically significant negative correlation between age and the gray matter measures (FDR corrected $p < .05$, Fig. 1). The effect of the age was significant after being corrected for the level of average hearing loss (Fig. 2). In addition, the results showed that there is a statistically

Table 2 Bivariate correlation between tinnitus-related measures and the collinearity in the linear prediction of MR measures

	Tinnitus-related measures					Collinearity statistics	
	Age	DUR	TQ	LOUD	HL	Tolerance	VIF
Age						.59	1.70
DUR	.24 **					.90	1.11
TQ	.15	.12				.72	1.40
LOUD	.17	.16	.47 **			.75	1.34
HL	.63 **	.31 **	.38 **	.34 **		.49	2.06

DUR duration of tinnitus, *TQ* tinnitus questionnaire, *LOUD* subjective loudness level represented by numeric rating scale (NRS), *HL* average hearing loss, *VIF* variance inflation factor

** $p < .01$

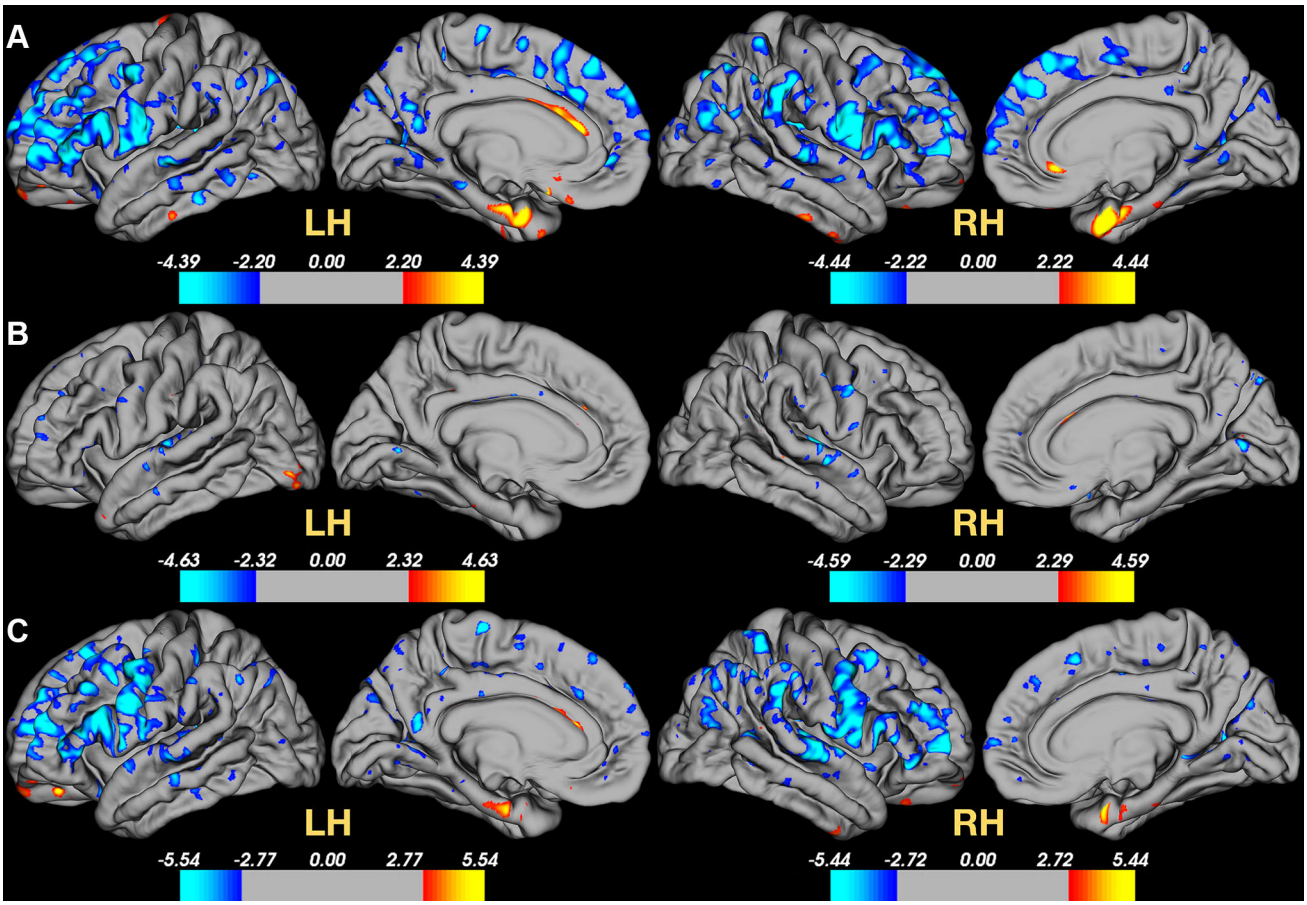

Fig. 1 The correlation of gray matter measures and the age corrected by the estimated intracranial volume on the whole brain (FDR corrected, $p < .05$). The *color bar* represents the tenth logarithm of *p* value, and the direction of the regression coefficients: *A* cortical thickness; *B* gray matter surface-based area; *C* gray matter volume

significant negative linear relationship of the degree of hearing loss with cortical thickness and volume (FDR corrected $p < .05$, Fig. 3), however the clusters of correlation were scattered smaller in size. This effect of the hearing loss was not statistically significant after correcting for both the age and intracranial volume. There were no significant correlations between gray matter measures and the subjective loudness of tinnitus tones, the duration of tinnitus, and the level of chronic stress induced by the tinnitus.

