



Gray Matter Volumes in Tinnitus and Hyperacusis



Punitkumar Makani



PROPOSITIONS

Accompanying the dissertation

GRAY MATTER VOLUMES IN TINNITUS AND HYPERACUSIS

- Tinnitus should be understood as a network-level disorder involving both auditory and non-auditory brain regions (Chapter 1).
- Inconsistent findings in tinnitus research largely arise from insufficient control of confounding factors, especially hearing status (Chapter 2).
- Tinnitus with hearing loss is associated with distinct structural brain differences in comparison with tinnitus with clinically normal hearing (Chapter 3).
- Tinnitus with hyperacusis is associated with distinct structural brain differences in comparison with tinnitus without hyperacusis (Chapter 4 & 5).
- The search for a single “tinnitus brain region” is overly simplistic and does not reflect the complicated nature of the condition (Chapter 6).
- Meaningful hearing occurs in the brain, not in the ear alone.
- Effective management of tinnitus requires integration of audiology and neuroscience fields.
- Future progress depends more on data sharing and methodological rigor than on new imaging technologies alone.
- Acoustic comfort is an overlooked form of accessibility in social settings.

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PhD thesis, University of Groningen

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Chapter 1

General introduction

Makani P. (2026)

1. GENERAL INTRODUCTION: TINNITUS AND NEUROIMAGING

Subjective tinnitus (here referred to as simply tinnitus), often described as the perception of ringing or buzzing in the ears or head in the absence of external auditory stimuli, is not just an auditory condition but also involves abnormal differences or changes in brain structure and function. Neuroimaging, one of the most exciting topics in neuroscience, plays a crucial role in understanding conditions like tinnitus. Neuroimaging is typically divided into structural and functional approaches, each serving distinct purposes in exploring this condition. Structural neuroimaging visualizes the brain's anatomical features, enabling the detection of any anatomical differences or changes associated with tinnitus. Functional neuroimaging goes a step further by capturing the brain's dynamic activities and its responses to various stimuli or tasks, offering insights into how brain function may be altered in tinnitus. Through the current and subsequent chapters, the aim was to demonstrate not only the practicality of structural neuroimaging, but also to highlight its inherent beauty in understanding conditions like tinnitus.

Tinnitus is thought to be associated with abnormal alterations in the auditory pathways that lead to characteristic structural and functional differences in the brain regions responsible for auditory processing. Therefore, earlier clinical understandings of tinnitus primarily focused on assessing these abnormalities within the auditory pathway [1–4]. However, more recent studies have provided growing evidence of tinnitus-related structural and functional differences extending beyond the auditory pathway [1–4]. This growing evidence has sparked great interest in understanding the interaction between auditory and non-auditory pathways in the pathophysiology of tinnitus, specifically regarding whether the differences observed in the auditory pathway are the primary cause or the secondary consequence of differences occurring beyond the auditory pathway.

Furthermore, tinnitus can vary greatly among individuals based on factors such as severity, duration, and loudness. It often coexists or overlaps with auditory conditions like hearing loss and hyperacusis, and/or psychological conditions such as anxiety, depression, and obsessive-compulsive disorder [5–9]. This observation further raises important questions about the role of structural and functional differences in the

brain concerning tinnitus and its related characteristics and comorbidities.

To date, numerous neuroimaging studies have explored structural differences in individuals with tinnitus. In particular, studies employing voxel-based morphometry (VBM) have revealed structural differences in gray matter within the auditory brain network and beyond, including the attentional, default mode, salience, emotional, memory, sensorimotor, and visual networks associated with tinnitus [1–3, 10, 11]. However, the structural mechanisms underlying tinnitus, particularly regarding its generation and perception, remain still unclear. Furthermore, despite these high-quality studies, the findings have often been inconsistent and sometimes contradictory.

Two major limitations contribute to the discrepant findings. The first major limitation is relatively small study sample size, which restricts the external validity of findings. The second major limitation is the insufficient matching or controlling for tinnitus-related comorbidities (such as hearing loss, hyperacusis, anxiety, and/or depression) and characteristics (such as tinnitus burden, tinnitus duration, and tinnitus loudness) in many previous neuroimaging studies. The influences of various tinnitus-related comorbidities and characteristics on differences in brain structure and function have already been observed [1–3, 10, 11]. Therefore, neuroimaging studies of tinnitus require relatively large study sample size that can be stratified according to clinically well-defined subgroups. Nevertheless, most existing neuroimaging studies of tinnitus have typically included around 20 participants per group, with rather broad inclusion criteria.

By acknowledging above two limitations, the main aim of this thesis was to yield more robust and clinically meaningful results on the tinnitus-related structural properties of the brain, ultimately advancing understanding of the structural mechanisms underlying tinnitus.

2. TINNITUS: TINNITUS-RELATED CHARACTERISTICS AND COMORBIDITIES

The central focus of this thesis revolves around tinnitus and its associated structural differences in the brain. It is thus important to introduce tinnitus and its associated characteristics and comorbidities. Tinnitus is characterized by the perception of sounds in the ears or head in the absence of any external auditory stimulus. Tinnitus is a common condition with a prevalence of about 5–40%, depending on the studied population and definition [5–9]. Tinnitus can have a significant impact on an individual's quality of life.

Tinnitus is often referred to as “ringing in the ears”, but it can also sound like a blowing, buzzing, clicking, falling tap water, hissing, humming, roaring, running engines, sizzling, water running, whistling, and so on. The characteristics of tinnitus can vary, including its frequency, duration, and intensity. Tinnitus can be intermittent or persistent and may occur in one ear (unilateral tinnitus) or both ears (bilateral tinnitus), or in the head. Tinnitus can be acute (occurring within the last 3 to 12 months) or chronic (persisting for over 12 months). Individuals with tinnitus may react differently to their tinnitus, this reaction to tinnitus (often referred as tinnitus severity) varies greatly among individuals. Validated tinnitus questionnaires are often used to assess the severity of tinnitus. Some well-known tinnitus severity questionnaires include the Tinnitus Functional Index (TFI) [12], Tinnitus Handicap Inventory (THI) [13], and Tinnitus Questionnaire (TQ) [14]. Each questionnaire has its own advantages and disadvantages, as capturing the multifaceted nature of tinnitus can be a challenge. Despite the challenge, there is still consensus that while the majority of individuals experience slight to mild tinnitus, a significant population (approximately 1–5%) struggle with severe tinnitus [7, 8]. Nonetheless, these questionnaires remain the most effective way to understand an individual's handicap associated with tinnitus.

Traditional theories assume that tinnitus is associated with age-related hearing loss. Approximately 90% of individuals with tinnitus have reported degree of hearing loss (hearing threshold $\geq 20 \pm 5$ decibel hearing level (dB HL)) [15, 16]. Interestingly, however, only around 30–40% of those with impaired hearing develop tinnitus [15, 16], indicating a complex relationship between two conditions. Furthermore, it is worth noting that not all individuals with tinnitus experience hearing loss. Ap-

proximately 8–10% of individuals with tinnitus actually have reported normal hearing (hearing threshold $\leq 20 \pm 5$ dB HL on conventional audiometry (or up to 8 kHz)) [17], which again highlights the multifaceted nature of tinnitus etiology.

Tinnitus, especially when persistent, can cause psychological impairments in individuals with tinnitus. These impairments can vary greatly among individuals. Such impairments include anxiety and depression. Validated anxiety and depression questionnaires are often used to assess the symptoms of anxiety and depression in individuals with tinnitus. Some well-known anxiety and depression questionnaires include the Hospital Anxiety and Depression Scale (HADS) [18], Beck Anxiety Inventory (BAI) [19], and Beck Depression Inventory (BDI-II) [20]. In the adult population, approximately 26% of individuals with tinnitus have reported problems with anxiety and depression [21], which highlights the significant psychological impairments often associated with tinnitus.

Another comorbidity of tinnitus is hypersensitivity to sound (referred as hyperacusis). Hyperacusis is characterized by an increased sensitivity to ordinary environmental sounds of mild to moderate intensity. Environmental sounds that are not uncomfortably loud for most people can be bothersome or even painful for individuals with hyperacusis and trigger avoidance behaviors. To assess the presence of hyperacusis in individuals with tinnitus, the validated Hyperacusis Questionnaire (HQ) [22, 23] is frequently used. Some other established measurements include Loudness Discomfort Levels (LDL) and psychoacoustic test [24]. In the adult population, hyperacusis is strongly associated with tinnitus and hearing loss. Approximately 90% of individuals with hyperacusis report tinnitus [25], up to 63% of individuals with tinnitus report hyperacusis [26], and up to 59% of individuals with hyperacusis report hearing loss [27]. This again highlights the multifaceted nature of tinnitus.

3. NEUROIMAGING: VOXEL-BASED MORPHOMETRY

Voxel-based morphometry (VBM) is a non-invasive structural neuroimaging technique that allows investigation of differences in gray matter (such as volume, and thickness) at a voxel-level, often comparing normal healthy brains with those suffering from pathology. Gray matter, a major component of the central nervous system, consists of neuronal cell bodies, neuropil (dendrites and unmyelinated axons), and glial cells (astrocytes and oligodendrocytes). Its primary function is to process sensory information or relay information between different regions of gray matter, such as guiding sensory stimuli to neurons and triggering appropriate responses.

VBM pre-processing steps include: 1) bias field correction: smooth out the intensity variations across brain image caused by scanner inhomogeneity or artifacts, 2) tissue segmentation: divide brain image into different tissue types, such as gray matter, white matter, and cerebrospinal fluid; 3) spatial registration/normalization: align segmented brain images to the standard template, 4) modulation: adapt to changes in brain volume due to spatial registration, 5) spatial smoothing: reduce noise and increases statistical power, and 6) quality assurance: detect outliers that might deviate significantly from the group mean after pre-processing. For a detailed description on VBM pre-processing steps, interested readers can consult to the current work by Gaser et al. [28].

In the context of tinnitus studies, VBM has already provided valuable cross-sectional insights into how structural gray matter differs between healthy individuals and those with tinnitus [1–4]. One of the key advantages of VBM is its capability to restrict the analysis to specific brain regions, allowing researchers to explore targeted hypotheses related to tinnitus. In addition, VBM can be further employed in longitudinal studies to track differences in gray matter over the course of tinnitus development [1–4], ultimately advancing understanding of the structural mechanisms underlying tinnitus.

4. THESIS OUTLINE

This thesis was an attempt to overcome the two major limitations outlined in the section 1 (General introduction: tinnitus and neuroimaging).

- **Chapter 01** provides a general introduction to the research topics and methods used in this thesis, laying the foundation for the subsequent chapters. It also outlines the primary aims of the research presented in this thesis.
- **Chapter 02** explores the application of the combined image- and coordinate-based meta-analysis on previous studies investigating tinnitus using whole-brain voxel-based morphometry (VBM) analysis. VBM is a non-invasive structural neuroimaging technique that allows investigation of differences in brain anatomy, particularly in gray matter. The primary aim of this study was to identify robust gray matter differences associated with tinnitus, while also exploring the potential influence of hearing loss on the results of the meta-analysis.
- **Chapter 03** presents a retrospective case-control VBM study that compared whole-brain gray matter morphology across four relatively large participant groups. This study re-analyzed data collected as part of four previous studies involving participants with 1) normal hearing with tinnitus, 2) normal hearing without tinnitus, 3) hearing loss with tinnitus, and 4) hearing loss without. Utilizing whole-brain VBM analysis, the primary aim of this study was to investigate the main effects of tinnitus and hearing loss, as well as their interaction effects on gray matter morphology across the four participant groups.
- **Chapter 04** presents the first retrospective case-control VBM study on tinnitus-related comorbidity, specifically focusing on hyperacusis. Hyperacusis is characterized by an increased sensitivity to ordinary environmental sounds of mild to moderate intensity. This study re-analyzed data collected as part of two previous studies involving participants with 1) hearing loss and tinnitus who scored above the threshold for hyperacusis based on the validated questionnaire, and 2) hearing loss and tinnitus who scored below the threshold for hyperacusis. Utilizing whole-brain VBM analysis, the primary aim of this study was to investigate gray matter differences associated with hyperacusis in participants with hearing loss and tinnitus.
- **Chapter 05** builds on the findings of chapter 04, which identified significant regional gray matter differences in participants with hearing loss and tinnitus affected by hyperacusis. Chapter 05 re-analyzed participant data from four previous studies, categorized into four groups: 1) normal hearing and tinnitus with hyperacusis, 2) normal hearing

and tinnitus without hyperacusis, 3) hearing loss and tinnitus with hyperacusis, and 4) hearing loss and tinnitus without hyperacusis. Utilizing volume-of-interest (VOI)-based VBM analysis, the primary aim was to investigate whether similar regional gray matter differences (identified in chapter 04) were present in participants with hyperacusis, regardless of their hearing levels.

- **Chapter 06** provides a general discussion of the results identified in this thesis within the broader context of our current understanding of the research topics. It also explores the possible clinical implications of these results and provides suggestions for future research directions.

PHD CANDIDATE CONTRIBUTION

In the chapters 2 to 5, The PhD candidate collected the data for analysis, designed and performed the data analysis, and wrote the manuscript under supervision.

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Chapter 2

A combined image- and coordinate-based meta-analysis of whole-brain voxel-based morphometry studies investigating subjective tinnitus

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ABSTRACT

Previous voxel-based morphometry (VBM) studies investigating tinnitus have reported structural differences in a variety of spatially distinct gray matter regions. However, the results have been highly inconsistent and sometimes contradictory. In the current study, we conducted a combined image- and coordinate-based meta-analysis of VBM studies investigating tinnitus to identify robust gray matter differences associated with tinnitus, as well as examine the possible effects of hearing loss on the outcome of the meta-analysis. The PubMed and Web of Science databases were searched for studies published up to August 2021. Additional manual searches were conducted for studies published up to December 2021. A whole-brain meta-analysis was performed using Seed-Based d Mapping with Permutation of Subject Images (SDM-PSI). Fifteen studies comprising 423 individuals with tinnitus and either normal hearing or hearing loss (mean age 50.94 years; 173 females) and 508 individuals without tinnitus and either normal hearing or hearing loss (mean age 51.59 years; 234 females) met the inclusion criteria. We found a small but significant reduction in gray matter in the left inferior temporal gyrus for groups of normal hearing individuals with tinnitus compared to groups of hearing-matched individuals without tinnitus. In sharp contrast, in groups with hearing loss, tinnitus was associated with increased gray matter levels in the bilateral lingual gyrus and the bilateral precuneus. Those results were dependent upon matching the hearing levels between the groups with or without tinnitus. The current investigation suggests that hearing loss is the driving force of changes in cortical gray matter across individuals with and without tinnitus. Future studies should carefully account for confounders, including hearing loss, hyperacusis, anxiety, and depression, to identify gray matter changes specifically related to tinnitus. Ultimately, the aggregation of standardized individual datasets with both anatomical and useful phenotypical information will permit a better understanding of tinnitus-related gray matter differences, the effects of potential comorbidities, and their interactions with tinnitus.

Keywords

Combined image- and coordinate-based meta-analysis

Hearing loss

Magnetic resonance imaging

Tinnitus

Voxel-based morphometry

1. INTRODUCTION

Subjective tinnitus (here referred to as simply tinnitus) is the perception of sounds in the ears or head in the absence of an external stimulus. It is a common condition with a prevalence of about 5 – 40%, depending on the studied population and definition [1–5]. Tinnitus commonly occurs with hearing loss, and the prevalence of tinnitus increases with age [6]. While the majority of the population reports slight to mild tinnitus, millions struggle with severe tinnitus, with a prevalence of about 1 – 5% [6, 7]. Severe tinnitus is associated with a significant impairment in quality of life and occurs in conjunction with anxiety, depression, distress, frustration, hyperacusis, mood swings, poor concentration, and sleep disturbances [1–7].

Despite its high prevalence and decades of clinical research, the pathophysiology of tinnitus is not well understood. Neuroimaging studies (both functional and anatomical) have revealed changes associated with tinnitus in both auditory and non-auditory pathways. To date, structural neuroimaging studies using voxel-based morphometry (VBM) to investigate tinnitus have reported structural abnormalities in a variety of spatially distinct gray matter regions, including the auditory network (inferior colliculus, medial geniculate body, and primary or secondary auditory cortices), the default mode network (parietal cortices and prefrontal cortices), the salience network (anterior cingulate cortex and anterior insula), the limbic network (accumbens nucleus, amygdala, hippocampus, and parahippocampus), the visual network (primary or secondary visual cortices), the sensorimotor network (supplementary motor area), and the cerebellum [8–11]. In addition, VBM studies investigating tinnitus have also explored the relationships between these gray matter regions and clinical conditions that occur in conjunction with tinnitus, e.g., anxiety, depression, hearing loss, hyperacusis, and tinnitus burden [12–14]. However, the results have been highly inconsistent and sometimes contradictory. The two main reasons for these discrepancies include the heterogeneity of the demographic factors (including the selection and characterization of groups, as well as the relatively small group sizes in general) and methodological factors (including the data analyses and statistical thresholds used) [10, 15].

A previous systematic review by Scott–Wittenborn et al. of VBM studies investigating tinnitus was unable to identify robust conclusions across studies [10]. This study reported that there were differences in the selection and characterization of groups with or without tinnitus across studies, making a direct comparison very difficult. In addition, the authors suggested that there is an increasing need to standardize the use of VBM to study gray matter differences associated with tinnitus.

Furthermore, a recent coordinate-based meta-analysis by Cheng et al. of VBM studies investigating tinnitus included eight VBM studies comparing gray matter differences between groups of individuals with tinnitus ($n = 242$) and groups of individuals without tinnitus ($n = 217$) using Anisotropic Effect Size Seed-Based d Mapping (AES-SDM) [15]. This meta-analysis reported significantly increased gray matter in the angular gyrus and the middle–superior temporal gyrus in the group with tinnitus compared to the group without tinnitus. In addition, the gray matter was significantly reduced in the caudate nucleus, the superior frontal gyrus, and the supplementary motor area in the group with tinnitus. This meta-analysis concluded that cortico-striatal circuits are involved in the pathophysiology of tinnitus. However, there were a few important limitations in this study. First, this meta-analysis combined both normal hearing as well as hearing loss groups with or without tinnitus, meaning it did not consider the possible effects of hearing loss. Second, this meta-analysis was thresholded at an uncorrected level of $p \leq 0.005$, which might be too liberal [16]. Lastly, this meta-analysis used only x , y , z -coordinates, although combining unthresholded statistical images with x , y , z -coordinates could have improved the sensitivity and specificity of the meta analysis [16].

To address these limitations, in the current study we conducted a combined image- and coordinate-based meta-analysis of whole-brain VBM studies investigating tinnitus. Using this approach, we aimed to identify robust gray matter differences associated with tinnitus, as well as examining the possible effects of hearing loss on the outcome of the meta-analysis.

2. MATERIALS AND METHODS

2.1. Search strategy

The PubMed and Web of Science databases were searched for studies published up to August 2021 with no restriction on the publication period. The following search terms were used: (1) “tinnitus”[All Fields] AND (“voxel-based morphometry”[All Fields] OR “voxel wise”[All Fields] OR “voxel-based”[All Fields]); and (2) “tinnitus”[All Fields] AND “gray matter”[All Fields] AND (“magnetic resonance imaging”[All Fields] OR “MRI”[All Fields]). To ensure that relevant papers were not missed, a second search was conducted in PubMed and Web of Science databases, replacing the term “gray matter” with “brain volume”: “tinnitus”[All Fields] AND “brain volume”[All Fields] AND (“magnetic resonance imaging”[All Fields] OR “MRI”[All Fields]). This search did not retrieve previously unidentified papers. Additional manual searches were conducted using BrainMap, Neurosynth, and NeuroVault websites for studies published up to December 2021. Finally, previous systematic reviews and meta-analyses were checked to verify that other English-language studies were not missed. The current meta-analysis followed PRISMA guidelines to identify studies [17, 18], as illustrated in the PRISMA flowchart in Figure 1.