Gray Matter Analysis in Correlation to the Age

In the younger subjects, cortical thickness was greater in widespread regions of the prefrontal cortices, including the bilateral dorsolateral and dorsomedial prefrontal cortices,

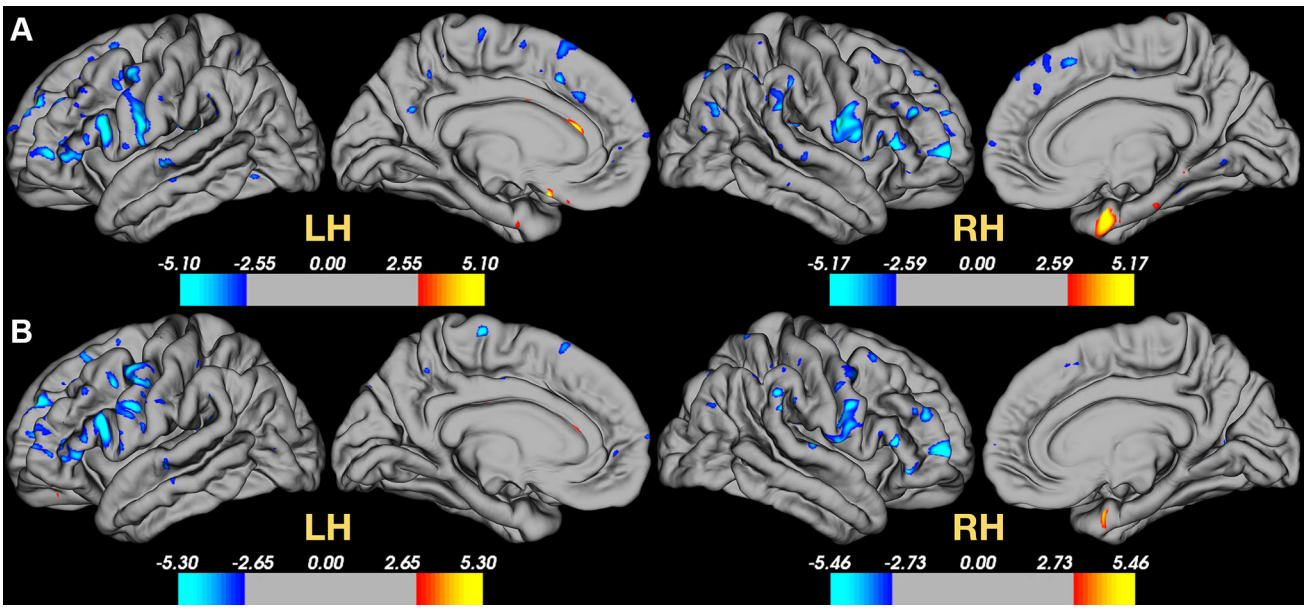

Fig. 2 The correlation of gray matter measures and the age corrected by the estimated intracranial volume and average hearing loss level on the whole brain: *A* cortical thickness; *B* gray matter volume. (FDR corrected, $p < .05$)

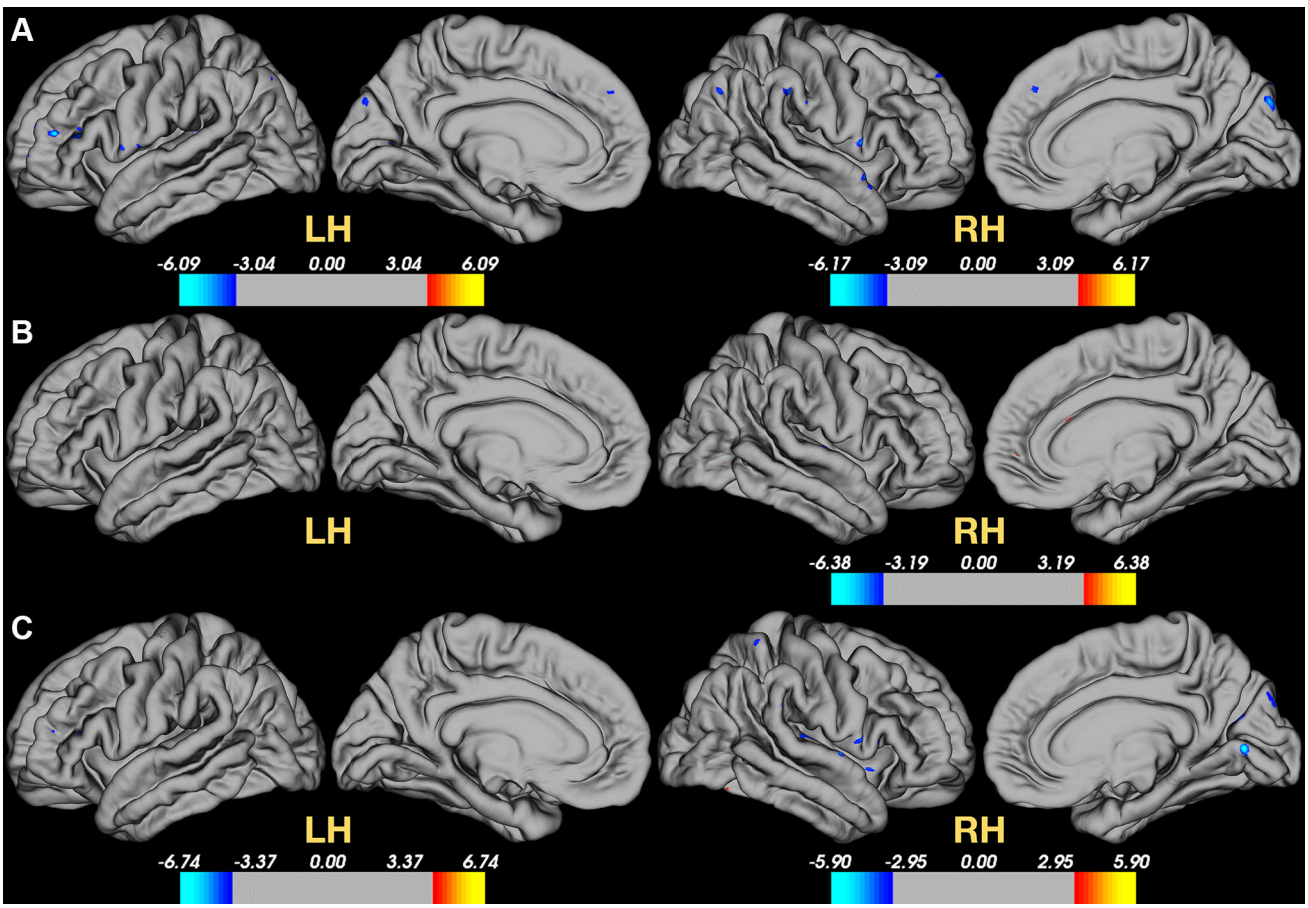

Fig. 3 The correlation of gray matter changes and the average hearing loss level corrected by the estimated intracranial volume on the whole brain: *A* cortical thickness; *B* gray matter surface-based area; *C* gray matter volume. (FDR corrected, $p < .05$) There were no significant clusters correlated to surface-based area in the left hemisphere

middle frontal cortices, temporal cortices, and the superior frontal cortices. The most extensive negative correlation of age to cortical thickness was found in the frontal region. However, part of the bilateral entorhinal cortices, medial orbitofrontal cortex, and the left rostral and bilateral subgenual anterior cingulate cortices were found to be positively correlated with age, as was part of the bilateral parahippocampal area (Fig. 1A). The significant region was smaller and limited for the gray matter surface area (Fig. 1B), while the same pattern was found in the correlation analysis of gray matter volume (Fig. 1C). There was a significant positive correlation of surface area to age in partial bilateral insular regions. The negative correlation in the frontal regions and some positive in the entorhinal cortex survived after being corrected by the influence of the average hearing loss level in cortical thickness and gray matter volume (Fig. 2A, B).

Gray Matter Analysis in Correlation to Hearing Loss

The correlation analysis of the average level of hearing loss revealed a significant negative correlation between hearing loss and cortical thickness in the scattered regions in the lateral and medial front, however the affected area was smaller than in correlation to age (Fig. 3A). In the surface area and grey matter volume, there were small clusters in right superior temporal cortex and right postcentral gyrus that showed positive correlation to the hearing loss (Fig. 3B, C). The correlation of the average level of hearing loss and cortical thickness when corrected for both estimated intracranial volume and age was not statistically significant.

Curvature Measure Analysis in Correlation to Age and Hearing Loss

One subject was excluded from the correlation due to a calculation failure, hence only 126 patients are analyzed. The mean curvature was negatively correlated to age in limited insular regions after being corrected by estimated intracranial volume (Fig. 4). The tendency was not statistically significant when corrected by average hearing loss level and in any other tinnitus-related measures.

Group Comparison of Gray Matter Measures in Different Tinnitus Phenotypes

The group effects for (i) pure tone versus narrow-band noise tinnitus and (ii) unilateral vs. bilateral tinnitus were not significant. When only unilateral tinnitus patients were counted (n = 94), the laterality of the tinnitus (left vs. right) did not show the significant difference (FDR corrected $p > .05$).

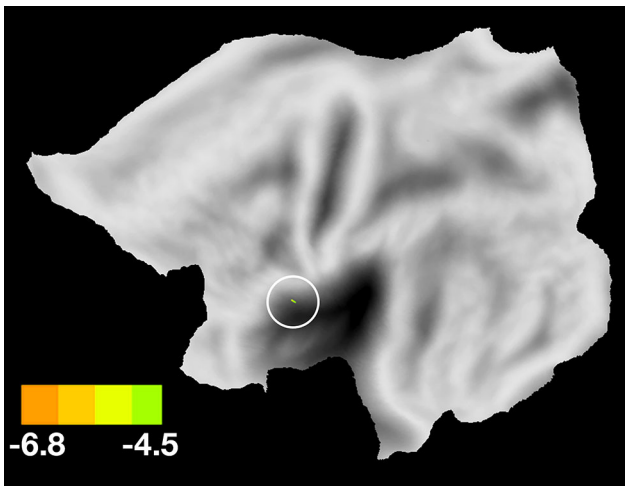

Fig. 4 The correlation of the age to the mean curvature values corrected by the estimated intracranial volume on the whole flattened brain; the highlighted region is insula. (FDR corrected, $p < .05$)