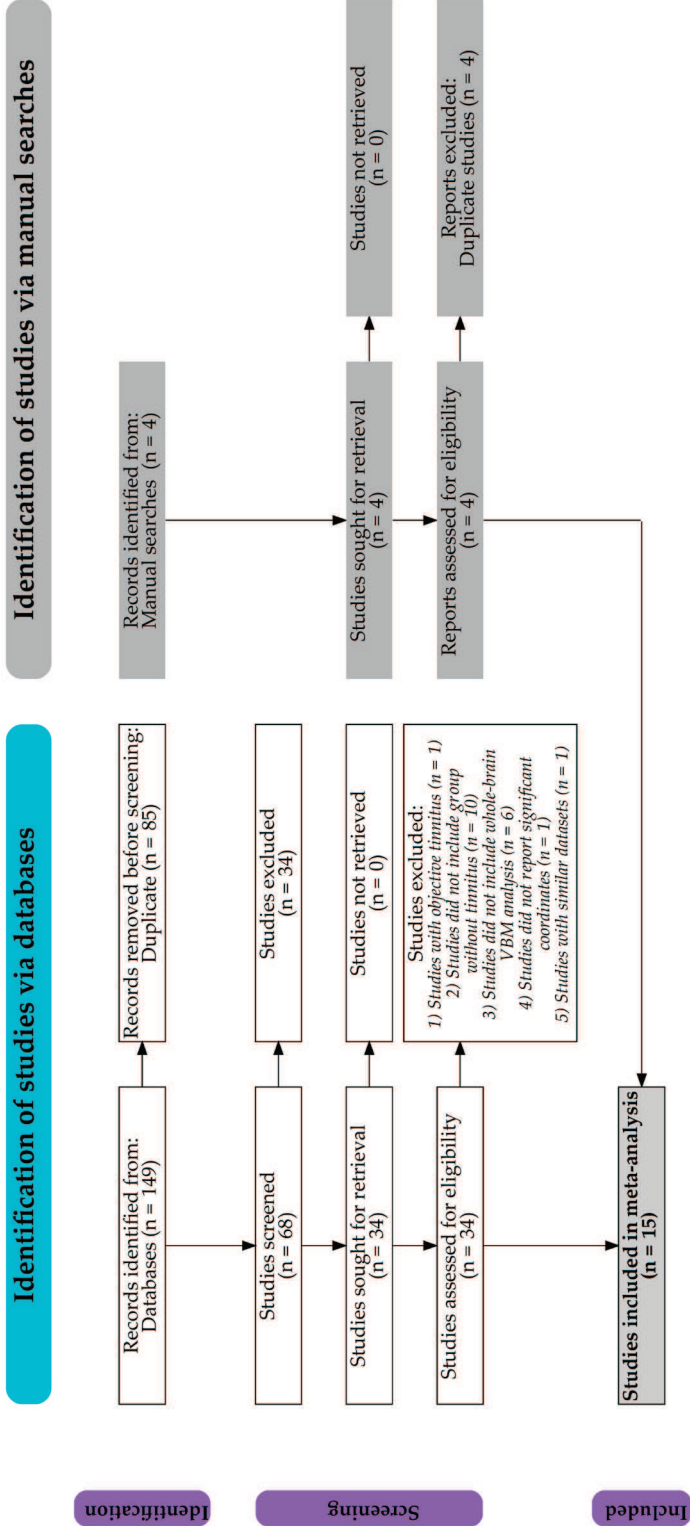


Figure 1. The PRISMA flowchart for studies included in the current meta-analysis.

2.2. Study selection

Studies were considered for inclusion if they (1) were published in a peer-reviewed journal in English; (2) investigated gray matter differences between a group with tinnitus (and either normal hearing or hearing loss) and a group without tinnitus (and either normal hearing or hearing loss); (3) involved a whole-brain VBM analysis; and (4) reported significant results as Montreal Neurological Institute (MNI) or Talairach (TAL) x, y, z -coordinates or indicated that significant results were not found. In addition, the corresponding authors were contacted via email by two investigators (P.M. and P.V.D.) to obtain unthresholded statistical images of the whole-brain VBM analysis. If two studies used similar datasets, then only the study with the larger dataset was included to avoid data duplication.

Studies were excluded if they (1) investigated gray matter differences associated with objective tinnitus, e.g., pulsatile tinnitus; (2) did not include a control group without tinnitus (and either normal hearing or hearing loss); (3) did not include a whole-brain VBM analysis or limited their analysis to specific region-of-interests; and (4) did not report significant results as MNI or TAL x, y, z -coordinates.

2.3. Data extraction

The following data from each study were extracted and included in an Excel spreadsheet: (1) the study information; (2) the demographic, audiometric, and questionnaire information; (3) the scanning information; (4) the methodological information; (5) the whole-brain VBM results (Supplementary Materials S1).

2.4. Quality assessment

The quality of the included studies was assessed using a 20-point checklist based on previous neuroimaging meta-analyses [19, 20], which were subsequently modified to reflect important variables for the current meta-analysis (Supplementary Materials S1). To improve the reliability and minimize errors or bias, the study selection, data extraction, and quality assessment were performed independently by two investigators (M.T. and P.M.). Disagreements were resolved by a third investigator (P.V.D.).

The current meta-analysis has been preregistered with the International Prospective Register of Systematic Reviews of the University of York (PROSPERO registration number = CRD42020208248; <https://www.crd.york.ac.uk/prospero/>, accessed on 10 June 2022).

2.5. Combined image- and coordinate-based meta-analysis

A combined image- and coordinate-based meta-analysis was performed using Seed-Based d Mapping with Permutation of Subject Images (SDM-PSI version 6.22; <https://www.sdmproject.com/>, accessed on 10 June 2022). Before preprocessing, input data were prepared for each study as recommended [21]. The detailed preprocessing steps for a combined image- and coordinate-based meta-analysis using SDM-PSI have been described previously [16, 21]. Firstly, images of the lower and upper bounds of unbiased possible effect sizes for each study were estimated within a gray matter mask by means of an anisotropic Gaussian kernel (full-width half-maximum (FWHM) = 20 mm; anisotropy = 1; voxel size = $2 \times 2 \times 2$ mm³). SDM-PSI uses multiple imputations (imputations = 50) of a Meta-Analysis of Studies with Non-Statistically Significant Unreported Effects (MetaNSUE) algorithms to estimate images of unbiased possible effect sizes for each study, and then adds the standard error [22]. Secondly, images of unbiased possible effect sizes from different imputations were combined using Rubin's rules to obtain combined images for each study [16]. The combined images were then meta-analyzed using a standard random effects model considering the sample size, inter-study variability, and between-study heterogeneity [16]. Thirdly, the results corrected for family-wise error (FWE) were calculated via the permutation of subject images (number of permutations = 500) [16]. Due to a software limitation, it was not possible to compute the FWE correction with more than 500 permutations (although applying 1000 permutations is more common in the field). Importantly, increasing the number of permutations would only narrow the null distribution, making the current finding even more significant. Thus, the findings reported here would remain unchanged.

Finally, to identify robust gray matter differences associated with tinnitus, as well as to examine the possible effects of hearing loss on the outcome of the meta-analysis, four whole-brain meta-analyses were conducted. The first meta-analysis included all studies retrieved by the online search. The second analysis excluded one study that did not match the groups for hearing level. The third and fourth analyses looked separately at gray matter changes associated with tinnitus in studies that included either individuals with normal audiograms or with hearing loss. This approach resulted in distinguishing four possible groups of individuals: individuals with (1) normal hearing with tinnitus (NH+T), (2) normal hearing without tinnitus (NH-T), (3) hearing loss with tinnitus (HL+T), and (4) hearing loss without tinnitus (HL-T). The results of the

whole-brain meta-analyses were corrected for multiple comparisons at the cluster level (FWE $p \leq 0.05$) using threshold-free cluster enhancement (TFCE) with an initial cluster size of $k \geq 10$ voxels.

2.6. Heterogeneity and publication bias

In cases of significant results, the effect sizes (Hedge's g) for each significant cluster were estimated for the included studies using SDM-PSI. The heterogeneity of the results was then calculated using the I^2 statistical test, in which $I^2 > 50\%$ is commonly agreed to indicate serious heterogeneity. In addition, Egger's test was used to assess the asymmetry of the funnel plot by examining the publication bias, where Egger's $p \leq 0.05$ indicates a significant publication bias. These analyses were performed using R version 4.1.3 (R Core Team, 2022; <https://www.R-project.org/>, accessed on 10 June 2022) with the metafor package (<https://www.metafor-project.org/>, accessed on 10 June 2022).

3. RESULTS

3.1. Studies included

Of the 153 identified studies (see Figure 1), a total of 15 studies met the inclusion criteria, resulting in the inclusion of a total of 423 individuals with tinnitus and either normal hearing or hearing loss (mean age 50.94 years; 173 females) and 508 individuals without tinnitus and either normal hearing or hearing loss (mean age 51.59 years; 234 females) [13, 23–36]. Unthresholded statistical images of whole-brain VBM analyses were obtained for 5 studies [23, 27, 31, 32, 36]. The demographic, audiometric, questionnaire, scanning, and methodological information as well as the whole-brain VBM results are shown in Table 1. More detailed information is provided in Supplementary Materials S1.

Table 1. Demographic, audiometric, questionnaire, scanning, methodologic information as well as whole-brain VBM results for studies included in the current meta-analysis.

Study	Groups	n (females)	Mean age (years)	Groups matched for			Mean tinnitus duration (months)	Mean tinnitus severity score	MRI scanner	Smoothing FWHM	Significant threshold	Whole-brain VBM results associated with tinnitus
				Age	Sex	HL						
Mühlau et al. [23]	NH+T	28 (15)	40	Yes	Yes	Yes	53 ± 52	TQ = 25	1.5T	8	FDR p < 0.05	↑GM: NS
	NH-T	28 (15)	39				-	-			voxel-level	↓GM: SCG
Landgrebe et al. [24]	NH+T	28 (13)	32.2	Yes	Yes	Yes	53.3	TQ = 32.9	1.5T	10	FDR p < 0.05	↑GM: NS
	NH-T	28 (13)	31.2				-	-			voxel-level	↓GM: NS
Husain et al. [25]	HL+T	8 (0)	56.13	Yes	Yes	No	NA	THI = 17.25			Uncorrected	↑GM: NS
	NH-T	11 (0)	48.09				-	-				↓GM: NS
	HL+T	8 (0)	56.13	Yes	Yes	Yes	NA	THI = 17.25	3T	8	p < 0.001	↑GM: MFG, SFG
	HL-T	7 (0)	51.38									↓GM: NS
Leaver et al. [26]	HL+T	23 (11)	47.4	Yes	Yes	Yes	149.9	NA	3T	6	Uncorrected	↑GM: NS
	HL-T	21 (13)	49				-	-			p < 0.002	↓GM: dmPFC, SMG, vmPFC
Boyen et al. [27]	HL+T	31 (11)	56	No	NA	No	NA	THI = 29				↑GM: MTG, SMG, STG
	NH-T	24 (8)	58				-	-			FWE p < 0.05	↓GM: HTH, OG, SFG
	HL+T	31 (11)	56	No	NA	Yes	NA	THI = 29	3T	8	voxel-level	↑GM: NS
	HL-T	16 (3)	63				-	-				↓GM: NS
Melcher et al. [28]	NH+T	24 (12)	46.9	Yes	Yes	Yes	NA	TRQ = 26.7	3T	8	Uncorrected	↑GM: NS
	NH-T	24 (12)	45.8				-	-			p < 0.001	↓GM: NS

Table 1. Demographic, audiometric, questionnaire, scanning, methodologic information as well as whole-brain VBM results for studies included in the current meta-analysis. (*continued*)

Study	Groups	n (females)	Mean age (years)	Groups matched for			Mean tinnitus duration (months)	Mean tinnitus severity score	MRI scanner	Smoothing FWHM	Significant threshold	Whole-brain VBM results associated with tinnitus
				Age	Sex	HL						
Allan et al. [29]	NH+T/ HL+T	73 (30)	58.38	Yes	Yes	Yes	NA	NA				↑GM: NS
	NH-T / HL-T	55 (25)	56.91				-	-	1.5T, 3T	10	FWE p < 0.05	↓GM: NS
	HL-T	15 (9)	47.6									↑GM: NS
	NH+T	15 (9)	50.2				NA	NA				↓GM: NS
	NH-T			Yes	Yes	Yes	-	-				
Schmidt et al. [13]	HL+T	15 (5)	55.13	NA	NA	No	NA	THI = 9.33				↑GM: ACC, SFG
	NH-T	13 (6)	52.93				-	-				↓GM: NS
	HL+T	15 (5)	55.13				NA	THI = 9.33	3T	10	Uncorrected p < 0.001 cluster-level	↑GM: NS
	HL-T	13 (8)	57.61	NA	NA	Yes	-	-				↓GM: NS
	HL+T	10 (5)	52	Yes	Yes	No	NA	NA				↑GM: NS
Luan et al. [30]	NH-T	35 (18)	55.97				-	-				↓GM: NS
	HL+T	10 (5)	52				NA	NA	3T	8	FDR p < 0.05 voxel-level	↑GM: NS
	HL-T	25 (9)	55.48	Yes	Yes	Yes	-	-				↓GM: NS
							NA	TQ = 42.2				↑GM: NS
Besteher et al. [31]	HL+T	59 (27)	50.6	NA	NA	No	NA	-	3T	8	Uncorrected p < 0.001	↓GM: IPC, PHPC, PCUN
	NH-T	66 (35)	45				-	-				
Koops et al. [32]	HL+T	39 (6)	59.2	No	NA	No	147.5	THI = 35				↑GM: NS
	NH-T	39 (21)	45.7				-	-	3T	8	TFCE FWE	↓GM: NS
	HL+T	39 (6)	59.2	Yes	NA	Yes	147.5	THI = 35			p < 0.05	↑GM: LG
	HL-T	22 (9)	62.6				-	-				↓GM: NS

Table 1. Demographic, audiometric, questionnaire, scanning, methodologic information as well as whole-brain VBM results for studies included in the current meta-analysis. (*continued*)

Study	Groups	n (females)	Mean age (years)	Groups matched for			Mean tinnitus duration (months)	Mean tinnitus severity score	MRI scanner	Smoothing FWHM	Significant threshold	Whole-brain VBM results associated with tinnitus
				Age	Sex	HL						
Wei et al. [33]	NH+T	27 (15)	46.4	Yes	Yes	Yes	23.4	THI = 4.0	3T	6	FDR $p < 0.05$ cluster-level	↑GM: NS ↓GM: CN, TH
	NH-T	27 (15)	46.6				-	-				
Lee et al. [34]	HL+T	12 (6)	73.27	Yes	Yes	Yes	4.2	THI = 38.5	3T	8	Uncorrected $p < 0.005$	↑GM: NS ↓GM: INS
	HL-T	11 (4)	74.83				-	-				
Wei et al. [35]	NH+T	33 (10)	48.2	Yes	Yes	Yes	NA	THI = 52.5	3T	6	FDR $p < 0.05$ cluster-level	↑GM: NS ↓GM: CAU, CAL, CUN, PHPC, STG
	NH-T	26 (11)	47.3				-	-				
Chen et al. [36]	NH+T	13 (7)	42.23	Yes	Yes	Yes	NA	THI = 53.38	3T	8	FWE $p < 0.001$ cluster-level	↑GM: NS ↓GM: MTG
	NH-T	18 (9)	45.33				-	-				

HL+T hearing loss group with tinnitus; HL-T hearing loss group without tinnitus; NH+T normal hearing group with tinnitus; NH-T normal hearing group without tinnitus; THI tinnitus handicap inventory; TQ tinnitus questionnaire; TRQ tinnitus reaction questionnaire; ↑GM increased gray matter; ↓GM decreased gray matter; ACC anterior cingulate cortex; CAL calcarine gyrus; CAU caudate nucleus; CN cochlear nucleus; CUN cuneus; dmPFC dorsomedial prefrontal cortex; HTH hypothalamus; INS insula; IPC inferior parietal cortex; LG lingual gyrus; OG occipital gyrus; MFG medial frontal gyrus, MTG middle temporal gyrus; PCUN precuneus; PHPC parahippocampus; SCG subcallosal gyrus; SFG superior frontal gyrus; SMG supramarginal gyrus; STG superior temporal gyrus; TH thalamus; vmPFC ventromedial prefrontal cortex; NA not available; NS not significant.

3.2. SDM-PSI meta-analyses

3.2.1. *Effect of tinnitus (all studies included)*

A total of 15 studies [13, 23–36] compared groups of individuals with tinnitus (NH+T and HL+T: $n = 423$; mean age 50.94 years; 173 females) to groups of individuals without tinnitus (NH-T and HL-T: $n = 508$; mean age 51.59 years; 234 females) (Table 1). These 15 studies either matched or did not match their groups for hearing loss (Table 1). The whole-brain meta-analysis showed that there was no significantly increased gray matter in the tinnitus group compared to the controls. However, tinnitus in this analysis was associated with significantly reduced gray matter in the left anterior cingulate cortex (with a cluster extending to the medial prefrontal cortex), the left interior temporal gyrus, and the left middle temporal gyrus (Table 2A; Figure 2A).

3.2.2. *Effect of tinnitus when groups are matched for hearing levels (one study excluded)*

One of the 15 retrieved studies did not match groups in terms of hearing levels [31]. Furthermore, this study intentionally included individuals with significant anxiety or depression (in both the tinnitus and the control groups). Therefore, in a second meta-analysis, in which this study was excluded (NH+T and HL+T vs. NH-T and HL-T excluding study [31]), we investigated the effect of tinnitus when the groups were matched for hearing levels. The 14 studies [13, 23–30, 32–36] included in this analysis compared 364 individuals with tinnitus (NH+T and HL+T excluding study [31]: mean age 50.96 years; 146 females) and 320 individuals without tinnitus (NH-T and HL-T excluding study [31]: mean age 51.86 years; 146 females) (Table 1). The results of the whole-brain meta-analysis showed that when matched for hearing levels, the groups with tinnitus had significantly higher gray matter levels in the right cuneus, the bilateral lingual gyrus (with a cluster extending to the cerebellum), the right posterior cingulate cortex, and the left precuneus compared to the groups without tinnitus (Table 2B; Figure 2B). No regions with significantly lower gray matter in the groups with tinnitus were found in this analysis.

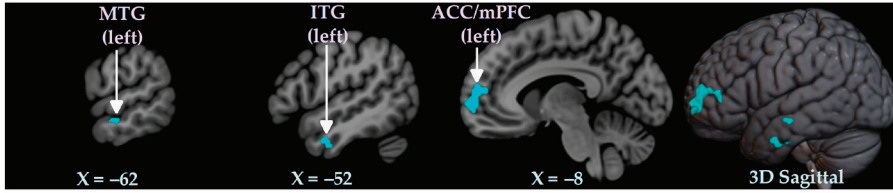
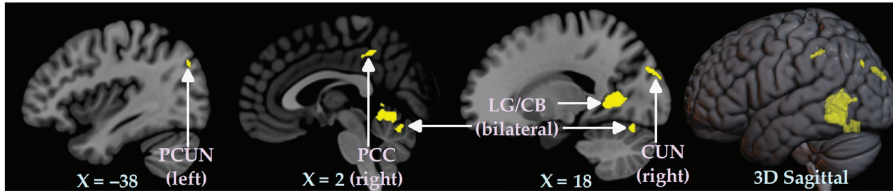
A. Effect of tinnitus –all studies included**B. Effect of tinnitus when groups are matched for hearing levels (one study excluded)**

Figure 2. Whole-brain meta-analyses of studies comparing individuals with and without tinnitus, (A) including all studies and (B) excluding one study [31] that did not match groups for hearing levels, using TFCE FWE-corrected statistics ($p \leq 0.05$, cluster size $k \geq 10$ voxels). (A) Lower gray matter levels in the left anterior cingulate cortex (with a cluster extending to the medial prefrontal cortex), the left inferior temporal gyrus, and the left middle temporal gyrus in the groups with tinnitus (NH+T and HL+T) compared to the groups without tinnitus (NH-T and HL-T), regardless of whether or not the groups were matched for hearing levels. (B) Higher gray matter levels in the right cuneus, the bilateral lingual gyrus (with a cluster extending to the cerebellum), the right posterior cingulate cortex, and the left precuneus in the groups with tinnitus (NH+T and HL+T excluding study [31]) compared to the groups without tinnitus (NH-T and HL-T excluding study [31]) and matched for hearing levels. Note: ACC, anterior cingulate cortex; CB, cerebellum; CUN, cuneus; ITG, inferior temporal gyrus; LG, lingual gyrus; mPFC, medial prefrontal cortex, MTG, middle temporal gyrus; PCC, posterior cingulate cortex; PCUN, precuneus.

Table 2. Whole-brain meta-analyses of studies comparing individuals with and without tinnitus (A) including all studies and (B) excluding one study that did not match groups for hearing levels.

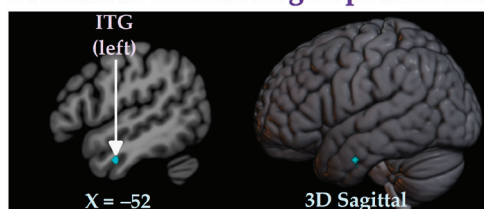
Gray matter regions	MNI X, Y, Z	SDM Z	FWE p TFCE	Voxels	Hedge's <i>g</i> cluster	<i>I</i> ² cluster	Egger's p cluster
A. Effect of tinnitus –all studies included							
NH+T & HL+T > NH-T & HL-T							
NS	-	-	-	-	-	-	-
NH+T & HL+T < NH-T & HL-T							
Left anterior cingu- late cortex/medial prefrontal cortex (ACC/mPFC)	-12, 44, 8 -8, 56, 10 -16, 66, -6 -8, 62, 0	-4.10 -4.01 -3.63 -3.60	0.028 0.018 0.036 0.026	224	-0.27	0 %	0.787
Left inferior tempo- ral gyrus (ITG)	-54, -10, -34 -54, -6, -34 -56, -16, -32	-3.80 -3.69 -3.56	0.036 0.036 0.046	45	-0.30	0 %	0.392
Left middle tempo- ral gyrus (MTG)	-62, -16, -14 -60, -12, -12	-4.06 -3.85	0.036 0.038	20	-0.30	0 %	0.614
B. Effect of tinnitus when groups are matched for hearing levels (one study excluded)							
NH+T & HL+T > NH-T & HL-T excluding study [31]							
Right cuneus (CUN)	16, -94, 24 18, -88, 30	4.16 3.90	0.016 0.028	67	0.38	0 %	0.738
Bilateral lingual gyrus/cerebellum (LG/CB)	20, -52, 2	4.39	0.002	948	0.33	0 %	0.789
	-4, -56, -8	4.26	0.006				
	12, -58, 0	3.96	0.004				
	16, -72, -18	3.19	0.016				
	4, -50, 0	3.15	0.012				
	2, -66, -20	3.13	0.020				
	-2, -64, -20	3.09	0.020				
	14, -72, -14	2.99	0.018				
	8, -68, -16	2.98	0.020				
Right posterior cin- gulate gyrus (PCC)	14, -72, 4	2.92	0.022	28	0.35	0 %	0.724
	0, -42, 46 0, -32, 42	4.24 3.80	0.036 0.038				
Left precuneus (PCUN)	-38, -78, 14	4.70	0.038	12	0.44	0 %	0.813
NH+T & HL+T < NH-T & HL-T excluding study [31]							
NS	-	-	-	-	-	-	-

HL+T hearing loss group with tinnitus; HL-T hearing loss group without tinnitus;
 NH+T normal hearing group with tinnitus; NH-T normal hearing group without
 tinnitus; NS not significant.

3.2.3. Effect of tinnitus in groups with normal hearing

Seven of the 15 studies [23, 24, 28, 29, 33, 35, 36] compared groups of normal hearing individuals with tinnitus (NH+T: $n = 168$; mean age 43.36 years; 81 females) and groups of normal hearing individuals without tinnitus (NH-T: $n = 166$; mean age 43.63 years; 84 females) (Table 1). The meta-analysis of these studies revealed a small area of decreased gray matter in the left inferior temporal gyrus in the group with tinnitus compared to the group without tinnitus (Table 3A; Figure 3A).

A. Effect of tinnitus in groups with normal hearing



B. Effect of tinnitus in groups with hearing loss

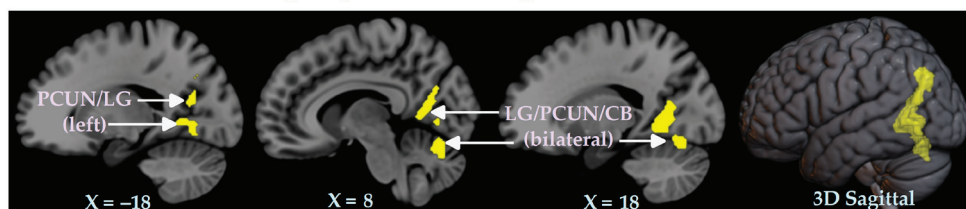


Figure 3. Whole-brain meta-analyses of studies comparing individuals with tinnitus and (A) normal hearing or (B) hearing loss, using TFCE FWE-corrected statistics ($p \leq 0.05$, cluster size $k \geq 10$ voxels). (A) Lower gray matter levels in the left inferior temporal gyrus in the groups with normal hearing and tinnitus (NH+T) compared to the groups with normal hearing but without tinnitus (NH-T). (B) Higher gray matter levels in the bilateral lingual gyrus (with a cluster extending to the precuneus and the cerebellum) and the left precuneus (with a cluster extending to the lingual gyrus) in the groups with hearing loss and tinnitus (HL+T) compared to the groups with hearing loss but without tinnitus (HL-T). Note: CB, cerebellum; ITG, inferior temporal gyrus; LG, lingual gyrus; PCUN, precuneus.

3.2.4. Effect of tinnitus in groups with hearing loss

Seven of the 15 studies [13, 25–27, 30, 32, 34] compared groups of hearing loss individuals with or without tinnitus (HL+T: $n = 138$; mean age 57.02 years; 44 females and HL-T: $n = 114$; mean age 59.14 years; 46 females) (Table 1). In all seven studies, the groups were well matched in terms of hearing levels. A whole-brain meta-analysis of these studies showed that the group with hearing loss and tinnitus had significantly increased gray matter levels in the bilateral lingual gyrus (with a cluster extend-

ing in the precuneus and the cerebellum) and the left precuneus (with a cluster extending in the lingual gyrus) (Table 3B; Figure 3B) compared to the group with hearing loss but without tinnitus.

Table 3. Whole-brain meta-analyses of studies comparing individuals with tinnitus and (A) normal hearing and (B) hearing loss.

Gray matter regions	MNI X, Y, Z	SDM Z	FWE p TFCE	Voxels	Hedge's g cluster	I ² cluster	Egger's P cluster
A. Effect of tinnitus in groups with normal hearing							
NH+T > NH-T							
NS	-	-	-	-	-	-	-
NH+T < NH-T							
Left inferior tempo- ral gyrus (ITG)	-52, -6, -30	-4.12	0.042	12	-0.59	0 %	0.517
B. Effect of tinnitus in groups with hearing loss							
HL+T > HL-T							
Right lingual gyrus/ precuneus/cerebel- lum (LG/PCUN/CB)	18, -56, 0	4.57	< 0.001	931	0.49	0 %	0.735
	18, -60, 10	3.56	0.008				
	4, -68, -20	3.44	0.012				
	8, -70, -18	3.38	0.012				
	18, -70, -16	3.26	0.012				
	8, -66, -24	3.00	0.016				
	28, -66, -10	2.90	0.042				

Table 3. Whole-brain meta-analyses of studies comparing individuals with tinnitus and (A) normal hearing and (B) hearing loss. (*continued*)

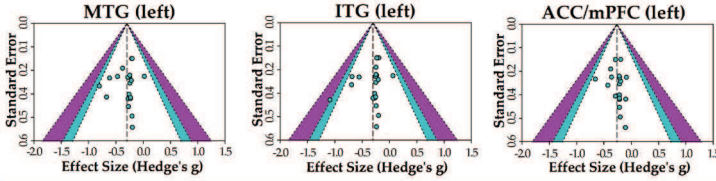
Gray matter regions	MNI X, Y, Z	SDM Z	FWE p TFCE	Voxels	Hedge's g cluster	I ² cluster	Egger's P cluster
Left precuneus/lin- gual gyrus (PCUN/ LG)	-14, -68, 38	3.66	0.012	466	0.46	0 %	0.768
	-14, -68, -2	3.21	0.018				
	-16, -66, -8	2.98	0.020				
	-18, -58, 0	2.94	0.020				
	-18, -66, 18	2.85	0.020				
	-12, -58, 8	2.61	0.030				
		HL+T < HL-T					
NS	-	-	-	-	-	-	-

HL+T hearing loss group with tinnitus; HL-T hearing loss group without tinnitus; NH+T normal hearing group with tinnitus; NH-T normal hearing group without tinnitus; NS not significant.

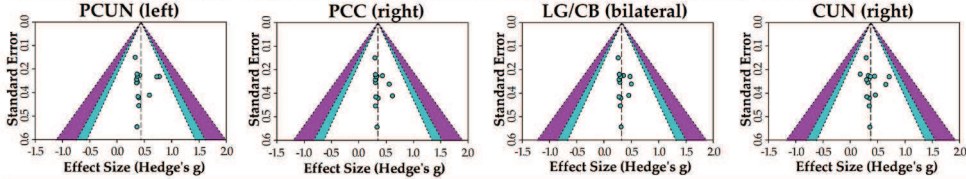
3.2.5. Heterogeneity and publication bias

The approximative effect sizes (Hedge's g), test of heterogeneity (I²) results, and Egger's probabilities of publication bias are provided for every significant group difference in Tables 2 and 3. The effect sizes were modest. The heterogeneity across studies was surprisingly low. Figure 4 shows the funnel plots for every significant finding. Egger's tests of funnel plot asymmetry were not significant for any of the findings across the four meta-analyses (all p > 0.05), suggesting no publication bias.

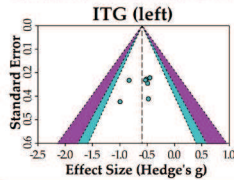
A. Effect of tinnitus –all studies included



B. Effect of tinnitus when groups are matched for hearing levels (one study excluded)



C. Effect of tinnitus in groups with normal hearing



D. Effect of tinnitus in groups with hearing loss

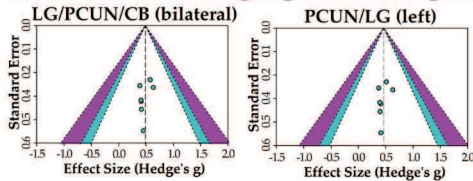


Figure 4. Heterogeneity and publication bias. Funnel plots for every significant finding obtained across the four meta-analyses. None of the funnel plots showed evidence of a reporting publication bias (Egger's tests: all $p > 0.05$).

4. DISCUSSION

The current study is the first combined image- and coordinate-based meta-analysis of VBM studies to examine gray matter differences associated with tinnitus and hearing loss. We found that matching for hearing levels affected the results of the meta-analyses. Depending on matching for hearing levels, we identified several areas with gray matter differences associated with tinnitus (including decreased gray matter in the left anterior cingulate and medial prefrontal cortex, decreased gray matter in the left inferior and middle temporal gyrus, and increased gray matter in the bilateral lingual gyrus and in the bilateral precuneus). These findings provide new insights into brain changes driven by tinnitus and hearing loss. Importantly, our findings support previous observations indicating that the major driver of gray matter changes in the brain is not tinnitus

but rather hearing loss and aging [32]. Our findings, moreover, motivate recommendations for future VBM studies of tinnitus.

4.1. Effects of matching hearing levels when identifying changes in gray matter associated with tinnitus

In this study, we conducted a series of four meta-analyses. The first analysis included all (15) retrieved studies and revealed three clusters of decreased gray matter in individuals with tinnitus compared to individuals without tinnitus (regardless of hearing level, i.e., NH+T and HL+T vs. NH-T and HL-T). One large cluster covered parts of the anterior cingulate cortex and medial prefrontal cortex. Two small clusters of decreased gray matter were further observed in the left inferior and middle temporal gyri (Table 2A; Figure 2A). No regions with significantly higher gray matter in individuals with tinnitus were found in this analysis.

To more carefully control for the hearing level in the meta-analysis, we performed a second meta-analysis excluding the one study [31] that did not match groups of individuals for hearing levels and included individuals with clinically significant depression and anxiety. In stark contrast to the result of the first meta-analysis, this second meta-analysis (NH+T and HL+T vs. NH-T and HL-T excluding study [31]), in which the hearing levels were now matched, found no areas of decreased gray matter in the individuals with tinnitus compared to the individuals without tinnitus. Moreover, several clusters of increased grey matter were found in the individuals with tinnitus. The largest clusters of increased gray matter were observed in the bilateral lingual gyrus with extensions into the cerebellum. Other smaller clusters were found in the right cuneus, the right posterior cingulate, and left precuneus (Table 2B; Figure 2B).

In light of these results, the third and fourth meta-analyses were conducted to test whether these clusters of gray matter changes in individuals with tinnitus compared to individuals without tinnitus were observed when specifically comparing subgroups with and without hearing loss. The third analysis (comparing NH+T vs. NH-T) found that in studies that included only individuals with normal hearing ($n = 7$ studies), the only difference between the individuals with and without tinnitus was a small area of decreased gray matter in the left inferior temporal gyrus of those with tinnitus (Table 3A; Figure 3A). The fourth and final analysis (comparing HL+T vs. HL-T), investigating the effects of tinnitus in groups of individuals with hearing loss ($n = 7$ studies), uncovered a series of clusters showing increased gray matter in individuals with tinnitus

compared to individuals without tinnitus. These clusters of increased gray matter were again found in the bilateral lingual gyrus extending into the precuneus and the cerebellum (Table 3B; Figure 3B).

The combined interpretation of these four meta-analyses indicates that matching for hearing levels dramatically affects the results. In particular, in our results, hearing loss was the driving force of changes in cortical gray matter across individuals with and without tinnitus. These findings disentangle the brain regions important in the pathology of tinnitus and hearing loss (see Section 4.2) and also motivate recommendations for future VBM studies of tinnitus (see Section 4.3).

The results of our investigation are not consistent with a recent meta-analysis that included eight VBM studies and also used Seed-Based d Mapping (AES-SDM) like our study [15]. The authors of this study reported significantly reduced gray matter levels in the caudate nucleus, the medial superior frontal gyrus, and the supplementary motor area, as well as increased gray matter in the angular and the middle temporal gyri in the groups with tinnitus compared to the groups without tinnitus [15]. Several reasons could explain the variability in findings between our study and this previous study. First, this previous study [15] did not distinguish between studies including individuals with and without hearing loss, which we found greatly impacted the results. Secondly, there were differences in study selection criteria (specifically, we did not include one study in the Chinese language). Finally, the validity of a meta-analysis is heavily dependent on the precision of the data under review. With the currently available studies, meta-analyses cannot control for the effects of important confounders like hyperacusis, anxiety, and depression, all known to be commonly associated with tinnitus and tinnitus burden [8–10]. Furthermore, the sample sizes are on average quite small and the employed image preprocessing methods, statistical models, and significance thresholds vary wildly across studies [10].

4.2. Brain regions associated with tinnitus and hearing loss

In the four meta-analyses performed in this study, we identified various brain regions with significant changes in gray matter associated with tinnitus and hearing loss. Specifically, we found a significant (1) decrease in gray matter in the anterior cingulate and medial prefrontal cortex in individuals with tinnitus (when not specifically matching for hearing loss, NH+T and HL+T vs. NH-T and HL-T); (2) decrease in gray matter in the inferior and middle temporal gyri in individuals with tin-

nitus (when comparing groups with normal hearing, NH+T vs. NH-T); and (3) increase in gray matter in the lingual gyrus and the precuneus in individuals with tinnitus (when comparing groups with hearing loss, HL+T vs. HL-T).

Our first key significant finding indicates decreased gray matter in the anterior cingulate and medial prefrontal cortex in participants with tinnitus (when not specifically matching for hearing loss, NH+T and HL+T vs. NH-T and HL-T). The anterior cingulate cortex is connected to the “cognitive” prefrontal cortex, the “emotional” limbic network, and the “sensory” auditory and visual networks [37–39]. Therefore, the anterior cingulate cortex plays a crucial role in integrating the neural circuits for affect regulation and sensory attention [37–39]. Affect regulation refers to the mechanisms by which unpleasant emotions are modulated or suppressed to achieve homeostasis and is an important concept in psychology regarding emotional regulation [37–39]. Similarly, the anterior cingulate cortex has been described as a key region of the salience network in the context of tinnitus [40,41]. The salience network is involved in modulating or suppressing unpleasant auditory stimuli and identifying relevant auditory stimuli for attention [40–42]. Thus, significantly reduced gray matter in the anterior cingulate cortex (with a cluster extending in the medial prefrontal cortex) might be related to a lack of modulation or suppression of unpleasant auditory stimuli in individuals with tinnitus and either normal hearing or hearing loss. This result is in agreement with a previous meta-analysis that found reduced gray matter in the frontal lobe areas in individuals with tinnitus and either normal hearing or hearing loss compared to individuals without tinnitus and either normal hearing or hearing loss [15].

Both the anterior cingulate cortex and the medial prefrontal cortex have been previously found to be important in the emotional aspects of tinnitus [42]. A previous magnetoencephalography study reported that the functional connectivity between the anterior cingulate cortex and prefrontal cortex correlated negatively with the tinnitus burden [43]. Furthermore, the inhibitory low-frequency repetitive transcranial magnetic stimulation of the anterior cingulate cortex–medial prefrontal cortex using a double cone coil has been investigated as an innovative approach for the treatment of tinnitus, and has been shown to reduce the tinnitus burden [44]. These findings suggest a link between the tinnitus burden and hyperactivity of the anterior cingulate–medial prefrontal cortex. Future research is needed to clarify the role of these brain regions in

the assessment and treatment of tinnitus, and especially regarding the tinnitus burden.

Our second key significant finding indicates decreased gray matter levels in a small area of the left inferior temporal gyrus in individuals with tinnitus (when comparing groups with normal hearing, NH+T vs. NH-T). The same finding was also observed when not specifically matching for hearing loss (i.e., NH+T and HL+T vs. NH-T and HL-T). The temporal lobe plays an important role in various cognitive functions, including auditory processing and language or speech comprehension [45]. Several studies have reported impairments in these domains in individuals with tinnitus [46–50]. Thus, our finding of reduced gray matter in the inferior temporal gyrus in tinnitus is not surprising. One study investigated speech perception in normal hearing individuals with tinnitus using a Mandarin version of the speech perception in noise test [51]. Compared to the normal hearing group without tinnitus, the normal hearing group with tinnitus displayed lower scores in perceiving sentences in a noisy environment [51]. Future studies in normal hearing individuals with tinnitus should explore the possible correlation between temporal lobe gray matter levels and scores on the speech in noise test.

Our third key significant finding indicates increased gray matter in individuals with tinnitus (when comparing groups with hearing loss, HL+T vs. HL-T). This difference was most prominent in the bilateral lingual gyrus and the bilateral precuneus. The same regions were found in the meta-analysis that included all studies that had matched their groups for hearing levels (i.e., NH+T and HL+T vs. NH-T and HL-T excluding study [31]). These results are in agreement with the previous conclusion that whereas hearing loss is associated with the loss of gray matter, hearing loss with the addition of tinnitus preserves gray matter [25,32]. In particular, the lingual gyrus and the precuneus are better preserved in individuals with hearing loss and tinnitus.

The lingual gyrus plays a role in encoding visual memories and is directly connected to the limbic network [52]. Damage to this region may lead to visual memory dysfunction [53]. In addition, previous studies have reported that the lingual gyrus is associated with better performance on memory tasks and cognitive functions [54–56]. Thus, our results suggest that hearing loss with additional tinnitus may be linked to better cognitive function than hearing loss without tinnitus. Supporting this conclusion, a recent cross-sectional study aimed at delineating the interaction

between tinnitus and cognition using a composite z-score from four cognitive tests found that in (non-Hispanic) groups with hearing loss, the additional presence of tinnitus was associated with better cognitive performance compared to the group without tinnitus [57]. Future studies are needed to investigate the contribution of the lingual gyrus (and other brain regions) to preserved cognitive performance in individuals with hearing loss with and without tinnitus.

The precuneus is part of the default mode network and plays an essential role in various complex cognitive functions, including conscious and internal awareness [58]. The default mode network is most active at wakeful rest and is functionally anticorrelated with the dorsal attention network [59]. A previous resting-state functional imaging study reported that tinnitus is associated with decreased functional connectivity within the default mode network and increased functional connectivity between the default mode network and the dorsal attention network [60]. Similarly, a previous diffusion imaging study, reporting reduced connectivity in the precuneus, speculated that the constant disturbance of tinnitus during wakeful rest may stimulate structural changes in the precuneus [61]. Future work is needed to investigate the mechanistic links between changes in attentional networks associated with hearing loss and additional tinnitus and preserved gray matter in the precuneus of individuals with hearing loss and additional tinnitus.

4.3. Recommendations for future VBM studies of tinnitus

Our study identifies the importance of matching individuals for hearing levels when investigating changes in gray matter associated with tinnitus. Our study also suggests that age and psychiatric comorbidities may influence the results. To investigate the interactions between tinnitus, hearing loss, age, anxiety, and depression when investigating brain changes associated with tinnitus, future VBM studies should carefully document the various demographic and methodological factors outlined in Table 4. Documenting comorbidities, such as depression and anxiety, is especially important, since it is not always feasible or desirable to exclude these groups. In addition, future studies should investigate the effects of the duration of tinnitus and the cause or duration of hearing loss on changes in brain volume. Two previous studies have researched the impacts of the tinnitus duration on gray matter levels in a larger tinnitus population [12,62]. To date, no tinnitus study has examined gray matter changes in response to the cause or duration of hearing loss (although the onset of hearing loss is often difficult to identify). Consid-

ering that our current results show that hearing loss is the main factor driving changes in gray matter, future research investigating the aspects of hearing loss that drive these changes is important. Finally, the effect of sex should also be considered in VBM studies. Here, we specifically recommend matching for the sex ratio when designing studies. We also recommend including the total intracranial volume (TIV), which is on average 12% larger in males [63] and can account for variations within as well as between sexes. We do not, however, recommend including both sex and TIV as covariates, since they are significantly correlated. We believe the best practical solution currently available is to match sex ratios between groups and include TIV as a covariate to control for interindividual variability in brain size or gray matter across sexes. However, if it is suspected that sex would influence the effect of the tinnitus or hearing loss on the regional gray matter, it would be important to design specific studies including sex, not as a confounder, but as a grouping variable. VBM studies should, furthermore, upload the unthresholded statistical images to a shared repository (e.g., NeuroVault, <https://neurovault.org/>, accessed on 10 June 2022). A concerted effort across researchers to curate a repository with a large number of individual data sets that include robust phenotypic data and T1 images is necessary to distinguish the brain changes associated specifically with tinnitus from the effects of hearing loss, age, hyperacusis, anxiety, and depression, and to investigate the interactions between these variables. We suspect that these recommendations are equally valid and important for other brain imaging approaches.