Region of Interest Correlation Analysis with Limbic Structures

The results of bivariate correlation analysis in the limbic structures' gray matter volumes and the factors related to the tinnitus pathologies are shown in Table 3. The influence of age is negatively correlated to the gray matter volume of bilateral amygdalae, hippocampi, nuclei accumbens, and thalami after being corrected by the effect of average hearing loss (uncorrected $p < .01$, Fig. 5). Hearing loss was significantly correlated to bilateral nucleus accumbens and thalamus, however not correlated to the volume of amygdala or the hippocampus. It is also noticeable that the volume of the left thalamus was significantly negatively correlated to age, average hearing loss, the subjective intensity of tinnitus tone, and the induced chronic stress. The duration and the age of the tinnitus patients are significantly correlated to each other (Table 2). However, the duration of tinnitus was not correlated to the gray matter volume of any regions of interest.

The hierarchical regression results analyzed the significance of the average hearing loss level in explaining the variance of the volumes of limbic regions over and above the main effect of age. The results showed that none of the volumes of limbic regions were explained by hearing loss significantly over the prediction by the age (Table 4).

Discussion

In this study, we analyzed the role of aging in chronic tinnitus patients for cortical thickness, surface-based areas, and the gray matter volume. The degree of mean curvature was also analyzed, however, the significant cluster was

Table 3 Pearson's bivariate correlations to limbic structures with tinnitus-related measures

Factors	Left				Right			
	Amyg	Hp	NAcc	Th	Amyg	Hp	NAcc	Th
Age	−.25**	−.28**	−.35**	−.59**	−.30**	−.31**	−.45**	−.59**
HL	−.04	−.14	−.26**	−.39**	−.15	−.15	−.31**	−.36**
LOUD	−.07	−.08	−.10	−.19*	−.02	−.04	−.11	−.18*
TQ	<.01	−.07	−.07	−.18*	.07	−.02	−.10	−.16
DUR	−.04	.05	−.10	−.10	.03	.02	−.15	−.08

Amyg Amygdala, *Hp* hippocampus, *NAcc* nucleus accumbens, *Th* thalamus, *HL* averaged hearing loss (dB SL), *LOUD* subjective loudness measured by numeric rating scale (NRS), *TQ* tinnitus questionnaire, *DUR* duration of tinnitus

** Uncorrected $p < .01$, * $p < .05$

small in size (number of vertices <50). Our data confirmed our hypothesis that aging seems to be the dominant factor explaining changes in widespread frontal regions including part of the temporal and entorhinal cortex. While the largest clusters of differences were centered on the frontal regions, the aging effect influenced the thicknesses of bilateral entorhinal cortices, subgenual anterior cingulate regions, left rostral anterior cingulate cortex, and right parahippocampal area. The effect of age was still significant in frontal regions when controlled for average hearing loss. However, the main effect of hearing loss was not statistically significant after controlling for age, suggesting age and not hearing loss is the predominant factor explaining the findings.

We have additionally performed a Sobel's test to verify the possible mediation effect of age on hearing loss in relation to the gray matter measures (Sobel 1987). Our data showed no significant mediation effect in cortical thickness, surface-based area, nor gray matter volume ($|Z| < .40$), indicating that the age directly influences both the level of hearing loss and the cortical thickness changes in tinnitus patients. This is in contrast to what we previously found for white matter, in which a significant mediation effect of hearing loss on white matter changes due to aging was demonstrated (Yoo et al. 2015). It is known that gray matter loss in time is linear, while white matter volume changes nonlinearly and its decrease is more accelerated in the elderly (Allen et al. 2005). We thus interpret from this data that the gray matter changes in chronic tinnitus due to aging is more consistent across time, although is also being influenced by the level of hearing loss (Fig. 1, 2).

In addition, the main effect of age was also visible in correlations to volumes of limbic structures including the amygdala, hippocampus, thalamus, and nucleus accumbens. The hierarchical regression analyses show that hearing loss cannot explain limbic structure volume changes significantly more over the aging effect. It is known that chronic tinnitus patients develop a connection between the auditory cortex and the amygdala (Crippa et al. 2010), and that the sensory and emotional perception of tinnitus are processed in the amygdala (De Ridder et al. 2006; Mirz et al. 2000b). However, further studies on white matter changes and functional connectivity are required to clarify the meaning of the volume changes in the limbic system.

Overall, the aging effect appears to be the most important predictor of gray matter structural changes in chronic tinnitus patients, not the average hearing loss level or the severity of tinnitus characteristics such as loudness, duration, or distress.

Current results showed a significant negative correlation of cortical thickness and gray matter volume to age in widespread frontal regions, and the tendency was the same after correcting for hearing loss. The pattern of correlation was more sporadic and the clusters were smaller for surface area. These correlations are in keeping with normal aging (Chao and Knight 1997; Fjell et al. 2014; Hutton et al. 2009; Pardo et al. 2007), suggesting that our results can indeed be explained by a general aging influence, and thus are not specific to the chronic tinnitus. Indeed, the morphometric changes seen in the dorsolateral or medial prefrontal cortices in tinnitus patients is shown to be negatively correlated to the age, as it is in the normal population (Fjell et al. 2014; Hutton et al. 2009).