Table 4. Recommendations for future (meta-analyses of) VBM studies investigating tinnitus.

Demographic factors
Match groups for age, sex ratio, and hearing loss
Provide clear definitions for hearing loss and/or provide audiograms for both ears
Report clinical information (anxiety, depression, hyperacusis, and tinnitus burden scores)
Methodological factors
Correct results for multiple comparisons at the cluster or voxel level (FWE, FWE-TFCE or FDR correction)
Control results for age and total intracranial volume
Report significant x, y, z -coordinates with t-values, z-values, and p-values
Data sharing
Make unthresholded statistical images available—e.g., via NeuroVault website (https://neurovault.org/), or create a shared initiative to aggregate a large number of data sets with minimal requirements on T1 brain imaging protocols and questionnaires for controlling for potential variables of interest like tinnitus duration, depression and anxiety.

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SUPPLEMENTARY MATERIALS

<https://www.mdpi.com/article/10.3390/brainsci12091192/s1>

DATA AVAILABILITY STATEMENT

The datasets analyzed in the current meta-analysis are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.



Chapter 3

**Gray matter differences associated
with tinnitus are dependent on hearing
status**

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ABSTRACT

Structural neuroimaging studies, particularly voxel-based morphometry (VBM), have revealed tinnitus-related gray matter differences in both auditory and non-auditory brain regions, implicating auditory, attentional, emotional, and memory-related processes. However, findings have been inconsistent, often due to small sample sizes and the heterogeneity of tinnitus comorbidities and characteristics, such as age, hearing status, hyperacusis, anxiety, depression, as well as tinnitus burden. To address these potential confounds, we conducted a retrospective case-control study with four relatively large groups, including participants with either normal hearing (as evidenced by normal audiograms) without tinnitus (NH, $n = 77$), normal hearing with tinnitus (NHT, $n = 35$), hearing loss without tinnitus (HL, $n = 46$), or hearing loss with tinnitus (HLT, $n = 70$). Using a whole-brain 2×2 ANOVA, adjusted for age, handedness, and total intracranial volume (TIV), we found significant interactions between the effects of tinnitus and hearing loss on several gray matter regions. Specifically, normal hearing individuals with tinnitus had smaller gray matter volumes in Heschl's gyrus (extending into the posterior insula and rolandic operculum) and the ventromedial prefrontal cortex compared to normal hearing individuals without tinnitus. In contrast, individuals with hearing loss and tinnitus had larger gray matter volumes in the lingual gyrus (extending into parahippocampal gyrus and precuneus) compared to individuals with hearing loss without tinnitus. We found no significant correlations between these gray matter regions and scores for hyperacusis, anxiety, depression, and tinnitus burden within the groups with tinnitus. In summary, our findings highlight distinct gray matter differences associated with tinnitus in individuals with normal audiograms versus those individuals with tinnitus and hearing loss, suggesting the presence of potentially different underlying mechanisms.

Keywords

Anxiety

Depression

Hyperacusis

Structural neuroimaging

Tinnitus burden

Voxel-based morphometry

Highlights

- Tinnitus and hearing loss interactions shape gray matter (GM) in brain regions (BRs).
- In normal hearing, tinnitus is linked to reduced GM in auditory and non-auditory BRs.
- In hearing loss, tinnitus is linked to increased GM in non-auditory BRs.
- GM changes are not explained by hyperacusis, anxiety, depression, or THI scores.

1. INTRODUCTION

Subjective tinnitus (here referred to as simply tinnitus) is the conscious perception of a sound in the absence of external auditory stimulus. It affects an estimated 5% to 40% of the population, depending on the specific population studied and the definitions applied [1–6]. The impact of severe tinnitus extends beyond auditory perception, significantly affecting quality of life. Individuals may experience difficulties with concentration, sleep, emotional well-being, and daily functioning.

Tinnitus is a heterogeneous condition with largely unknown underlying mechanisms. Despite its heterogeneous nature, certain factors are consistently associated with tinnitus, such as hearing loss and hyperacusis (an increased sensitivity to environmental sounds of mild to moderate intensity). About 90% of individuals with tinnitus have some degree of hearing loss [7], and about 63% report hyperacusis [8]. However, tinnitus is not exclusive to those with hearing deficits. About 10% of individuals with tinnitus demonstrate normal hearing thresholds on standard audiograms (frequencies up to 8 kHz) [9].

Structural neuroimaging studies using voxel-based morphometry (VBM) have provided evidence of structural gray matter differences associated with tinnitus. These differences are observed in both auditory and non-auditory brain regions. The brain regions identified as associated with tinnitus include those responsible for auditory processing in the temporal lobe, attentional control and conscious perception in the parietal lobe and prefrontal cortex, emotional processing and memory functions within the limbic lobe, sensorimotor integration in the sensorimotor cortex, and visual processing in the occipital lobe [6, 10–13]. Overall, these findings highlight the complex multidimensional and multisensory nature of tinnitus.

Despite these findings, the results of previous VBM studies are highly inconsistent. These inconsistencies may result from several factors, including small sample sizes and differences among groups investigated, such as age, hearing status, and tinnitus burden [6, 12–14]. Additionally, the presence of coexisting conditions like hyperacusis, anxiety, and depression in conjunction with tinnitus may further explain the divergent results observed across studies [6, 15, 16]. To address these limitations, we aggregated data from four previous neuroimaging studies conducted at the Department of Otorhinolaryngology, University Medical Center

Groningen (UMCG) in the Netherlands [17–20]. This approach enabled us to include four relatively large participant groups, including individuals with 1) normal hearing without tinnitus (NH, $n = 77$), 2) normal hearing with tinnitus (NHT, $n = 35$), 3) hearing loss without tinnitus (HL, $n = 46$), and 4) hearing loss with tinnitus (HLT, $n = 70$). Our study aimed to explore the effects of tinnitus and hearing loss, and their interaction, on gray matter volumes across these groups (NH, NHT, HL, and HLT). The available data further allowed us to explore the impact of hyperacusis, anxiety, depression, and tinnitus burden within the tinnitus groups (NHT and HLT).

2. MATERIALS AND METHODS

2.1. Ethical approvals

The data from the four studies [17–20] were collected in adherence to the principles of the Declaration of Helsinki, and each study received approval from the UMCG Medical Ethics Committee (METc). Written informed consent was obtained from all participants. The committee also approved the reanalysis of the data (METc number 2020/347).

2.2. Participants

Our study included 240 participants, divided into four groups: normal hearing without tinnitus (NH, $n = 80$), normal hearing with tinnitus (NHT, $n = 35$), hearing loss without tinnitus (HL, $n = 52$), and hearing loss with tinnitus (HLT, $n = 73$). Pure tone audiometry was conducted for both ears of each participant at octave frequencies ranging from 0.25 to 8 kHz to assess hearing thresholds. The mean threshold (pure tone average, PTA) across these frequencies were calculated for each participant. Hearing loss was defined by the mean PTA from 0.25 to 8 kHz for both ears ≥ 25 dB HL (decibels Hearing Level). All participants with PTA below 25 dB HL were referred to as normal hearing, even though the presence of tinnitus in the NHT group obviously indicates an auditory pathology. The presence of hyperacusis was evaluated using the Hyperacusis Questionnaire (HQ) [21]. Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS) [22]. Handedness was measured using the Edinburgh Handedness Inventory (EHI) [23]. For participants with tinnitus, tinnitus burden was assessed using the Tinnitus Handicap Inventory (THI) [24]. Twelve participants were later excluded due to the poor quality of the T1-weighted anatomical brain images (more details in

Section 2.4). Therefore, the main analyses included 228 participants: 77 NH, 35 NHT, 46 HL, and 70 HLT.

2.3. T1-weighted anatomical brain imaging

For each participant, high-resolution 3-dimensional T1-weighted anatomical brain images were obtained using a 3T Philips Intera MRI scanner (Philips Medical System, Best, the Netherlands) equipped with a SENSE phase-array head coil (Philips Medical System, Best, the Netherlands). Fast-field echo images were acquired at a reconstructed voxel size of 1 x 1 x 1 mm. Detailed scanning parameters of the four included studies can be found in Supplementary Table S1.

2.4. Data preprocessing and image quality control

T1-weighted anatomical brain images were initially converted from REC/PAR Philips files to NIFTI files using dcm2niix v1.0.2021 (<https://github.com/rordenlab/dcm2niix>). Subsequently, the images were preprocessed, and statistical parametric maps were derived using CAT 12.7 toolbox (Structural Brain Mapping Group, University of Jena, Germany, <http://dbm.neuro.uni-jena.de/cat12/>) for SPM 12 (Wellcome Center for Human Neuroimaging, University College London, <https://www.fil.ion.ucl.ac.uk/spm>) in MATLAB R2020a (The MathWorks, Natick, MA, USA). The preprocessing steps followed the recommendations of CAT 12.7. First, the images were corrected for bias, noise, and global intensity. Then, they were segmented into gray matter, white matter, and cerebrospinal fluid (CSF). The gray matter images were normalized to the Montreal Neurological Institute (MNI152) template using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTTEL) algorithm, which involves affine and non-linear registration [25, 26]. During normalization, the voxel size of the gray matter images was set to 1.5 x 1.5 x 1.5 mm. To account for volumetric deformation, the normalized gray matter images were modulated using the Jacobian determinant of the deformation field [25, 26]. Quality control was performed by visually inspecting the normalized modulated gray matter images and automatically assessing their homogeneity based on overall image quality and voxel-based correlation between images (see Supplementary Figure S1 for more details). Based on visual and automatic quality assessment, 12 images were excluded from further analyses. The total intracranial volume (TIV) was calculated by summing the volumes of gray matter, white matter, and CSF, and used in the analyses to correct for brain size differences. Finally, the normalized modulated gray matter images were

smoothed using a Gaussian smoothing kernel with a full-width half-maximum (FWHM) of 12 mm.

2.5 Data analyses

2.5.1. Demographic, audiometric, and questionnaires data

Statistical analyses of the demographic, audiometric, and questionnaire data were conducted using IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA). To assess group differences for continuous variables, Welch's unequal variances one-way ANOVA was utilized, followed by Bonferroni-corrected post-hoc t-test analyses. For categorical variables, the Chi-square test was employed to examine group differences.

2.5.2. Whole-brain voxel-based analysis

The preprocessed gray matter images underwent voxel-wise statistical analysis using a General Linear Model (GLM). Voxels with a gray matter intensity below 0.1 were excluded to avoid potential edge effects at the gray and white matter boundary. The main effects of tinnitus and hearing loss, as well as their interaction effects on gray matter volumes were investigated through whole-brain voxel-wise 2 x 2 ANOVA, considering the two factors of tinnitus and hearing loss in the four participant groups (NH, NHT, HL, and HLT). Age, handedness scores, and TIV were included as covariates. The statistical threshold was set at $p \leq 0.05$ family-wise error (FWE) corrected at the voxel-level and cluster-size $k > 5$. To interpret the significant interaction effects, post-hoc tests were conducted on the contrast estimates of the model comparing the four groups pairwise using MarsBar 0.44 toolbox (<https://marsbar-toolbox.github.io/about.html>). The statistical threshold was set at $p \leq 0.003$ (0.05/16) to correct for the number of tests.

2.5.3. Volume of interest analysis within the participant groups with tinnitus

To evaluate the potential influence of the presence of hyperacusis, anxiety, depression, or tinnitus burden within the participant groups experiencing tinnitus (NHT and HLT), volume of interest (VOI) analysis was carried out on each significant VOI derived from the whole-brain 2 x 2 ANOVA. Specifically, the "get_totals" MATLAB function (freely available from, http://www0.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m/) was used to extract gray matter volumes for each VOI. Partial correlation coefficients were then computed between each VOI and the HQ hyperacusis, HADS anxiety and depression, or THI tinnitus burden scores, while con-

trolling for total intracranial volume. The statistical threshold for partial correlation analysis was set at $p \leq 0.001$ ($p \leq 0.05/48$), corrected for the number of tests.

3. RESULTS

3.1. Demographic, audiometric, and questionnaires data

The groups in the study differed significantly in terms of several variables. The statistical analyses revealed significant differences between the four groups when comparing age ($F(3,103.9) = 41.0$, $p < 0.001$), hearing thresholds ($F(3,100.7) = 161.6$, $p < 0.001$), sex distribution ($X^2(3) = 18.5$, $p < 0.001$), HADS anxiety and depression scores (anxiety: $F(3,98.9) = 6.7$, $p < 0.001$; depression: $F(3,96.5) = 8.4$, $p < 0.001$), as well as HQ hyperacusis scores ($F(3,100.5) = 28.3$, $p < 0.001$). Both groups with hearing loss (HL and HLT) were older compared to both groups with normal audiograms (NH and NHT). Mean hearing thresholds for both ears at octave frequencies ranging from 0.25 to 8 kHz per group are shown in Figure 1. The average profile indicates an increase in thresholds in the high frequencies. Both groups with tinnitus (NHT and HLT) scored significantly higher on the HQ scale compared to both groups without tinnitus (NH and HL), with the NHT group scoring higher than the HLT group. Similarly, the NHT group scored significantly higher on the HADS anxiety and depression scale compared to both groups without tinnitus (NH and HL) and significantly higher on the HADS anxiety subscale compared to the HLT group. Summarized data for the four groups are shown in Table 1 (more details in Supplementary Table S2).

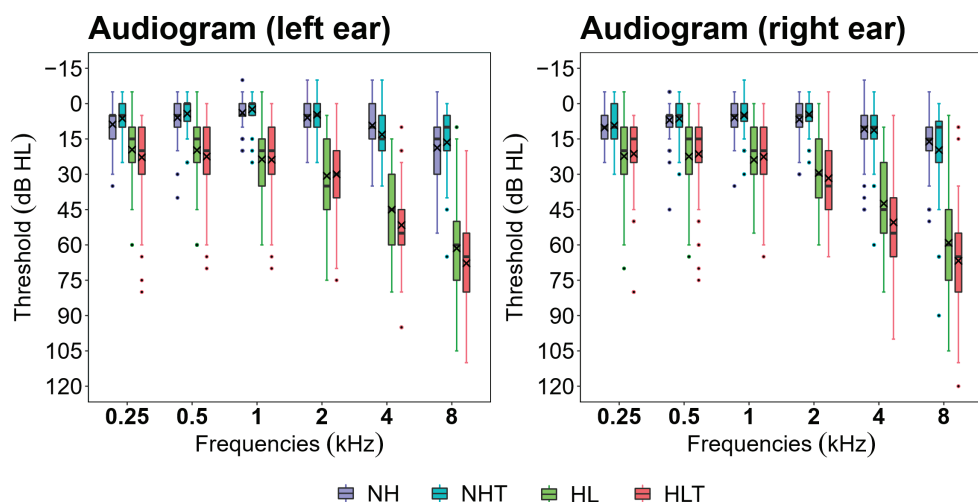


Figure 1. Mean hearing thresholds for the four participant groups with their standard deviations. Both groups with hearing loss (HL and HLT) had worse hearing thresholds compared to both groups with normal hearing (NH and NHT) [$F(3,100.7) = 161.6$, $p < 0.001$]. HL hearing loss without tinnitus; HLT hearing loss with tinnitus; NH normal hearing without tinnitus; NHT normal hearing with tinnitus.

Table 1. Summarized demographic, audiometric, and questionnaires data for the four participant groups.

Groups	Normal Hearing		Hearing Loss	
	without Tinnitus (NH)	with Tinnitus (NHT)	without Tinnitus (HL)	with Tinnitus (HLT)
Demographic				
n	77	35	46	70
Mean Age (years)	40.2 ± 13.7 (18 – 67) ^{c,d}	44.6 ± 11.7 (19 – 62) ^{c,d}	59.6 ± 10.6 (33 – 80) ^{a,b}	58.3 ± 9.6 (27 – 76) ^{a,b}
Sex (male female)	31 46	18 17	31 15	51 19
Audiometric (for both ears 0.25 to 8 kHz)				
Mean PTA (dB HL)	9.1 ± 6.7 ^{c,d}	8.6 ± 6.7 ^{c,d}	33.3 ± 12.6 ^{a,b}	36.1 ± 9.9 ^{a,b}
Questionnaires				
HQ	11.7 ± 6.0 (3 – 27) ^{b,d}	23.2 ± 6.5 (10 – 35) ^{a,c,d}	13.8 ± 8.3 (2 – 33) ^{b,d}	18.2 ± 8.0 (0 – 33) ^{a,b,c}
HADS-Anxiety	3.9 ± 2.7 (0 – 11) ^b	6.6 ± 3.7 (0 – 16) ^{a,c,d}	3.3 ± 3.1 (0 – 11) ^b	4.6 ± 3.7 (0 – 16) ^b

Table 1. Summarized demographic, audiometric, and questionnaires data for the four participant groups. (*continued*)

Groups	Normal Hearing		Hearing Loss	
	without Tinnitus (NH)	with Tinnitus (NHT)	without Tinnitus (HL)	with Tinnitus (HLT)
HADS-Depression	2.2 ± 2.5 (0 – 11) ^{b,d}	4.9 ± 3.7 (0 – 15) ^{a,c}	2.8 ± 3.5 (0 – 13) ^b	4.5 ± 4.0 (0 – 16) ^a
THI	–	40.4 ± 20.8 (4 – 88)	–	32.7 ± 20.8 (4 – 82)

Mean ± Standard Deviation (Range). ^athe group differed significantly ($p \leq 0.05$) from the normal hearing without tinnitus (NH) group; ^bthe group differed significantly ($p \leq 0.05$) from normal hearing with tinnitus (NHT) group; ^cthe group differed significantly ($p \leq 0.05$) from hearing loss without tinnitus (HL) group; ^dthe group differed significantly ($p \leq 0.05$) from hearing loss with tinnitus (HLT) group.

3.2. Whole-brain voxel-based analyses

3.2.1. Main effects of tinnitus and hearing loss on gray matter volumes

Whole-brain voxel-wise 2 x 2 ANOVA controlling for age, handedness, and TIV showed significant main effects of tinnitus on the gray matter volumes of the left inferior occipital gyrus (IOG) with a cluster extending into left fusiform gyrus (FFG) (Table 2). Tinnitus was associated with higher gray matter volumes in the IOG/FFG, irrespective of hearing status (Figure 2A). The analysis also revealed a significant main effect of hearing loss on the gray matter volumes of the bilateral mediodorsal thalamic nuclei (Thal_MD), left gyrus rectus (REC), and left posterior cerebellum (CERpost) with a cluster extending into left cerebellum crus-II (CER-CRU2) (Table 2). Hearing loss was associated with smaller gray matter volumes of the Thal_MD and REC; and higher gray matter volumes of the CERpost/CERCPU2, irrespective of tinnitus status (Figure 2B).

Table 2. Results of the main effects of tinnitus and hearing loss on gray matter volumes using the whole-brain voxel-wise 2 x 2 ANOVA controlling for age, handedness, and TIV (FWE $p \leq 0.05$ at the voxel-level and cluster-size $k > 5$).

Area	FWE p cluster-level	FWE p peak-level	Peak MNI			Cluster- size k	F-value	Z-value
			X	Y	Z			

Main Effects of Tinnitus (height threshold FWE $F_{\text{-value}} = 20.8$)

IOG / FFG (left)	0.038	0.034	-44	-68	-14	12	21.8	4.4
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Main Effects of Hearing Loss (height threshold FWE $F_{\text{-value}} = 20.8$)

Thal_MD (bi-lateral)	0.002	0.001	-6	-8	-2	509	31.8	5.3
REC (left)	0.012	0.009	-9	15	-24	138	25.2	4.7
CERpost / CERCRU2 (left)	0.015	0.008	-16	-88	-46	101	25.4	4.8

Automated Anatomical Atlas 3 (AAL3) labelling: CERpost posterior cerebellum; CERCRU2 cerebellum crus-II; FFG fusiform gyrus; IOG inferior occipital gyrus; REC gyrus rectus; Thal_MD mediodorsal thalamic nuclei.

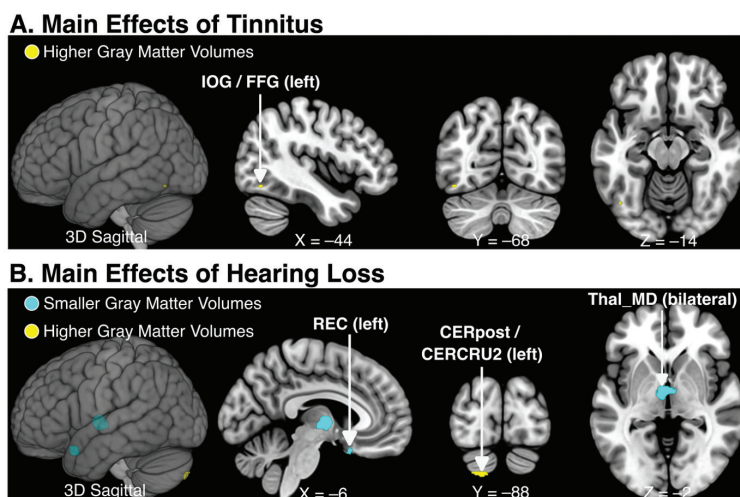


Figure 2. Main effects of tinnitus (panel A) and hearing loss (panel B) on gray matter volumes using whole-brain voxel-wise 2 x 2 ANOVA including age, handedness score, and TIV as covariates (FWE $p \leq 0.05$ at the voxel-level and cluster-size $k > 5$). (A) Tinnitus was associated with higher gray matter volumes of the left inferior occipital gyrus (IOG) with a cluster extending into the left fusiform gyrus (FFG), irrespective of hearing thresholds. (B) Hearing loss was associated with lower gray matter volumes of the bilateral mediodorsal thalamic nuclei (Thal_MD), the left gyrus rectus (REC); and higher gray matter volumes of the left posterior cerebellum crus-II (CERpost / CERCRU2).

3.2.2.Interaction effects of tinnitus and hearing loss on gray matter volumes

The same whole-brain voxel-wise 2 x 2 ANOVA controlling for age, handedness, and TIV showed significant interaction between effects of tinnitus and hearing loss on gray matter volumes in the several cortical areas: bilateral Heschl’s gyrus (HES, extending into posterior insula (INSpost) and rolandic operculum (ROL)), lingual gyrus (LING, extending into parahippocampal gyrus (PHG) and precuneus (PCUN)), ventromedial prefrontal cortex (PFCventmed), right inferior frontal gyrus (IFGoperc), and ventral posterolateral thalamic nuclei (Thal_VPL, extending into lateral globus pallidum (PALlat) and medial geniculate thalamic nuclei (Thal_MGN)) (Table 3 & Figure 3).

Table 3. Results of the interaction between effects of tinnitus and hearing loss on gray matter volumes using the whole-brain voxel-wise 2 x 2 ANOVA controlling for age, handedness, and TIV (FWE p ≤ 0.05 at the voxel-level and cluster-size k > 5).

Area	FWE p cluster-level	FWE p peak-level	Peak MNI			Cluster- size k	F-value	Z-value
			X	Y	Z			
Interaction Effects of Hearing Loss and Tinnitus (height threshold FWE F-value = 20.8)								
HES / INSpost / ROL (left)	< 0.001	0.001	-34	-24	16	780	30.7	5.2
HES / INSpost / ROL (right)	< 0.001	< 0.001	38	-21	12	1263	40.2	6.0
LING / PHG / PCUN (left)	0.006	0.005	-14	-52	-2	238	26.6	4.9
LING / PHG / PCUN (right)	0.003	0.005	9	-57	2	392	26.4	4.9
		0.008	24	-44	-3		25.4	4.8
PFCventmed (bilateral)	0.005	0.007	3	42	-12	262	25.6	4.8
IFGoperc (right)	0.019	0.018	45	6	30	73	23.4	4.6
Thal_VPL / PALlat / Thal_MGN (left)	< 0.001	< 0.001	-18	-14	-2	941	41.5	6.1
Thal_VPL / PALlat / Thal_MGN (right)	0.001	0.003	16	-16	-2	512	28.1	5.0

Automated Anatomical Atlas 3 (AAL3) labelling: HES Heschl’s gyrus; IFGoperc inferior frontal gyrus (opercular part); INSpost posterior insula; LING lingual gyrus; PALlat lateral globus pallidum (lenticular nucleus); PCUN precuneus; PFCventmed ventromedial prefrontal cortex; PHG parahippocampal gyrus; ROL rolandic operculum; Thal_MGN medial geniculate thalamic nuclei; Thal_VPL ventral posterolateral thalamic nuclei.

Interaction Effects of Tinnitus and Hearing Loss

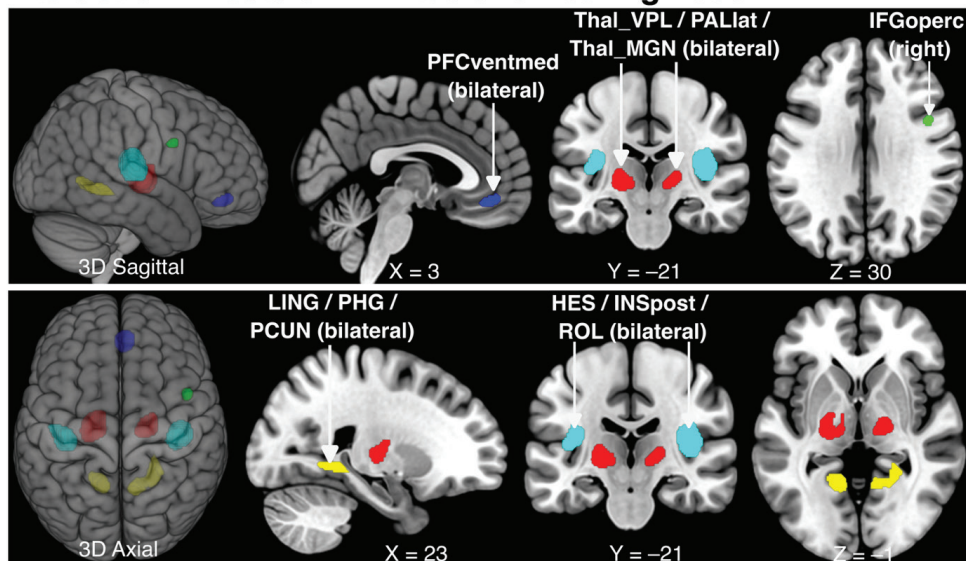


Figure 3. Brain regions with interaction between the effects of tinnitus and hearing loss on gray matter volumes. These regions were determined using whole-brain voxel-wise 2×2 ANOVA including age, handedness score, and TIV as co-variables (FWE $p \leq 0.05$ at the voxel-level and cluster-size $k > 5$). Significant interaction occurred in several brain regions, including bilateral Heschl's gyrus (HES) (cluster extending into posterior insula (INSpost) and bilateral rolandic operculum (ROL)), bilateral lingual gyrus (LING) (cluster extending into parahippocampal gyrus (PHG) and precuneus (PCUN)), bilateral ventromedial prefrontal cortex (PFCventmed), right inferior frontal gyrus (IFGoperc), and bilateral ventral posterolateral thalamic nuclei (Thal_VPL) (cluster extending into lateral globus pallidum (PALlat) and medial geniculate thalamic nuclei (Thal_MGN)). The interactions are further illustrated in Figure 4.

Figure 4 shows the model contrast estimates for each brain region demonstrating a significant interaction. Post-hoc tests comparing the four groups pairwise (NH vs. NHT and HL vs. HLT) revealed several significant differences (Bonferroni corrected $p \leq 0.003$). Compared to the NH group, the NHT group had smaller gray matter volumes in the left and right Heschl's gyrus (extending into INSpost and ROL, $p < 0.001$), the ventromedial prefrontal cortex ($p = 0.002$), and the right inferior frontal gyrus ($p = 0.002$). The NHT group also had higher gray matter volumes in the left and right ventral posterolateral thalamic nuclei (extending into PALlat and Thal_MGN, $p < 0.001$) compared to the NH group. On the other hand, compared to the HL group, the HLT group had higher gray matter volumes in the left and right lingual gyrus (extending into PHG and PCUN) ($p < 0.001$). Finally, the HLT groups group also had a

trend towards higher gray matter volumes in the right Heschl's gyrus ($p = 0.004$) as well as in the ventromedial prefrontal cortex ($p = 0.004$) compared to the HL group.

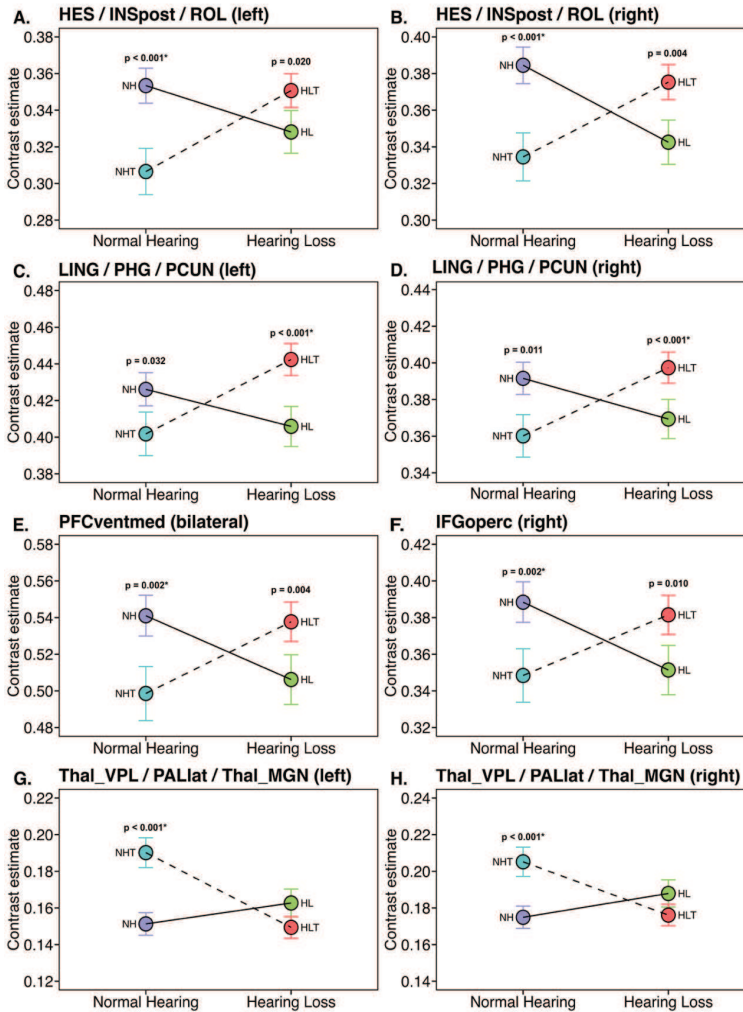


Figure 4. Volume of interest (VOI) analyses on the results of the interaction between the effects of tinnitus and hearing loss on gray matter volumes using MarsBar. The corresponding VOIs are shown in Figure 3. The graphs show the mean contrast estimate and 90% confidence interval (CI). The statistical threshold was set at $p \leq 0.003$ (0.05/16) to correct for the number of tests. HL hearing loss without tinnitus; HLT hearing loss with tinnitus; NH normal hearing without tinnitus; NHT normal hearing with tinnitus.

3.2.3. *Effects of hyperacusis, anxiety, depression, or tinnitus burden on gray matter volumes*

We found no significant correlation between VOIs derived from the whole-brain 2 x 2 ANOVA (shown in Figure 2 and 3) and either the HQ hyperacusis, HADS anxiety and depression, or THI tinnitus burden scores within the participant groups experiencing tinnitus (NHT and HLT) (Supplementary Table S3). These variables, therefore, do not appear to drive the observed differences in gray matter volumes within the context of the whole-brain 2 x 2 ANOVA.

4. DISCUSSION

Subjective tinnitus is often associated with hearing loss. A subset of tinnitus sufferers, however, have normal audiograms. The aim of this retrospective case-control study was to investigate the effect of hearing loss on gray matter differences associated with tinnitus. To this end, we compared whole-brain voxel-wise gray matter volumes across four large participant groups (Table 1 & Figure 1), including individuals with normal hearing without tinnitus (NH), normal hearing with tinnitus (NHT), hearing loss without tinnitus (HL), and hearing loss with tinnitus (HLT). A 2 x 2 ANOVA analysis, controlling for age, handedness, and total intracranial volume, revealed significant effects of tinnitus and hearing loss on gray matter volumes (Table 2 & Figure 2) as well as interactions between tinnitus and hearing loss on gray matter volumes in several brain regions (Table 3 & Figure 3). Notably, among individuals with normal hearing (NH vs. NHT), tinnitus was associated with smaller gray matter volumes in Heschl's gyrus (extending into posterior insula and rolandic operculum) and ventromedial prefrontal cortex, as well as larger gray matter volumes in the ventral posterolateral thalamic nuclei (extending into lateral globus pallidum and medial geniculate thalamic nuclei). In contrast, among individuals with hearing loss (HL versus HLT), tinnitus was associated with larger gray matter volumes in the lingual gyrus (extending into parahippocampal gyrus and precuneus). Within these participants, these structural differences were not associated with tinnitus comorbidities such as hyperacusis, anxiety, depression, or tinnitus burden.

4.1. Interaction of tinnitus and hearing loss in the auditory cortex

The interaction of tinnitus and hearing loss in Heschl's gyrus showed effects that differed between individuals with normal hearing and those with hearing loss (Figure 4A and 4B). Individuals with tinnitus despite normal audiograms (NHT) showed significantly smaller gray matter volumes compared to those individuals with tinnitus and hearing loss (HLT). The reverse trend was seen for participants with hearing loss: gray matter volumes were slightly larger in the tinnitus subjects with a trend towards significance in the right hemisphere. The vast majority of studies investigating gray matter levels in individuals with tinnitus do not report any group difference in the primary auditory cortex [6, 11]. One study reported lower gray matter volumes in tinnitus across participants with and without hearing loss [27]. Another study found larger gray matter volumes in individuals with tinnitus and hearing loss relative to controls with normal hearing, but not relative to controls with hearing loss [18]. Age and hearing loss are likely key factors determining decreases in auditory cortices gray matter [6, 13, 20, 27]; however, our investigation suggests that, in individuals with normal hearing levels, tinnitus is associated with lower gray matter volumes in the auditory cortex.

4.2. Interaction of tinnitus and hearing loss beyond the auditory cortex

We also found significant interactions between tinnitus and hearing loss in non-auditory regions, including the lingual gyrus bilaterally (extending into the parahippocampal gyrus and precuneus), the ventromedial prefrontal cortex, the inferior frontal gyrus, and the ventral posterolateral thalamic nuclei. These results are in agreement with previous studies and meta-analyses, linking tinnitus with structural differences in non-auditory brain regions, including areas involved in cognitive, emotional, and sensory processing [6, 10, 11, 13]. Similar to auditory regions, our findings suggest, however, that the patterns of gray matter differences associated with tinnitus vary depending on hearing status.

The ventromedial prefrontal cortex (PFCventmed) is a central hub in the brain's cognitive, emotional, and sensory networks and has been implicated in reward-based decision making, self-related information processing, and emotional regulation and sensory attention [28–31]. The PFCventmed exerts inhibitory control over the amygdala and helps modulate or suppress unpleasant emotions [28–31]. In individuals with

tinnitus, this region may fail to direct attention to relevant sounds and filter distressing auditory stimuli [32–34]. Gray matter differences in the PFCventmed have been described in several previous studies. Leaver et al. [35] reported decreased gray matter volume in tinnitus participants with mixed hearing levels compared to younger normal-hearing controls, although Koops et al. [20] found that differences in this region were better explained by aging rather than tinnitus. In contrast, Husain et al. [36] observed larger PFCventmed volumes in tinnitus participants with hearing loss, supporting accumulating evidence that tinnitus may protect against gray matter decline associated with hearing loss. The results of the current study further refine this hypothesis, suggesting that tinnitus itself (that is, in the absence of hearing loss) is not protective against gray matter volume changes.

The lingual gyrus is part of the ventral visual stream and is involved in visual processing, including letter and pattern recognition, visual imagery, and visual memory [37, 38]. Nevertheless, a previous study from our group reported larger gray matter volumes in participants with tinnitus and hearing loss in this region, within a cluster that also included the fusiform gyrus, parahippocampal gyrus, and precuneus [20]. Furthermore, as part of a recent meta-analysis including 14 VBM studies, we found that tinnitus was associated with larger gray matter volumes in the lingual gyrus (as well as parahippocampal gyrus and precuneus) in participants with hearing loss only [6]. Future work is needed to understand the link between changes in visual processing and tinnitus.

4.3. Tinnitus comorbidities and gray matter regions

Contrary to expectation, our findings did not reveal a significant association between changes in gray matter volumes in limbic regions and measures of anxiety, depression (HADS scores), and tinnitus burden (THI scores, Supplementary Table S3). Tinnitus, particularly when loud or persistent, is often associated with emotional distress. Evidence suggests that shared neural mechanisms involving the limbic network, critical for emotional regulation and stress response [39], also play a role in the perception and processing of tinnitus [11, 32, 40] and may contribute to the emotional stress, including anxiety and depression, associated with tinnitus. The lack of association may be due to the limited number of participants in our study with severe to catastrophic symptoms, defined as HADS scores >15 or THI scores >58. While additional studies directly comparing individuals with more versus less severe tinnitus symptoms are needed to examine the effects of tinnitus severity on gray matter vol-

umes [15,41], the lack of associations in our work, nevertheless, suggests that tinnitus-related structural brain differences are not merely secondary to emotional distress but instead reflect the direct neural pathology of tinnitus.

4.4. Strengths and limitations

Despite the strengths of this study, including relatively large, well-characterized cohorts and the examination of the interactions between tinnitus and hearing loss rather than collapsing across hearing status, two limitations should be noted. First, participants were drawn from four different studies, resulting in anatomical images acquired with varying imaging protocols. Although all the images were acquired on the same MRI scanner, with an MPRAGE sequence and 1-mm resolution, images for participants with tinnitus and normal hearing (NHT group) came from two studies with a relatively short acquisition time and 8-channel head coil (Supplementary Table S1), which might have influenced the results of our analysis. To address differences in imaging, data were pre-processed to maximize the correlation between the images, and scans that deviated from the rest were discarded. Second, as expected from the general population, participants with hearing loss (both the HL and HLT groups) were on average older than those without hearing loss. To mitigate this potential confound, age was included as a covariate in the analyses with one single regressor for all groups. This procedure controls for between-groups age differences. However, possible differential effects of age on gray matter volumes in individuals with or without hearing loss could complicate the interpretation of the observed interaction effects. To minimize this confound, we concentrated our interpretation on the comparisons between groups with comparable hearing levels.

5. CONCLUSION

In conclusion, our findings confirm that structural gray matter differences are associated with tinnitus in both auditory and non-auditory regions, including areas involved in sensory, cognitive, and limbic processing. Importantly, we identified distinct gray matter differences linked to tinnitus in individuals with normal hearing (normal audiograms) versus those with hearing loss, suggesting differences in tinnitus mechanisms in the presence or absence of hearing loss.

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SUPPLEMENTARY MATERIALS

Supplementary Table S1. Acquisition parameters for T1-weighted anatomical brain imaging.

	<i>Langers et al. 2012</i>	<i>Boyen et al. 2013</i>	<i>Amaral and Langers 2015</i>	<i>Koops et al. 2020</i>
SENSE Coil Type	8-channel head coil	8-channel head coil	8-channel head coil	32-channel head coil
Repetition Time TR (ms)	9	9	9	10.4
Echo Time (ms)	3.6	3.5	3.6	5.7
Field of View (mm)	192 x 176	232 x 256	232 x 256	256 x 224
Flip Angle (°)	8	8	8	8
Number of Slices	144	170	170	160
Slice Thickness (mm)	1	1	1	1
Slice Gap (mm)	0	0	0	0
Scan Time (sec)	160	251	251	614

Supplementary Table S2. Overview of demographic, audiometric, questionnaires, morphometric data for the four groups.