There is a positive correlation of cortical thickness and gray matter volume in the left rostral anterior cingulate cortex, the bilateral entorhinal cortices, the subgenual anterior cingulate, and the parahippocampal area. It is known that rostral and subgenual anterior cingulate cortical thicknesses are considered to be more preserved than the other regions (Tisserand et al. 2002), and the bilateral entorhinal cortices notably suffer from prominent aging-related atrophies (Fjell et al. 2014). Our results show the unique tendency in chronic tinnitus patients: they seem to be less affected by the normal gray matter degeneration in the rostral anterior cingulate and entorhinal cortex and may even significantly preserve the gray matter as they become older. However, since the gray matter measures in the same

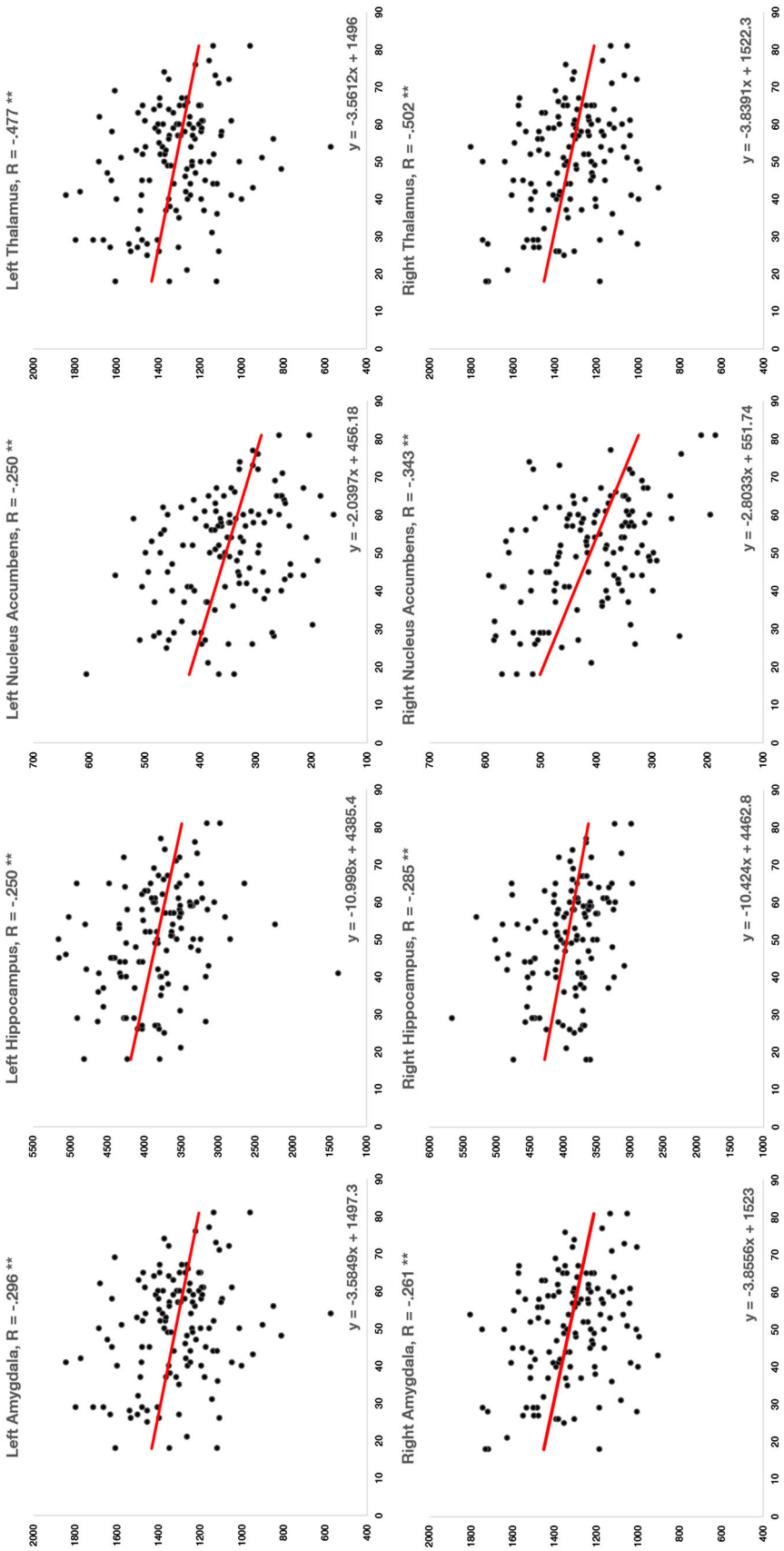

Fig. 5 The partial correlation of the age and the gray matter volumes of limbic structures corrected by average hearing loss; regions of interest are bilateral amygdalae, hippocampi, nuclei accumbens and thalami respectively (uncorrected $p < .01$)

Table 4 Hierarchical regression for limbic structures volume using average hearing loss level over and above age

Factors	ΔR^2	F (2, 124)	MSE	p Value
Left Amyg	.024	6.040	195.936	.070
Left Hp	.003	5.408	549.335	.561
Left NAcc	.002	8.746	80.041	.599
Left Th	.001	32.615	828.234	.754
Right Amyg	.002	6.133	179.223	.603
Right Hp	.004	7.014	458.884	.464
Right NAcc	.001	15.793	80.878	.671
Right Th	.000	33.204	816.984	.800

Amyg Amygdala, *Hp* hippocampus, *NAcc* nucleus accumbens, *Th* thalamus, *MSE* mean squared error

region were not correlated to the duration or the subjective loudness of tinnitus, the changes do not seem to be elevated as the tinnitus becomes chronic or more severe and are not likely to be compensatory in one way or another.