Group	Normal Hearing without Tinnitus	Normal Hearing with Tinnitus	Hearing Loss without Tinnitus	Hearing Loss with Tinnitus	Statistic
<i>Demographic</i>					
n	77	35	46	70	-
Mean Age (years)	40.2 ± 13.7 ^{c,d} (18 – 67)	44.6 ± 11.7 ^{c,d} (19 – 62)	59.6 ± 10.6 ^{a,b} (33 – 80)	58.3 ± 9.6 ^{a,b} (27 – 76)	F(3,103.9) = 41.0, p < 0.001
Sex (male female)	31 46	18 17	31 15	51 19	X ² (3) = 18.5, p < 0.001
Handedness (L R NP NA)	9 63 0 5	3 32 0 0	4 41 0 1	5 52 2 11	-
<i>Audiometric (for both ears 0.25 to 8 kHz)</i>					
Mean PTA (dB HL)	9.1 ± 6.7 ^{c,d}	8.6 ± 6.7 ^{c,d}	33.3 ± 12.6 ^{a,b}	36.1 ± 9.9 ^{a,b}	F(3,100.7) = 161.6, p < 0.001
Mean LF-PTA (dB HL)	7.0 ± 5.9 ^{c,d}	5.6 ± 6.3 ^{c,d}	21.9 ± 13.7 ^{a,b}	22.4 ± 13.8 ^{a,b}	F(3,40.7) = 98.3, p < 0.001
Mean HF-PTA (dB HL)	11.3 ± 8.8 ^{c,d}	11.6 ± 9.4 ^{c,d}	44.7 ± 15.6 ^{a,b}	49.7 ± 12.6 ^{a,b}	F(3,192.1) = 99.8, p < 0.001
<i>Questionnaires</i>					
HQ	11.7 ± 6.0 ^{b,d} (3 – 27)	23.2 ± 6.5 ^{a,c,d} (10 – 35)	13.8 ± 8.3 ^{b,d} (2 – 33)	18.2 ± 8.0 ^{a,b,c} (0 – 33)	F(3,100.5) = 28.3, p < 0.001
HQ-Attentional	4.1 ± 2.4 ^b (0 – 10)	7.1 ± 2.2 ^{a,c,d} (3 – 12)	3.9 ± 2.3 ^b (0 – 9)	5.1 ± 2.4 ^b (0 – 9)	F(3,102.3) = 16.9, p < 0.001
HQ-Social	3.1 ± 2.4 ^{b,c,d} (0 – 11)	8.7 ± 3.9 ^{a,c} (1 – 16)	5.6 ± 3.6 ^{a,b,d} (0 – 14)	7.5 ± 4.0 ^{a,c} (0 – 16)	F(3,92.4) = 32.8, p < 0.001
HQ-Emotional	4.3 ± 2.8 ^{b,d} (0 – 11)	7.3 ± 2.3 ^{a,c} (2 – 12)	4.3 ± 3.3 ^{b,d} (0 – 10)	5.9 ± 2.9 ^{a,c} (0 – 12)	F(3,102.7) = 14.1, p < 0.001
HADS-Anxiety	3.9 ± 2.7 ^b (0 – 11)	6.6 ± 3.7 ^{a,c,d} (0 – 16)	3.3 ± 3.1 ^b (0 – 11)	4.6 ± 3.7 ^b (0 – 16)	F(3,98.9) = 6.7, p < 0.001
HADS-Depression	2.2 ± 2.5 ^{b,d} (0 – 11)	4.9 ± 3.7 ^{a,c} (0 – 15)	2.8 ± 3.5 ^b (0 – 13)	4.5 ± 4.0 ^a (0 – 16)	F(3,96.5) = 8.4, p < 0.001
THI	-	40.4 ± 20.8 (4 – 88)	-	32.7 ± 20.8 (4 – 82)	t(69.2) = 1.8, p = 0.081
THI-Functional	-	22.6 ± 11.3 (0 – 44)	-	19.3 ± 11.6 (0 – 44)	t(71.0) = 1.4, p = 0.176
THI-Emotional	-	9.9 ± 8.0 (0 – 30)	-	7.7 ± 6.9 (0 – 26)	t(60.6) = 1.3, p = 0.183
THI-Catastrophic	-	7.9 ± 4.6 ^d (0 – 16)	-	5.7 ± 4.0 ^c (0 – 20)	t(61.4) = 2.5, p = 0.016
<i>Morphometric</i>					
WOIQ (%)	81.1 ± 5.3 ^b	76.1 ± 1.9 ^{a,c,d}	80.1 ± 5.5 ^b	82.2 ± 4.0 ^b	F(3,115.8) = 43.2, p < 0.001
TIV (cm ³)	1463.6 ± 145.1	1422.6 ± 149.7	1472.7 ± 133.2	1481.8 ± 126.4	F(3,103.0) = 1.4, p = 0.256

Mean ± Standard Deviation (Range). dB HL decibel hearing loss; HADS hospital anxiety depression scale; HF-PTA high frequencies pure tone average (2 to 8 kHz); HQ hyperacusis questionnaires; L left; LF-PTA low frequencies pure tone average (0.25 to 1 kHz); NA not available; NP no preference; PTA pure tone average; R right; THI tinnitus handicap inventory; WOIQ weighted overall image quality. ^aThe group differed significantly (p ≤ 0.05) from the normal hearing group without tinnitus; ^bThe group differed significantly (p ≤ 0.05) from the normal hearing group with tinnitus; ^cThe group differed significantly (p ≤ 0.05) from the hearing loss group without tinnitus; ^dThe group differed significantly (p ≤ 0.05) from the hearing loss group with tinnitus

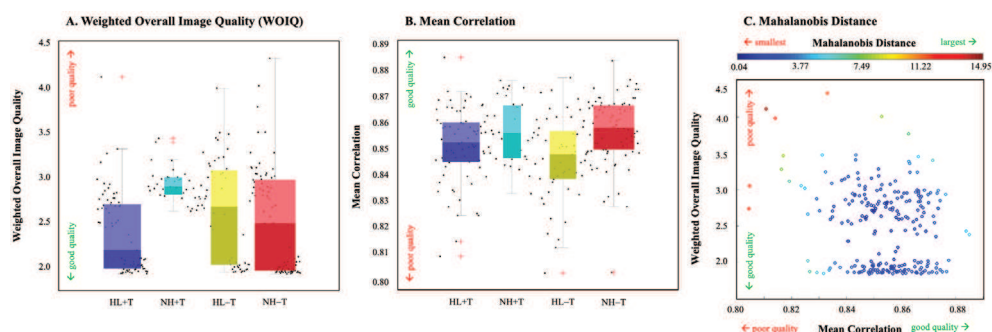
Supplementary Table S3. Results of the partial correlation coefficients between each VOI and the HADS anxiety and depression, HQ hyperacusis, or THI tinnitus burden scores, including total intracranial volume (TIV) as a covariate ($p \leq 0.001$ or $p \leq 0.05/48$, corrected for the number of tests).

Area	HADS Anxiety	HADS Depression	HQ Hyperacusis	THI Tinnitus Burden
IOG / FFG (left)	$r = 0.09, p = 0.384$	$r = 0.01, p = 0.925$	$r = 0.18, p = 0.074$	$r = 0.12, p = 0.256$
Thal_MD (bilateral)	$r = 0.17, p = 0.101$	$r = 0.02, p = 0.815$	$r = 0.19, p = 0.054$	$r = 0.07, p = 0.508$
REC (left)	$r = 0.18, p = 0.081$	$r = 0.04, p = 0.710$	$r = 0.03, p = 0.797$	$r = 0.08, p = 0.416$
CERpost / CERCRU2 (left)	$r = -0.01, p = 0.938$	$r = 0.06, p = 0.565$	$r = 0.07, p = 0.514$	$r = 0.16, p = 0.102$
HES / INSpst / ROL (left)	$r = 0.06, p = 0.561$	$r = -0.01, p = 0.942$	$r = -0.02, p = 0.838$	$r = 0.18, p = 0.067$
HES / INSpst / ROL (right)	$r = 0.13, p = 0.189$	$r = 0.06, p = 0.552$	$r = 0.07, p = 0.482$	$r = 0.20, p = 0.042$
LING / PHG / PCUN (left)	$r = 0.15, p = 0.136$	$r = 0.16, p = 0.113$	$r = 0.07, p = 0.521$	$r = 0.14, p = 0.182$
LING / PHG / PCUN (right)	$r = 0.05, p = 0.596$	$r = 0.03, p = 0.750$	$r = 0.01, p = 0.956$	$r = 0.06, p = 0.555$
PFCventmed (bilateral)	$r = -0.07, p = 0.474$	$r = -0.11, p = 0.290$	$r = 0.01, p = 0.919$	$r = 0.02, p = 0.847$
IFGoperc (right)	$r = 0.03, p = 0.751$	$r = 0.001, p = 0.989$	$r = 0.10, p = 0.324$	$r = 0.03, p = 0.793$
Thal_VPL / PALlat / Thal_MGN (left)	$r = 0.08, p = 0.452$	$r = 0.001, p = 0.992$	$r = 0.23, p = 0.010$	$r = 0.11, p = 0.264$
Thal_VPL / PALlat / Thal_MGN (right)	$r = -0.01, p = 0.917$	$r = -0.06, p = 0.536$	$r = 0.13, p = 0.202$	$r = -0.13, p = 0.310$

Automated Anatomical Atlas 3 (AAL3) labelling: CERpost posterior cerebellum; CERCRU2 cerebellum crus-II; FFG fusiform gyrus; HES Heschl's gyrus; IFGoperc inferior frontal gyrus (opercular part); INSpst posterior insula; IOG inferior occipital gyrus; LING lingual gyrus; PALlat lateral globus pallidum (lenticular nucleus); PCUN precuneus; PFCventmed ventromedial prefrontal cortex; PHG parahippocampal gyrus; REC gyrus rectus; ROL rolandic operculum; Thal_MD mediodorsal thalamic nuclei; Thal_MGN medial geniculate thalamic nuclei; Thal_VPL ventral posterolateral thalamic nuclei.

Image Quality Control

Preprocessing of voxel-based morphometry (VBM) analysis is highly dependent on image quality. In particular, multi-centre data sharing studies need to take into account varying image properties due to different scanners, sequences and protocols. However, even images acquired with one scanner using the same sequence and protocol may vary due to disparate artifacts (e.g., motion artifacts). In this study, image quality control was performed at both subject and group levels using CAT12.7 toolbox. At subject level, the normalized modulated gray matter images were visually inspected to quickly check whether the normalization was successful for all images. At group level, the normalized modulated gray matter images were automatically inspected for homogeneity on the basis of a weighted overall image quality (WOIQ) and a mean voxel-based correlation between gray matter images, thus allowing to identify any outliers of the entire study sample (Supplementary Figure S1).



Supplementary Figure S1. Group level image quality control using CAT12.7 toolbox. (A) The weighted overall image quality (WOIQ) ratio combines measurements of noise and spatial resolution of the images before preprocessing. A low ratio indicates good image quality before preprocessing. (B) Mean voxel-based correlation score measures the homogeneity of the entire study sample after preprocessing. Mean correlation score for most images is above 80%, representing these images are more similar to each other. (C) Mahalanobis distance matrix on the basis of a WOIQ ratio and a mean voxel-based correlation score makes it possible to combine above two measures of image quality before and after preprocessing. Outliers indicate either a problem with image quality (e.g., motion artifacts), or a variation in the brain anatomy (e.g., enlarged ventricles). HL+T hearing loss group with tinnitus; HL-T hearing loss group without tinnitus; NH+T normal hearing group with tinnitus; NH-T normal hearing group without tinnitus.

DATA AVAILABILITY STATEMENT

The unthresholded statistical parametric map supporting the main finding of this study is openly available at neurovault.org (<https://identifiers.org/neurovault.collection:22787>).

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CONFLICTS OF INTEREST

The authors report no competing interests.



Chapter 4

Hyperacusis is associated with smaller gray matter volumes in the supplementary motor area

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ABSTRACT

Hyperacusis is a disorder in loudness perception characterized by increased sensitivity to ordinary environmental sounds and associated with otologic conditions, including hearing loss and tinnitus (the phantom perception of sound) as well as neurologic and neuropsychiatric conditions. Hyperacusis is believed to arise centrally in the brain; however, the underlying causes are unknown. To gain insight into differences in brain morphology associated with hyperacusis, we undertook a retrospective case-control study comparing whole-brain gray matter morphology in participants with sensorineural hearing loss and tinnitus who either scored above or below the threshold for hyperacusis based on a standard questionnaire. We found that participants reporting hyperacusis had smaller gray matter volumes and cortical sheet thicknesses in the right supplementary motor area (SMA), independent of anxiety, depression, tinnitus burden, or sex. In fact, the right SMA volumes extracted from an independently defined volume of interest could accurately classify participants. Finally, in a subset of participants where functional data were also available, we found that individuals with hyperacusis showed increased sound-evoked responses in the right SMA compared to individuals without hyperacusis. Given the role of the SMA in initiating motion, these results suggest that in hyperacusis the SMA is involved in a motor response to sounds.

Keywords

Audition

Hearing loss

Hypersensitivity

Sound-evoked functional MRI

Surface-based morphometry

Tinnitus

Voxel-based morphometry.

Highlights

- Hyperacusis is characterized by hypersensitivity to ordinary environmental sounds.
- Gray matter in the supplementary motor area is reduced in hyperacusis.
- Sound-evoked responses are increased in the same brain region.
- Our results suggest impaired motor responses to sound in hyperacusis.

1. INTRODUCTION

Hyperacusis is characterized by an increased sensitivity to ordinary environmental sounds of mild to moderate intensity [1–4]. Environmental sounds that are not uncomfortably loud for most people can be bothersome or even painful for individuals with hyperacusis and trigger avoidance behaviors [3, 4]. Hyperacusis is distinguished from misophonia, which involves strong emotional reactions to specific sounds, like someone chewing or whispering [5, 6]. The overall prevalence of hyperacusis in the general population is estimated to be about 17% [7]. While hyperacusis can occur as a primary complaint, it is frequently comorbid with conditions such as Williams syndrome and autism (reported prevalence of 95% and 63%, respectively) [7] and can occur following acute head trauma, neck injury, and various otologic conditions, many involving stapedial reflex dysfunctions [2, 3, 5, 8–10]. In the adult population, hyperacusis is strongly associated with hearing loss and tinnitus [7, 11]. Indeed, up to 90% of individuals with hyperacusis report concurrent tinnitus [1], up to 63% of individuals with tinnitus report hyperacusis [7], and up to 59% of individuals with hyperacusis have hearing loss [12]. In addition, hyperacusis has been associated with anger, anxiety, depression, frustration, insomnia, and stress, resulting in a significant reduction in the quality of life [2, 3, 13].

Electrophysiological studies in animals with temporary hearing loss induced by salicylate exposure or noise-overexposure suggest that hyperacusis could be the consequence of increased neural gain along the auditory pathway [14–17]. In this model, increased central gain compensates for the loss of peripheral auditory input and results in an enhanced cerebral response to external sounds. In support of this hypothesis, functional neuroimaging studies in humans have found hyperacusis to be associated with enhanced sound-evoked responses in the auditory cortex and the inferior colliculus [18, 19]. Interestingly, although decreased sound tolerance is likely an auditory phenomenon, a previous investigation using resting-state electroencephalography concluded that non-auditory brain regions might also be involved in the pathophysiology of hyperacusis, with the orbitofrontal cortex, the anterior cingulate cortex, and the supplementary motor area showing enhanced beta waves at rest [20].

In this retrospective case-control study, we examined gray matter differences associated with hyperacusis in a group of adults with sensorineural

hearing loss and tinnitus who had taken part in previous neuroimaging studies in our laboratory. We provide convincing evidence that hyperacusis is associated with structural and functional differences in the right supplementary motor area (SMA). These findings provide new insights into the structural and functional mechanisms that might underlie hyperacusis.

2. MATERIALS AND METHODS

The data for this study were gathered from two previous neuroimaging studies conducted between 2004 and 2019 at the Department of Otorhinolaryngology of the University Medical Centre Groningen (UMCG) [19, 21]. Studies were conducted in accordance with the principles of the Declaration of Helsinki and approved by the UMCG medical ethical committee (METc), which also gave approval for the reanalysis of the data (METc number: 2020/347). All participants gave written informed consent. Participants structural MRI data and hearing thresholds were available from both studies, as well as responses to standard questionnaires about anxiety, depression, handedness, hyperacusis, and tinnitus burden [19, 21]. Sound-evoked functional MRI data from one of the original studies were also re-investigated in a supplementary analysis [19].

2.1. Participants

In total, 73 participants with bilateral sensorineural hearing loss and tinnitus were included from the two studies [19, 21]. Hearing thresholds were assessed with pure tone audiometry at octave frequencies ranging from 0.25 to 8 kHz. Hyperacusis was evaluated with the Hyperacusis Questionnaire (HQ) [22]. In addition, tinnitus burden was measured with the Tinnitus Handicap Inventory (THI) [23], the presence of anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HADS) [24], and handedness was measured with the Edinburgh Handedness Inventory (EHI) [25]. Four participants were excluded from the current investigation due to missing data, and three additional participants were excluded as a result of low image quality (see section 2.3. MRI data preprocessing and quality control). Sixty-six participants were therefore included in the main analyses.

A cut-off score of ≥ 22 on the 14-item Hyperacusis Questionnaire was used to define the presence or absence of hyperacusis. This cut-off ensures that 95% of the participants classified with hyperacusis have an

Uncomfortable Loudness Level below or equal to 77 dB Hearing Level (dB HL) [26]. Participants who score below 22, on the other hand, can tolerate pure tones of 86 ± 12 dB HL [26]. With these criteria, 25 (38%) of the 66 participants in our group were classified as having hyperacusis (mean age $\pm$ standard deviation = 59.5 ± 7.9 years, 10 females), and 41 participants who scored below the cut-off score were included in the control group (mean age $\pm$ standard deviation = 58.3 ± 10.5 years, 9 females).

2.2. Brain imaging

For each participant, high-resolution 3-dimensional T1-weighted anatomical images were obtained using a 3-Tesla Philips Intera scanner (Philips Medical System, Best, The Netherlands) equipped with a phase-array head coil (SENSE) with either 8-channel [21] or 32-channel [19]. Fast-field echo images were acquired with the following parameters for the two studies [19/21]: repetition time = 9/10.4 ms, echo time = 3.5/5.7 ms, field of view = $232 \times 256/256 \times 224$ mm, flip angle = 8° , number of slices = 170/160, slice thickness = 1 mm, slice gap = 0 mm, scan time = 251/614 sec, reconstructed voxel size = $1 \times 1 \times 1$ mm.

In a supplementary analysis, sound-evoked functional MRI data from one of the original studies were also re-investigated and included [19]. In this study, the cerebral response to pure tones of various frequencies (0.25 to 8 kHz, loudness matched to a 1 kHz tone at 40 dB SPL) was recorded with echo-planar imaging in a sparse-sampling experiment (repetition time = 10 sec, acquisition time = 2 sec, stimulus duration = 7.5 sec, reconstructed voxel size = $2 \times 2 \times 2$ mm, full brain coverage). Activation evoked by the pure tones was contrasted with a silence condition. During both the tone and silence conditions, participants had to perform a concurrent visual valence task, which involved a motor response on a keypad. Statistical parametric maps of the contrasts between sound and silence were retrieved from this study for each available participant and used to compare the sound-evoked cerebral response between two groups under investigation here.

2.3. MRI preprocessing and quality control

T1-weighted anatomical images were preprocessed using CAT12.7 (v.1653, Structural Brain Mapping Group, University of Jena, Germany, <http://dbm.neuro.uni-jena.de/cat12/>) and SPM12 (v.7487, Wellcome Centre for Human Neuroimaging, University College London, <https://www.fil.ion.ucl.ac.uk/spm/>), in MATLAB R2020a (The MathWorks, Natick, MA, USA). Prior to preprocessing, REC/PAR Philips images were converted to NIFTI

(Neuroimaging Informatics Technology Initiative) format using dcm2niix (v.1.2.20200331, <https://github.com/rordenlab/dcm2niix/>). Preprocessing steps followed the recommendations of CAT12.7. Briefly, T1-weighted anatomical images were initially corrected for bias, noise, and global intensity, and then segmented into gray matter, white matter, and cerebrospinal fluid (CSF) images. Subsequently, gray matter images were normalized to the Montreal Neurological Institute (MNI152) template using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) algorithm, using affine followed by non-linear registration [27]. Normalized gray matter images were resampled to $1.5 \times 1.5 \times 1.5$ mm voxels using a nearest neighbour interpolation. To account for volume changes induced by normalization, the normalized gray matter images were modulated by the resulting Jacobian determinant [27].

The quality of the normalized modulated gray matter images was assessed in two steps. First, gray matter images were visually inspected to detect potential segmentation issues (e.g., tissue misclassification). Second, gray matter image homogeneity was assessed in CAT12.7 based on the weighted overall image quality index, and the average voxel-wise correlation between images. This second step led to the removal of three datasets with a Mahalanobis distance larger than 1.5 IQR compared to the rest of the data. Finally, the normalized modulated gray matter images were smoothed using a Gaussian smoothing kernel of 12 mm full-width half-maximum (FWHM).

2.4. Statistical analyses

2.4.1. Demographic, audiometric, and questionnaire data

Statistical analyses of demographic, audiometric, and questionnaires data were performed using SPSS (v.27, IBM Corp., Armonk, New York, USA). For the categorical variable sex, a Chi-square test was used to test whether groups differed in sex distribution. Based on the outcome of the Shapiro-Wilk normality test, continuous variables were tested for between-group comparisons using either independent samples Welch's t-tests or independent samples Mann-Whitney U tests. Lastly, Spearman's Rank correlation coefficients were computed to assess the strength and direction of relationships between pairs of questionnaire scores. A statistical threshold of $p \leq 0.05$ was used for all analyses.

2.4.2. Whole-brain gray matter volume analyses

Between-group differences in gray matter volumes were investigated voxel-wise with a General Linear Model (GLM). Two whole-brain two-sample t-test analyses were conducted with different sets of covariates to test for the effect of possible confounds. The total intracranial volume (TIV; computed as the sum of the gray, white, and CSF compartments) was entered as a covariate in the model in both analyses to correct for differences in brain sizes [28]. In the first analysis, TIV was the only covariate. In the second analysis, age, handedness scores, and hearing thresholds were entered into the model with TIV. Hearing thresholds were calculated as the pure tone average (PTA) in dB HL across all tested frequencies (0.25, 0.5, 1, 2, 4, and 8 kHz) for both ears. A statistical threshold of $p \leq 0.05$ family-wise error (FWE) corrected at the voxel-level was used for both analyses. Although very strict, this statistical threshold is recommended for voxel-based morphometry (VBM) analyses [29]. It greatly reduces the reporting of false positive findings and allows us drawing conclusions at the voxel by opposition to cluster level.

2.4.3. Volume-of-interest analyses in the right SMA

Considering the primary results, several supplementary analyses were conducted in a volume-of-interest (VOI) restricted to the right supplementary motor area (SMA). The right SMA VOI was obtained from a published meta-analysis of functional MRI data, which used both task-related activation coordinates and anatomical boundaries to delineate motor cortex areas (freely available as the Human Motor Area Template (HMAT), <http://lrnlab.org/>) [30]. Gray matter volumes were extracted in this right SMA VOI for each participant using `get_totals` MATLAB script (freely available from http://www0.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m/) and further analysed in SPSS.

2.4.3.1. Effect of anxiety, depression, tinnitus burden

To assess the possible impact of anxiety, depression, or tinnitus burden on the results, Spearman's Rank correlation coefficients between the right SMA gray matter volumes (HMAT VOI) and either the HADS or the THI scores were computed across all participants using a statistical threshold of $p \leq 0.05$.

2.4.3.2. Effect of sex

2×2 between-groups ANOVA was conducted to examine the possible interaction between the effect of sex and hyperacusis on the right SMA gray matter volumes (HMAT VOI) using a statistical threshold of $p \leq 0.05$.

2.4.3.3. Receiver operating characteristic curve

The receiver operating characteristic (ROC) curve was obtained from the data to evaluate the possibility of classifying participants with hyperacusis based on their right SMA gray matter volumes (HMAT VOI). The ROC analysis was conducted in SPSS using logistic regressions.

2.4.3.4. Cortical thickness

Cortical thickness was estimated in CAT12.7 by surface-based morphometry using topological correction and spherical mapping [31, 32]. Cortical thickness VOI of the right SMA was extracted from the Human Connectome Project Multi-Model Parcellation (HCP-MMP1) Atlas [33] and compared between groups with independent samples Welch's t-test using a statistical threshold of $p \leq 0.05$.

2.4.3.5. Sound-evoked activity

The Blood Oxygen Level Dependent (BOLD) response to sounds was compared between participants with and without hyperacusis. The analysis was conducted within the right SMA (HMAT VOI). The average BOLD response to sounds was compared between groups using SPM12 ($p \leq 0.05$ FWE corrected at the voxel level), corrected for age and sex. statistical threshold of $p \leq 0.05$ was used for all analyses.

3. RESULTS

3.1. Group characteristics

The groups of participants with and without hyperacusis did not differ significantly in terms of age ($U = 507$, $Z = -0.1$, $p = 0.942$), sex distribution [$\chi^2(1) = 2.5$, $p = 0.116$], total intracranial volume [$t(62.9) = -1.5$, $p = 0.135$], or weighted overall image quality ($U = 392.5$, $Z = -1.6$, $p = 0.113$). Pure tone hearing thresholds were well matched between groups across all tested frequencies (Figure 1), and the pure tone averages (PTA 0.25–8 kHz, PTA 0.25–1 kHz, and PTA 2–8 kHz) for both ears did not differ between groups [PTA 0.25–8 kHz: $U = 401.5$, $Z = -1.2$, $p = 0.218$; PTA 0.25–1 kHz: $U = 449.5$, $Z = -0.6$, $p = 0.563$; PTA 2–8 kHz: $t(46.2) = 0.9$, $p = 0.381$]. However, compared to controls, participants with hyperacusis scored significantly higher on measures of depression (HADS depression: $U = 303$, $Z = -2.7$, $p = 0.007$) as well as tinnitus burden (THI: $U = 280.5$, $Z = -2.8$, $p = 0.006$). There was also a trend towards higher scores on measures of anxiety (HADS anxiety: $U = 365$, $Z = -1.8$, $p = 0.067$) in participants with hyperacusis compared to controls. Demographic, au-

diometric, and questionnaires data for both groups are summarized in Table 1 (more details: Supplementary Table S1).

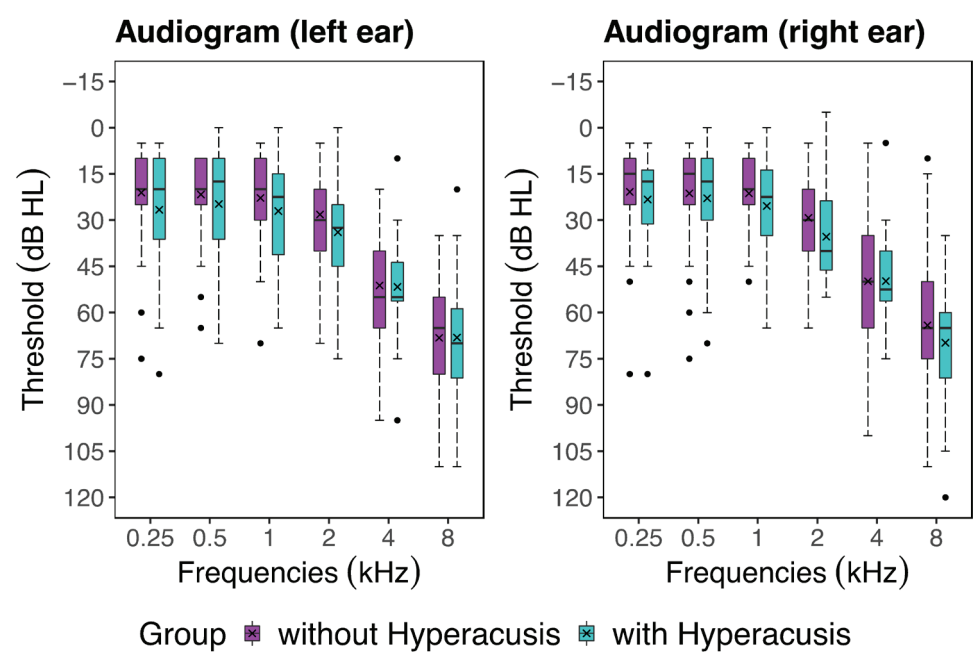


Figure 1. Audiometric assessments for the groups with and without hyperacusis. All participants had sensorineural hearing loss and tinnitus. There was no group difference in hearing thresholds (dB HL) at any of the tested frequencies.

Table 1. Summarized demographic, audiometric, and clinical data for the groups with and without hyperacusis.

	Group without Hyperacusis	Group with Hyperacusis
Demographic		
<i>n</i>	41	25
Age (years)	58.3 ± 10.5 (27 – 76)	59.5 ± 7.9 (41 – 73)
Sex (male / female)	(32 / 9)	(15 / 10)
Audiometric (PTA 0.25 – 8 kHz for both ears)		
Hearing levels dB HL	35 ± 8.6	38.2 ± 12.3
Questionnaires		
HQ Hyperacusis	13.2 ± 5.2 (0 – 21)	26.3 ± 3.7 (22 – 33)*
HADS Anxiety	3.7 ± 3 (0 – 11)	5.9 ± 4.6 (0 – 16)
HADS Depression	3.3 ± 3.1 (0 – 10)	6.2 ± 4.7 (0 – 16)*
THI Tinnitus burden	27.4 ± 19.4 (4 – 80)	41.8 ± 20.9 (6 – 82)*
Mean ± Standard Deviation (Range). * <i>p</i> ≤ 0.05.		

3.2. Whole-brain VBM reveals smaller right SMA gray matter volumes in participants with hyperacusis

Gray matter volume differences between groups were assessed voxel-wise across the whole brain in two analyses controlling for different sets of potential confounds ($p_{FWE} \leq 0.05$ at the voxel level). In the first analysis, the total intracranial volume (TIV) was the only covariate. This analysis revealed a single cluster (244 voxels) of smaller gray matter volumes in the right supplementary motor area of participants with hyperacusis (Supplementary Figure S1, Supplementary Table S2). In the second analysis, age, handedness scores, and hearing thresholds (PTA 0.25–8 kHz for both ears) were added as covariates in the model together with TIV. This analysis returned very similar results, identifying a single cluster (265 voxels) of smaller gray matter volumes in the right supplementary area of participants with hyperacusis (Figure 2, Table 2). No any brain region showed a significant increase in gray matter volumes associated with hyperacusis. In addition, we re-ran the analysis using the less conservative threshold free cluster enhancement technique (TFCE with FWE correction $p \leq 0.05$ using peak-cluster-level). We found a similar albeit larger cluster (14,276 voxels), showing smaller gray matter volumes in participants with hyperacusis compared to those without hyperacusis (Supplementary Figure S2, Supplementary Table S3). This larger cluster encompassed the SMA, pre-supplementary motor area (Pre-SMA), and precentral gyrus (PrC) bilaterally. However, we did not observe any other significant gray matter differences between two groups with and without hyperacusis. Furthermore, we observed that our finding was replicated when treating hyperacusis as a continuous rather than a categorical variable and that the negative correlation between the right SMA gray matter volumes (HMAT VOI) and the hyperacusis scores was largely driven by the attentional subscale of the Hyperacusis Questionnaire (Supplementary Table S4, Supplementary Table S5).

3.3. Anxiety, depression, or tinnitus burden do not drive smaller gray matter volumes in hyperacusis

The average scores on scales evaluating depression and tinnitus burden differed significantly between participants with and without hyperacusis (Table 1). However, we found no significant correlation between the right SMA gray matter volumes (HMAT VOI) and either the HADS anxiety [$r_s(63) = -0.04$, $p = 0.765$], the HADS depression [$r_s(63) = -0.06$, $p = 0.614$], or the THI tinnitus burden [$r_s(62) = -0.07$, $p = 0.607$] scores. These variables, therefore, do not appear to drive the gray matter vol-

umes difference observed in the right SMA between participants with and without hyperacusis.

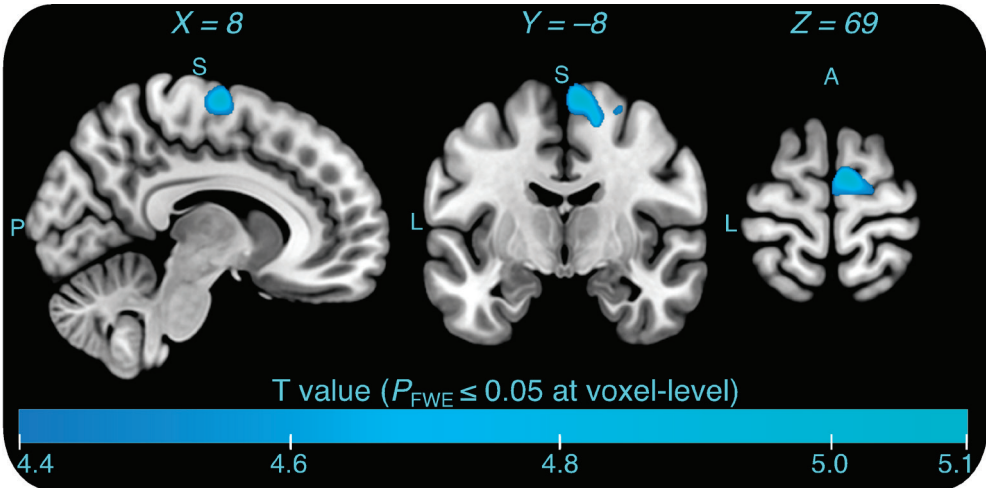


Figure 2. Results of the whole-brain between groups comparison showing smaller gray matter volumes in the right supplementary motor area (SMA) of participants with hyperacusis compared to those without hyperacusis. The analysis accounted for age, handedness score, hearing thresholds (PTA 0.25 – 8 kHz for both ears), and total intracranial volume (TIV) as confounding variables. One single cluster (265 voxels) of gray matter volumes difference was found in the entire brain, revealing smaller gray matter volumes in the right SMA of participants with hyperacusis, shown in cold color map (peak MNI X, Y, Z – coordinates = 8, –8, 69, T = 5.1, pFWE = 0.016 at the voxel level).

Table 2. Results of whole-brain two-sample t-test analysis between the groups with and without hyperacusis including age, handedness score, hearing thresholds (PTA 0.25 – 8 kHz for both ears), and total intracranial volume (TIV) as covariates (pFWE ≤ 0.05 at the voxel-level, height threshold T = 4.4).

Area	pFWE voxel-level	pFWE cluster-level	MNI coordinates			Cluster size k	T-value	Z-value
			X	Y	Z			
SMA (right)	Group without Hyperacusis > Group with Hyperacusis							
	0.016		8	−8	69		5.1	4.6
	0.027	0.004	15	−6	58	265	4.5	4.5
	0.038		21	−15	64		4.4	4.4
	Group without Hyperacusis < Group with Hyperacusis							
NS	−	−	−	−	−	−	−	−

NS not significant.

3.4. No interaction between effect of sex and hyperacusis

Since a previous meta-analysis reported differences in the gray matter volumes of the SMA between males and females [34], effect of sex could, therefore, potentially interact with effect of hyperacusis. However, 2×2 between-groups ANOVA on the right SMA gray matter volumes (HMAT VOI) revealed a significant effect of hyperacusis [$F(1,62) = 18.4$, $p < 0.001$], a trend towards an effect of sex [$F(1,62) = 3.6$, $p = 0.064$], but no significant interaction between effect of sex and hyperacusis [$F(1,62) = 0.2$, $p = 0.641$].

3.5. Right SMA gray matter volumes can accurately classify participants with hyperacusis

The receiver operating characteristic (ROC) curve was computed to verify that participants with hyperacusis could reliably be classified based on their right SMA gray matter volumes (HMAT VOI). The area under the curve (AUC) was 0.82 ± 0.1 ($p < 0.001$), 95% CI = (0.7, 0.9), demonstrating a very good classification performance (Figure 3). Almost 60% of participants with hyperacusis (identified by their questionnaire scores) could be classified with <5% false positives based on their right SMA gray matter volumes.

3.6. Thinner right SMA cortical sheet in participants with hyperacusis

To test whether differences in the right SMA cortical sheet thickness could explain the smaller cortical volumes in this area, thickness values in the right SMA (HPC-MMP1 VOI) were compared between groups. In addition to smaller cortical volumes, the analysis revealed that the right SMA cortical sheet was thinner in individuals with hyperacusis compared to controls [$t(42.9) = -2.7$, $p = 0.010$, 95% CI = (-0.3, -0.04)] (Figure 4). Therefore, it appears that differences in gray matter volumes associated with hyperacusis are at least partly driven by differences in cortical thickness.

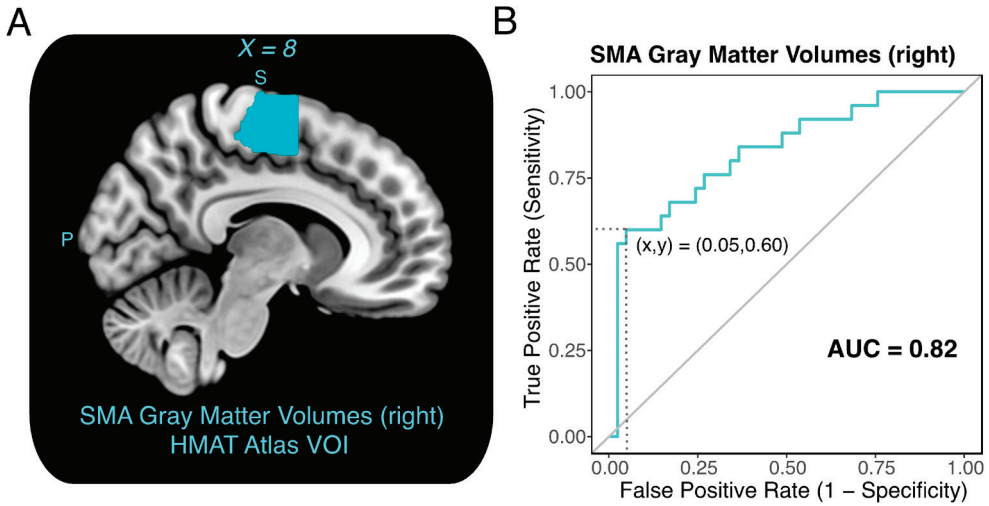


Figure 3. A receiver operating characteristic (ROC) analysis demonstrated the capability to classify patients with hyperacusis based on the gray matter volumes in an independently defined right SMA volume-of-interest. (A) The right SMA volume-of-interest (VOI in cyan color) was defined according to the Human Motor Area Template (HMAT) atlas, derived from anatomical and functional landmarks. (B) ROC curve for the detection of participants with hyperacusis based on right SMA gray matter volumes; a classifier was able to distinguish participants with or without hyperacusis with an area under the curve of 0.82 ($p < 0.001$, 95%CI 0.72–0.93). About 60% of participants with hyperacusis could be classified based on their right SMA gray matter volumes with less than 5% false positives.

3.7. Sound-evoked activity in the right SMA is elevated in participants with hyperacusis

In a final analysis, we tested the hypothesis that the right SMA would show increased responsiveness to sounds in individuals with hyperacusis, which would suggest a link between sound-evoked cerebral activity and decreased gray matter volumes and thickness in the right SMA. Sound-evoked functional MRI data were available from a subset of 10 participants with hyperacusis (mean age $\pm$ standard deviation = 58.8 ± 9.1 years, 1 female) and 18 controls (mean age $\pm$ standard deviation = 58.9 ± 10.9 years, 3 females). The two groups were well-matched in terms of age [$U = 83.5$, $Z = -0.3$, $p = 0.755$], sex distribution [$\chi^2(1) = 0.2$, $p = 0.629$], and hearing thresholds [PTA 0.25–8 kHz: $t(16.2) = -0.5$, $p = 0.645$]. The cerebral activity in the right SMA (HMAT VOI) in response to the presentation of pure tones relative to the activity during a silence condition was compared between the two groups of participants. We found a higher BOLD response ($p\text{FWE} \leq 0.05$ at the voxel level) in the

right SMA of participants with hyperacusis relative to controls, corrected for age and sex (Figure 5).

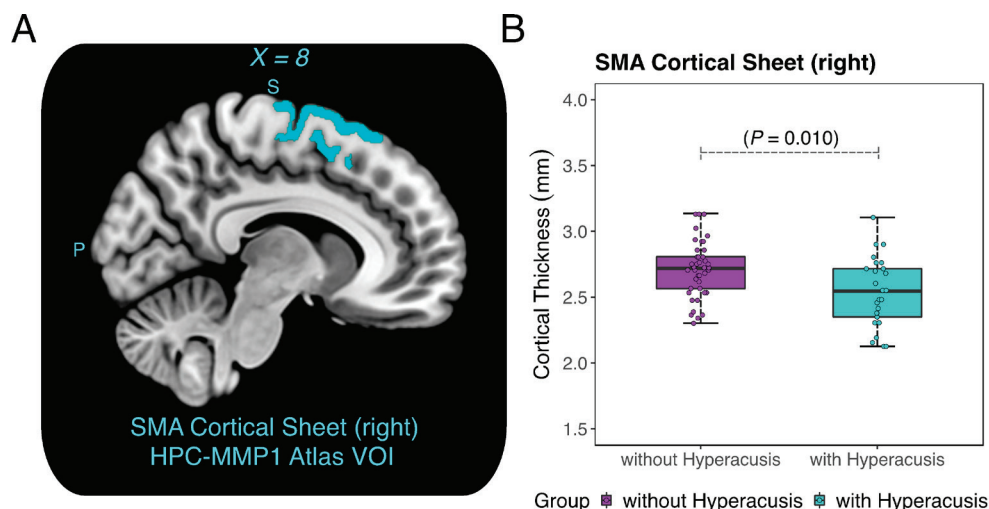


Figure 4. Thinner right supplementary motor area (SMA) cortical sheet in individuals with hyperacusis. (A) A sagittal view of the right SMA volume-of-interest (VOI in cyan color) of cortical sheet according to the Human Connectome Project Multi-Model Parcellation (HPC-MMP1) Atlas. (B) The surface-based VOI analysis revealed that the right SMA cortical sheet was thinner in participants with hyperacusis relative to those without hyperacusis [$t(42.9) = -2.7$, $p = 0.010$].

4. DISCUSSION

The aim of this retrospective study was to investigate potential differences in gray matter associated with hyperacusis. Sixty-six participants with sensorineural hearing loss and tinnitus were included in the final analysis, of whom twenty-five (38%) met the criteria for hyperacusis [22, 26]. The two groups were well-matched in terms of age, sex distribution, and hearing thresholds. We found that individuals with hyperacusis had smaller gray matter volumes in the right supplementary motor area (SMA) even when considering the potentially confounding effects of age, handedness scores, and hearing thresholds. We found no significant effect of anxiety, depression, or tinnitus burden, and no significant interaction between sex and hyperacusis on the right SMA gray matter volumes. In fact, we found that the right SMA gray matter volumes could accurately classify participants with hyperacusis versus controls with an 82% area under the curve. Moreover, we found that, in

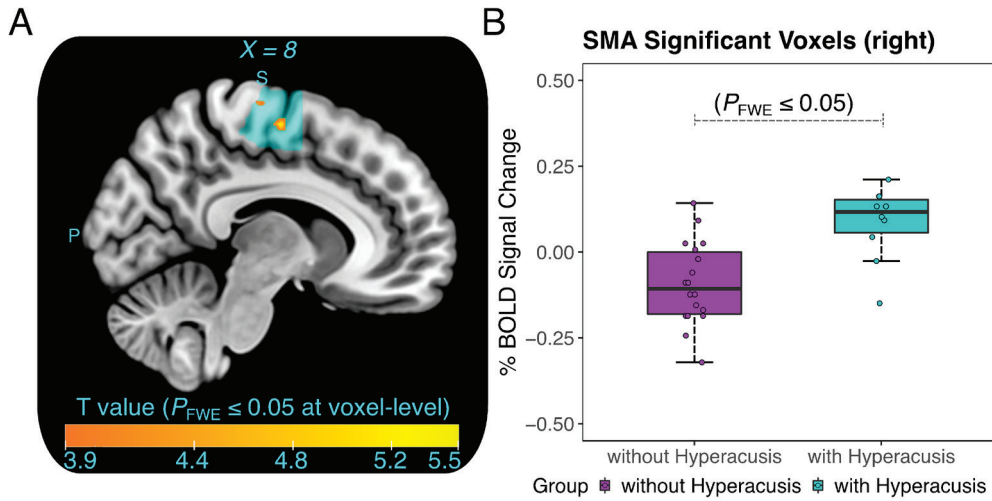


Figure 5. Higher sound-evoked activity in the right supplementary motor area (SMA) of participants with hyperacusis compared to those without hyperacusis. (A) The analysis conducted in the right SMA volume-of-interest (VOI in cyan colour, identical to the VOI in Figure 3) showed increased BOLD response to sounds versus baseline in participants with hyperacusis compared to those without hyperacusis (shown in warm color map: cluster size = 30 voxels, peak MNI X, Y, Z – coordinate = 8, -10, 60, $T = 5.5$, $p_{FWE} < 0.001$ at the voxel-level). The analysis accounted for age and sex as confounding variables. (B) Box plots of the average % BOLD signal change in the right SMA voxels showing significantly higher response to 40dB HL pure tones in participants with hyperacusis.

the hyperacusis group, the right SMA cortical sheet was thinner, which can partially explain smaller gray matter volumes in this group.