Many previous studies on structural changes in tinnitus patients reported inconsistent results (Boyen et al. 2013; Husain et al. 2011; Landgrebe et al. 2009; Mühlau et al. 2006; Schecklmann et al. 2013), possibly due to small sample sizes, the selection of different tinnitus subgroups, and other methodological issues. However, there appears to be some similarities in reporting that the level of hearing loss is an important factor that drives structural changes in tinnitus patients. Our results specify that the main effect of hearing loss is not as widespread when controlled for age, and the main effect of age is more important. Since our results included a group of patients with a wide range of hearing loss across multiple ages, it is unlikely that such a correlation with gray matter changes is biased depending on whether a tinnitus patient carries a pathological hearing loss or whether one is more affected by presbycusis. The results also suggest that most of the major gray matter measures including the degree of curvedness are significantly explained by age, not by hearing loss level or tinnitus characteristics.

The current study has analyzed changes in gray matter using various measures, primarily cortical thickness, gray matter area, volume, and the degree of mean curvature. Among the different methods in analyzing gray matter changes, volume-based analysis is previously found to be more influenced by changes in surface area than the cortical thickness (Winkler et al. 2010), for which we have controlled by performing a surface-based analysis. While there are possible variances caused by different normalization and segmentation methods, all of the measures we have analyzed were not correlated to tinnitus behavioral measures to any significant extent, and the aging effect was significant after correcting for the hearing loss.

We conclude that gray matter changes in chronic tinnitus patients are predominantly explained by age, more so than hearing loss, and not by tinnitus characteristics. Structural changes related to hearing loss overlap with regions influenced by age, and those changes were not statistically significant when they were controlled for the aging effect, suggesting the changes were related to aging and not hearing loss. Some of the aging-related cortical thickness and gray matter volume increases were exclusive to tinnitus patients. These increases could possibly be related to the functional compensation of hearing loss. The aging effect in chronic tinnitus appears to affect non-auditory regions and consequently might be more related to higher-level modulation rather than being related to peripheral auditory dysfunction, i.e. presbycusis.

References

Abbena E, Salamon S, Gray A (2006) Modern differential geometry of curves and surfaces with Mathematica. CRC Press, Boca Raton

Aldhafeeri FM, Mackenzie I, Kay T, Alghamdi J, Sluming V (2012) Neuroanatomical correlates of tinnitus revealed by cortical thickness analysis and diffusion tensor imaging. Neuroradiology 54:883–892. doi:10.1007/s00234-012-1044-6

Allen JS, Bruss J, Brown CK, Damasio H (2005) Normal neuroanatomical variation due to age: the major lobes and a parcellation of the temporal region. Neurobiol Aging 26:1245–1260

Audiology BSO (2008) Recommended procedure: pure tone air and bone conduction threshold audiometry with and without masking and determination of uncomfortable loudness levels

Boyen K, Langers DR, de Kleine E, van Dijk P (2012) Gray matter in the brain: differences associated with tinnitus and hearing loss. Hear Res. doi:10.1016/j.heares.2012.02.010

Boyen K, Langers DR, de Kleine E, van Dijk P (2013) Gray matter in the brain: differences associated with tinnitus and hearing loss. Hear Res 295:67–78

Brozoski T, Odintsov B, Bauer C (2012) Gamma-aminobutyric acid and glutamic acid levels in the auditory pathway of rats with chronic tinnitus: a direct determination using high resolution point-resolved proton magnetic resonance spectroscopy (H-MRS). Front Syst Neurosci 6:9. doi:10.3389/fnsys.2012.00009

Chao L, Knight R (1997) Prefrontal deficits in attention and inhibitory control with aging. Cereb Cortex 7:63–69

Chung DY, Gannon RP, Mason K (1984) Factors affecting the prevalence of tinnitus. Audiology 23:441–452

Collins DL, Neelin P, Peters TM, Evans AC (1994) Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 18:192–205

Crippa A, Lanting CP, van Dijk P, Roerdink JB (2010) A diffusion tensor imaging study on the auditory system and tinnitus. Open Neuroimaging J 4:16–25. doi:10.2174/1874440001004010016

Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis: I. Segmentation and surface reconstruction. Neuroimage 9:179–194

De Ridder D, Fransen H, Francois O, Sunaert S, Kovacs S, Van De Heyning P (2006) Amygdalohippocampal involvement in tinnitus and auditory memory. Acta Otolaryngol 126:50–53

De Ridder D, Vanneste S, Congedo M (2011) The distressed brain: a group blind source separation analysis on tinnitus. PLoS One 6:e24273. doi:10.1371/journal.pone.0024273

Eggermont JJ, Roberts LE (2004) The neuroscience of tinnitus. Trends Neurosci 27:676–682

Elgoyhen AB, Langguth B, De Ridder D, Vanneste S (2015) Tinnitus: perspectives from human neuroimaging. Nat Rev Neurosci 16:632–642. doi:10.1038/nrn4003

Fischl B, Dale AM (2000) Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci 97:11050–11055

Fischl B, Sereno MI, Dale AM (1999) Cortical surface-based analysis: II: inflation, flattening, and a surface-based coordinate system. Neuroimage 9:195–207

Fischl B et al (2002) Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355

Fjell AM et al (2009) One-year brain atrophy evident in healthy aging. J Neurosci 29:15223–15231

Fjell AM, McEvoy L, Holland D, Dale AM, Walhovd KB, AsDN Initiative (2014) What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. Prog Neurobiol 117:20–40

Gates GA, Mills JH (2005) Presbycusis. The Lancet 366:1111–1120

Gilles A, De Ridder D, Van Hal G, Wouters K, Kleine Punte A, Van de Heyning P (2012) Prevalence of leisure noise-induced tinnitus and the attitude toward noise in university students. Otol Neurotol 33:899–906. doi:10.1097/MAO.0b013e31825d640a

Hagler DJ, Saygin AP, Sereno MI (2006) Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. Neuroimage 33:1093–1103

Helfer TM (2011) Noise-induced hearing injuries, active component, U.S. Armed Forces, 2007–2010. MSMR 18:7–10

Hiller W, Goebel G (1992) A psychometric study of complaints in chronic tinnitus. J Psychosom Res 36:337–348

Hiller W, Goebel G, Rief W (1994) Reliability of self-rated tinnitus distress and association with psychological symptom patterns. Br J Clin Psychol 33(Pt 2):231–239

Huang Q, Tang J (2010) Age-related hearing loss or presbycusis. Eur Arch Otorhinolaryngol 267:1179–1191

Husain FT, Medina RE, Davis CW, Szymko-Bennett Y, Simonyan K, Pajor NM, Horwitz B (2011) Neuroanatomical changes due to hearing loss and chronic tinnitus: a combined VBM and DTI study. Brain Res 1369:74–88. doi:10.1016/j.brainres.2010.10.095

Hutton C, Draganski B, Ashburner J, Weiskopf N (2009) A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. Neuroimage 48:371–380

Kaltenbach JA, Afman CE (2000) Hyperactivity in the dorsal cochlear nucleus after intense sound exposure and its resemblance to tone-evoked activity: a physiological model for tinnitus. Hear Res 140:165–172

Kreuzer PM, Landgrebe M, Vielsmeier V, Kleinjung T, De Ridder D, Langguth B (2014) Trauma-associated tinnitus. J Head Trauma Rehabil 29:432–442. doi:10.1097/HTR.0b013e31829d3129

Landgrebe M et al (2009) Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas. Neuroimage 46:213–218. doi:10.1016/j.neuroimage.2009.01.069

Langguth B, Kreuzer PM, Kleinjung T, De Ridder D (2013) Tinnitus: causes and clinical management. Lancet Neurol 12:920–930. doi:10.1016/S1474-4422(13)70160-1

Leaver AM, Renier L, Chevillet MA, Morgan S, Kim HJ, Rauschecker JP (2011) Dysregulation of limbic and auditory networks in tinnitus. Neuron 69:33–43. doi:10.1016/j.neuron.2010.12.002

Lin FR, Ferrucci L, Metter EJ, An Y, Zonderman AB, Resnick SM (2011) Hearing loss and cognition in the Baltimore Longitudinal Study of Aging. Neuropsychology 25:763

MacDonald D, Kabani N, Avis D, Evans AC (2000) Automated 3-D extraction of inner and outer surfaces of cerebral cortex from MRI. NeuroImage 12:340–356

Mahoney CJ, Rohrer JD, Goll JC, Fox NC, Rossor MN, Warren JD (2011) Structural neuroanatomy of tinnitus and hyperacusis in semantic dementia. J Neurol Neurosurg Psychiatry 82:1274–1278. doi:10.1136/jnnp.2010.235473

McCombe A, Baguley D, Coles R, McKenna L, McKinney C, Windle-Taylor P (2001) Guidelines for the grading of tinnitus severity: the results of a working group commissioned by the British Association of Otolaryngologists, Head and Neck Surgeons, 1999. Clin Otolaryngol Allied Sci 26:388–393

Meeus O, Blaivie C, Van de Heyning P (2007) Validation of the Dutch and the French version of the Tinnitus Questionnaire. B-Ent 3(Suppl 7):11–17

Meeus O, Heyndrickx K, Lambrechts P, De Ridder D, Van de Heyning P (2009) Phase-shift treatment for tinnitus of cochlear origin. Eur Arch Otorhinolaryngol. doi:10.1007/s00405-009-1145-y

Meeus O, De Ridder D, Van de Heyning P (2011) Administration of the combination clonazepam-Deanxit as treatment for tinnitus. Otol Neurotol. doi:10.1097/MAO.0b013e31820e737c

Melcher JR, Knudson IM, Levine RA (2012) Subcallosal brain structure: correlation with hearing threshold at supra-clinical frequencies (>8 kHz), but not with tinnitus. Hear Res 295:79–86. doi:10.1016/j.heares.2012.03.013

Mirz F, Gjedde A, Ishizu K, Pedersen CB (2000a) Cortical networks subserving the perception of tinnitus–a PET study. Acta Otolaryngol Suppl 543:241–243

Mirz F, Gjedde A, Sdkilde-Jrgensen H, Pedersen CB (2000b) Functional brain imaging of tinnitus-like perception induced by aversive auditory stimuli. NeuroReport 11:633–637

Mühlau M et al (2006) Structural brain changes in tinnitus. Cereb Cortex 16:1283–1288

Muhr P, Rosenhall U (2010) Self-assessed auditory symptoms, noise exposure, and measured auditory function among healthy young Swedish men. Int J Audiol 49:317–325. doi:10.3109/14992020903431280

Nondahl DM, Cruickshanks KJ, Huang G-H, Klein BE, Klein R, Tweed TS, Zhan W (2012) Generational differences in the reporting of tinnitus. Ear Hear 33:640

Pakkenberg B, Gundersen HJG (1997) Neocortical neuron number in humans: effect of sex and age. J Comp Neurol 384:312–320

Pardo JV et al (2007) Where the brain grows old: decline in anterior cingulate and medial prefrontal function with normal aging. Neuroimage 35:1231–1237

Pereira JB, Ibarretxe-Bilbao N, Marti MJ, Compta Y, Junqué C, Bargallo N, Tolosa E (2012) Assessment of cortical degeneration in patients with Parkinson's disease by voxel-based morphometry, cortical folding, and cortical thickness. Hum Brain Mapp 33:2521–2534

Pilgramm M, Rychlick R, Lebisch H, Siedentop H, Goebel G, Kirchhoff D (1999) Tinnitus in the Federal Republic of Germany: a representative epidemiological study. In: Proceedings of the sixth international tinnitus seminar, 1999. The Tinnitus and Hyperacusis Society Cambridge, United Kingdom, pp 64–67

Salat DH et al (2004) Thinning of the cerebral cortex in aging. Cereb Cortex 14:721–730

Salvi RJ, Wang J, Ding D (2000) Auditory plasticity and hyperactivity following cochlear damage. Hear Res 147:261–274

Schecklmann M et al (2013) Auditory cortex is implicated in tinnitus distress: a voxel-based morphometry study. Brain Struct Funct 218:1061–1070. doi:10.1007/s00429-013-0520-z

Schlee W, Mueller N, Hartmann T, Keil J, Lorenz I, Weisz N (2009) Mapping cortical hubs in tinnitus. BMC Biol 7:80. doi:10.1186/1741-7007-7-80

Shargorodsky J, Curhan GC, Farwell WR (2010) Prevalence and characteristics of tinnitus among US adults. Am J Med 123:711–718. doi:10.1016/j.amjmed.2010.02.015

Smits M, Kovacs S, de Ridder D, Peeters RR, van Hecke P, Sunaert S (2007) Lateralization of functional magnetic resonance imaging (fMRI) activation in the auditory pathway of patients with lateralized tinnitus. Neuroradiology 49:669–679. doi:10.1007/s00234-007-0231-3

Sobel ME (1987) Direct and indirect effects in linear structural equation models. Sociol Methods Res 16:155–176

Tisserand DJ, Pruessner JC, Arigita EJS, van Boxtel MP, Evans AC, Jolles J, Uylings HB (2002) Regional frontal cortical volumes decrease differentially in aging: an MRI study to compare volumetric approaches and voxel-based morphometry. Neuroimage 17:657–669

van der Loo E, Congedo M, Vanneste S, De Heyning PV, De Ridder D (2011) Insular lateralization in tinnitus distress. Auton Neurosci 165:191–194. doi:10.1016/j.autneu.2011.06.007

Van Essen DC (2005) A population-average, landmark-and surface-based (PALS) atlas of human cerebral cortex. Neuroimage 28:635–662

Van Essen DC, Drury HA, Dickson J, Harwell J, Hanlon D, Anderson CH (2001) An integrated software suite for surface-based analyses of cerebral cortex. J Am Med Inf Assoc 8:443–459

Vanneste S, Plazier M, der Loo E, de Heyning PV, Congedo M, De Ridder D (2010) The neural correlates of tinnitus-related distress. Neuroimage 52:470–480. doi:10.1016/j.neuroimage.2010.04.029

Vanneste S, de Heyning PV, Ridder DD (2011) Contralateral parahippocampal gamma-band activity determines noise-like tinnitus laterality: a region of interest analysis. Neuroscience 199:481–490

Vanneste S, Van De Heyning P, De Ridder D (2015) Tinnitus: a large VBM-EEG correlational study. PLoS One 10:e0115122. doi:10.1371/journal.pone.0115122

Winkler AM et al (2010) Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. NeuroImage 53:1135–1146. doi:10.1016/j.neuroimage.2009.12.028

Winkler AM et al (2012) Measuring and comparing brain cortical surface area and other areal quantities. Neuroimage 61:1428–1443

Yoo HB, De Ridder D, Vanneste S (2015) White matter changes in tinnitus: is it all age and hearing loss? Brain connectivity 6(1):84–93