In light of the above results, we predicted that hyperacusis would be associated with enhanced sound-evoked responses in the right SMA. This pattern of larger stimulus-evoked activity and smaller gray matter volumes has been reported for instance in war veterans who developed posttraumatic stress disorder (PTSD), showing reduced gray matter volumes in the amygdala [35, 36] along with enhanced amygdala BOLD response to fearful stimuli [37]. According to expectations, we found increased BOLD responses to sounds in the right SMA of participants with hyperacusis compared to controls. The result of this complimentary analysis suggests a link between the right SMA gray matter volumes and hyper-responsiveness of this region to sound stimuli. Although we included sex as a covariate, generalizing findings to both sexes should be done cautiously given the limited number of female participants in

this analysis. Future studies should aim to include larger and more sex-balanced groups.

The discovery of hyperacusis-related differences in the right SMA gray matter morphology is, at first, perhaps surprising: the SMA is remote from the auditory system and is known to be involved in action preparation [38]. However, previous work indicates that the SMA, in fact, contributes to auditory processing and auditory imagery, and a review concluded that the supplementary motor and pre-motor areas play a role in facilitating motor responses to sounds [39]. Accordingly, electrophysiological recordings in monkeys showed that some SMA neurons fire only when a movement is preceded by a specific sensory cue, for instance, an auditory stimulus but not a visual or tactile stimulus [40, 41]. These neurons do not respond to the sole presentation of the sensory cue but rather respond only if the cue is followed by appropriate action. These findings suggest that, in hyperacusis, sounds of moderate intensity might trigger motor programs that correspond to an aversive reaction to environmental sounds. Interestingly, Fried et al. [42] reported a left-right specialization of the SMA whereby bilateral movements were elicited exclusively by stimulation of the non-dominant (right) hemisphere. This finding could potentially explain why in the current study hyperacusis was associated with lower gray matter volumes in the right SMA, as the most natural reaction to perceiving a very loud noise is probably to cover one's ears.

Although the current investigation cannot confirm a causal link between hyperacusis and smaller SMA gray matter volumes and cortical thickness, our findings suggest testable hypotheses. One hypothesis could be that, in participants with hyperacusis, smaller gray matter volumes and cortical thickness and yet enhanced sound-evoked activity in the right SMA reflect the tendency to initiate movements in response to sounds perceived as too loud. In support of this hypothesis, Auerbach et al. [14] reported that inducing hearing loss and hyperacusis in rats through salicylate injection resulted in faster response latencies to sounds. The effect was dependent on sound intensity and was correlated with an increased sound-evoked neuronal response in the auditory cortex and inferior colliculus at an individual level [14]. These findings, together with our results, suggest that the initiation of a motor reaction to sound stimuli may be facilitated in participants with hyperacusis as a result of peripheral damage and increased central gain along the auditory pathway. Given that some SMA neurons are modality specific, examining whether participants with hyperacusis have shorter reaction times specifically

to auditory as opposed to visual or tactile stimuli would suggest a more direct link between increased central gain in the auditory pathway and gray matter alterations in the SMA. Furthermore, differentiating between different types of behavioural reactions to hyperacusis might increase our understanding of the causality between hyperacusis and the SMA gray matter differences. Recent studies in PTSD, for instance, have shown that gray matter changes are dependent on the type of trauma [43] as well as the type of behavioural strategy deployed (or not deployed) to avoid trauma [44]. Such an investigation would, however, require a more complete assessment of individuals with hyperacusis than afforded by the instruments available in this study [3, 45, 46].

An alternative hypothesis could be that hyperacusis results from reduced SMA gray matter volumes and thickness. In this scenario, reduced SMA gray matter volumes would allow hyperacusis to develop following sensorineural hearing loss. This hypothesis is consistent with the observation that, in general, hyperacusis is slightly more prominent in females [47], who on average have smaller SMA gray matter volumes than males [48]. However, the current study found that the effect of hyperacusis on the right SMA gray matter volumes was much larger than the effect of sex and that there was no interaction between sex and hyperacusis. Ultimately, longitudinal studies will be necessary to provide insight into the complex relationship between peripheral damage, hyperacusis susceptibility, and behavioural adaptation/reactivity.

In conclusion, hyperacusis in individuals with sensorineural hearing loss and tinnitus appears to be associated with smaller gray matter volumes and reduced cortical thickness of the right supplementary motor area (SMA). This neuroanatomical difference is robust enough to be noticeable in the relatively small number of participants investigated in this study and, in fact, can successfully classify participants with hyperacusis. Our finding of increased evoked (BOLD) responses to sound stimuli in this area supports the hypothesis that increased central gain associated with hyperacusis occurs not only along the auditory pathway but also in the non-auditory SMA and could lead to changes in SMA gray matter volume and thickness. Further research investigating the link between hyperacusis and the structure and function of the SMA are warranted, not only in individuals with sensorineural hearing loss and tinnitus but also in children who develop hyperacusis following repeated otitis media episodes and individuals with conditions such as Williams syndrome and autism.

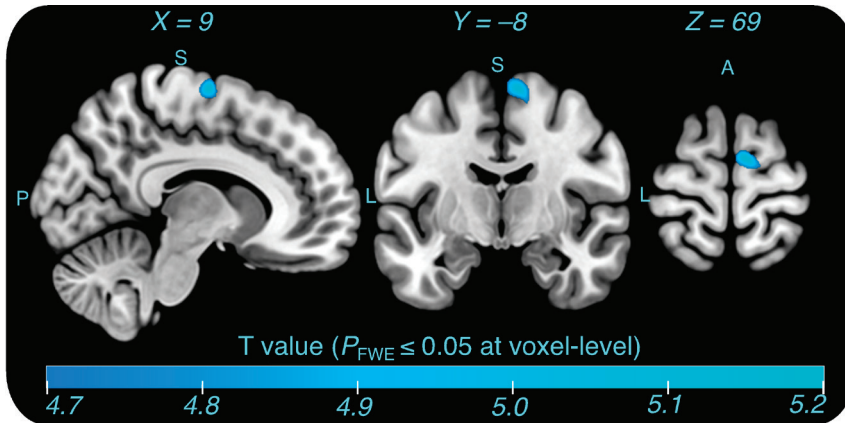
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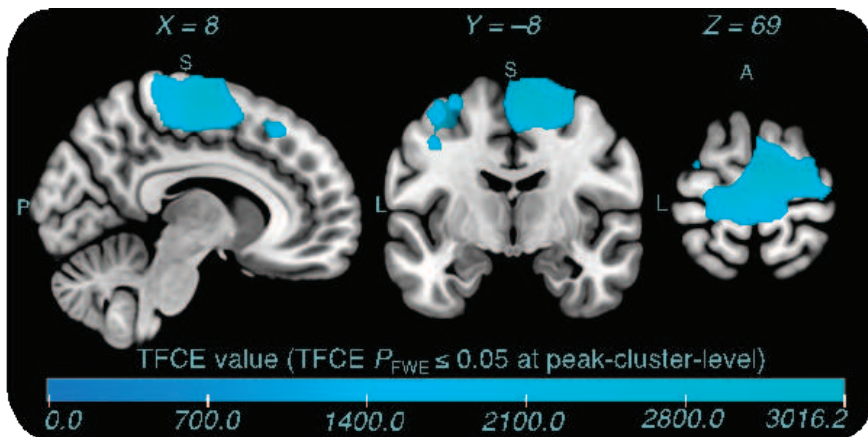
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SUPPLEMENTARY MATERIALS



Supplementary Figure S1. Results of the whole-brain between groups comparison showing smaller gray matter volumes in the right supplementary motor area (SMA) of participants with hyperacusis compared to those without hyperacusis. The analysis accounted for total intracranial volume (TIV) as a confounding variable. One single cluster (244 voxels) of gray matter volumes difference was found in the entire brain, shown in cold color map (peak MNI X, Y, Z - coordinates = 9, -8, 69, $T = 5.2$, $P_{FWE} = 0.012$ at the voxel-level).



Supplementary Figure S2. Results of the whole-brain between groups comparison showing smaller gray matter volumes in the right supplementary motor area (SMA) of participants with hyperacusis compared to those without hyperacusis. The analysis accounted for age, handedness score, hearing thresholds (PTA 0.25 - 8 kHz for both ears), and total intracranial volume (TIV) as confounding variables. One single cluster (14,276 voxels) of gray matter volumes difference was found in the entire brain (shown in cold color map), however, cluster was extended into the SMA, pre-supplementary motor area, and precentral gyrus bilaterally (peak MNI X, Y, Z - coordinates = 8, -8, 69, $TFCE = 3016.2$, $TFCE PFWE = 0.001$ at the peak-cluster-level, permutation = 5000).

Supplementary Table S1. Overview of demographic, audiometric, questionnaires, and morphometric data for the groups with and without hyperacusis.

	Group without Hyperacusis	Group with Hyperacusis	Statistics
Demographic			
<i>n</i>	41	25	–
Age (years)	58.3 ± 10.5 (27 – 76)	59.5 ± 7.9 (41 – 73)	$U = 507, Z = -0.1, P = 0.942$
Sex (male / female)	(32 / 9)	(15 / 10)	$\chi^2(1) = 2.5, P = 0.116$
Handedness (L / R / NP / NA)	(5 / 29 / 0 / 7)	(0 / 23 / 2 / 0)	–
Audiometric (for both ears)			
Hearing levels dB HL (PTA 0.25 – 8 kHz)	35 ± 8.6 (20.4 – 57.9)	38.2 ± 12.3 (11.2 – 66.3)	$U = 401.5, Z = -1.2, P = 0.218$
Hearing levels dB HL (PTA 0.25 – 1 kHz)	21.5 ± 12.4 (7.5 – 68.3)	25 ± 16.6 (5 – 71.7)	$U = 449.5, Z = -0.6, P = 0.563$
Hearing levels dB HL (PTA 2 – 8 kHz)	48.5 ± 12.6 (28.3 – 15.8)	51.5 ± 13.3 (17.5 – 88.3)	$t(46.2) = 0.9, P = 0.381$
Questionnaires			
HQ Hyperacusis	13.2 ± 5.2 (0 – 21)	26.3 ± 3.7 (22 – 33)	$t(62.6) = 11.9, P < 0.001$
HQ-attentional	3.7 ± 1.7	7.3 ± 1.4	$U = 52.2, Z = -5.7, P < 0.001$
HQ-social	5.4 ± 3.4	10.5 ± 2.4	$t(56.9) = 6.8, P < 0.001$
HQ-emotional	4.1 ± 1.9	8.4 ± 2.1	$t(46.6) = 7.9, P < 0.001$
HADS Anxiety	3.7 ± 3 (0 – 11)	5.9 ± 4.6 (0 – 16)	$U = 365, Z = -1.8, P = 0.067$
HADS Depression	3.3 ± 3.1 (0 – 10)	6.2 ± 4.7 (0 – 16)	$U = 303, Z = -2.7, P = 0.007$
THI Tinnitus burden	27.4 ± 19.4 (4 – 80)	41.8 ± 20.9 (6 – 82)	$U = 280.5, Z = -2.8, P = 0.006$
THI-functional	15.6 ± 10.2	25.6 ± 11.8	$U = 255.5, Z = -3.1, P = 0.002$
THI-emotional	6.7 ± 6.8	9.6 ± 6.9	$U = 345.5, Z = -1.9, P = 0.060$
THI-catastrophic	5.2 ± 4.1	6.7 ± 3.9	$U = 365.5, Z = -1.8, P = 0.081$
Morphometric			
TIV (cm ³)	1494.9 ± 139.1	1451 ± 96.3	$t(62.9) = -1.5, P = 0.135$
WOIQ (%)	83 ± 3.7	80.9 ± 4.2	$U = 392.5, Z = -1.6, P = 0.113$

Mean ± Standard Deviation (Range). NA not available; NP no preference; L left; R right.

Supplementary Table S2. Results of the whole-brain two-sample t-test analysis between the groups with and without hyperacusis including total intracranial volume (TIV) as a covariate ($P_{\text{FWE}} \leq 0.05$ at the voxel-level, height threshold $T = 4.7$).

Area	P_{FWE} voxel- level	P_{FWE} cluster- level	MNI coordinates			Cluster size k	T-value	Z-value
			X	Y	Z			
SMA (right)	Group without Hyperacusis > Group with Hyperacusis							
	0.012	0.005	9	−8	69	244	5.2	4.7
	0.049	0.047	20	−15	64	1	4.7	4.3
NS	Group without Hyperacusis < Group with Hyperacusis							
	−	−	−	−	−	−		−

NS not significant.

Supplementary Table S3. Results of whole-brain two-sample t-test analysis between the groups with and without hyperacusis including age, handedness score, hearing thresholds (PTA 0.25 – 8 kHz for both ears), and total intracranial volume (TIV) as covariates (TFCE $P_{FWE} \leq 0.05$ at the peak-cluster-level).

Area	TFCE P_{FWE} peak-cluster-level	MNI coordinates			Cluster size k	TFCE-value	
		X	Y	Z			
Group without Hyperacusis > Group with Hyperacusis							
SMA	0.001	8	-8	69	14276	3016.2	
Pre-SMA		15	-6	58		2987.8	
PrC (bi-lateral)		-18	-33	74		2949.3	
Group without Hyperacusis < Group with Hyperacusis							
NS	-	-	-	-	-	-	-

NS not significant. PrC precentral gyrus; Pre-SMA pre-supplementary motor area.

Supplementary Table S4. Results of the regression analysis between hyperacusis scores and whole-brain gray matter volumes including age, handedness scores, hearing thresholds, and total intracranial volume (TIV) as covariates ($P_{\text{FWE}} \leq 0.05$ at the cluster-level, cluster forming threshold $P \leq 0.001$, height threshold $T = 3.2$).

Area	P_{FWE} cluster- level	P uncor- rected	MNI coordinates			Cluster size k	T-value	Z-value
			X	Y	Z			
Negative effect of Hyperacusis								
SMA (right)	< 0.001	< 0.001	6	-15	52	5326	4.1	3.9
		< 0.001	3	-14	68		4.1	3.9
PreCG (left)		< 0.001	-18	-33	74		4.3	4.0
Positive effect of Hyperacusis								
NS	-	-	-	-	-	-	-	-

NS not significant. PreCG precentral gyrus.

Supplementary Table S5. Person's correlation coefficients between the different subscales of the HQ and the right SMA gray matter volumes (HMAT VOI).

	HQ-attentional	HQ-social	HQ-emotional
	Khalfa et al.		
SMA (right)	$r = -0.51$	$r = -0.33$	$r = -0.34$
	$P < 0.001$	$P = 0.011$	$P = 0.009$
	$n = 59$	$n = 59$	$n = 59$
	Fackrell et al.		
SMA (right)	$r = -0.44$	$r = -0.26$	-
	$P < 0.001$	$P = 0.049$	-
	$n = 59$	$n = 59$	-

Correlation between the right SMA gray matter volumes and scores on the Hyperacusis Questionnaire

Regression analyses were conducted in order to test whether using the Hyperacusis Questionnaire (HQ) scores as a continuous variable (instead of relying on a cut-off score) would produce different results, and to test whether a subset of items from the HQ drove the correlation.

Methods

- 1 A General Linear Model (GLM) was built with the HQ scores as a co-variate of interest, also controlling for the effects of age, handedness, hearing thresholds, and total intracranial volume (TIV). The analysis was performed in SPM12/CAT12.7 in the whole brain with two different statistical thresholds: FWE correction at the voxel level ($P \leq 0.05$), and FWE correction at the cluster level ($P \leq 0.05$, cluster forming threshold $P \leq 0.001$).
- 2 Gray matter volumes extracted from the right SMA HMAT VOI (see main text) were correlated with the scores on the different subscales of the HQ. Two sets of correlation analyses were conducted: (a) taking into account the original classification into three principal components¹, and (b) using a newer decomposition² which proposed a two factors structure based on 10 of the 14 original questions.

Results

- 1 There was no significant correlation between hyperacusis scores and whole-brain gray matter volumes at the strictest threshold ($P_{\text{FWE}} \leq 0.05$). However, relaxing the threshold to $P \leq 0.001$ (with correction at the cluster size level, $P_{\text{FWE}} \leq 0.05$) showed a negative correlation between hyperacusis scores and gray matter volumes with main peaks in the right SMA extending into the left precentral gyrus (Supplementary Table S4).
- 2 Regressing the right SMA gray matter volumes on the different subscales of the HQ showed significant negative correlations with every subscale of the questionnaire (Supplementary Table S5). The stronger correlations were found for the attentional subscale independently of the manner of grouping the items.

DATA AVAILABILITY STATEMENT

The unthresholded statistical parametric map supporting the main finding of this study is openly available at neurovault.org (<https://identifiers.org/neurovault.collection:13313>).

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We dedicate this work to the memory of Prof. David Baguley, who will remain an inspiration to us and many others in the field of tinnitus and hyperacusis research. We would like to thank the UMCG tinnitus research group for sharing their critical input. We also want to show our gratitude to Shahin Safazadeh for his help with the data collection.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Chapter 5

Hyperacusis in tinnitus individuals is associated with smaller gray matter volumes in the supplementary motor area regardless of hearing levels

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ABSTRACT

Recent evidence suggests a connection between hyperacusis and the motor system of the brain. For instance, our recent study reported that hyperacusis in participants with tinnitus and hearing loss is associated with smaller gray matter volumes in the supplementary motor area (SMA). Given that hearing loss can affect gray matter changes in tinnitus, this study aimed to determine if the changes reported in our previous findings of smaller SMA gray matter volumes in hyperacusis persist in the absence of hearing loss. Data for this study were gathered from four prior studies conducted between 2004 and 2019 at the University Medical Centre Groningen (UMCG). A total of 101 participants with tinnitus and either clinically normal hearing (normal hearing with tinnitus or NHT, $n = 35$) or bilateral sensorineural hearing loss (hearing loss with tinnitus or HLT, $n = 66$) were included across four studies. Hyperacusis was determined by a score of ≥ 22 on the Hyperacusis Questionnaire (HQ). In the NHT group, 22 (63%) participants scored ≥ 22 on the HQ (NHT with hyperacusis: mean age 44.1 years, 12 females), while in the HLT group, 25 (38%) participants scored ≥ 22 on the HQ (HLT with hyperacusis: mean age 59.5 years, 10 females). The 2×2 between-group ANOVAs revealed that hyperacusis is associated with smaller SMA gray matter volumes, regardless of hearing levels. Notably, the smaller SMA gray matter volumes in hyperacusis were primarily influenced by the attentional subscales of the HQ. The association between hyperacusis and the motor system may indicate a constant alertness to sounds and a readiness for motor action.

Keywords

Gray matter

Hearing loss

Hyperacusis

Supplementary motor area

Tinnitus

Voxel-based morphometry

1. INTRODUCTION

Hyperacusis is a condition characterized by an increased sensitivity to everyday environmental sounds of mild to moderate intensity. Hyperacusis can significantly impact the quality of life of those affected [1–3]. While the exact prevalence of hyperacusis remains unknown, it is estimated to affect up to 17% of the general adult [4] and pediatric [5] population. It occurs significantly more often in individuals with hearing disorders, such as tinnitus and hearing loss. In fact, approximately 90% of individuals with hyperacusis report suffering from tinnitus [1], and, vice versa, approximately 63% of individuals with tinnitus report concurrent hyperacusis [4]. Additionally, an estimated 59% of individuals with hyperacusis also suffer from hearing loss [6]. In addition to tinnitus and hearing loss, there are other factors that can contribute to hyperacusis in both adults and children, such as head injuries, Williams syndrome, upper respiratory tract infections, chronic otitis media, and obsessive-compulsive disorder [2, 7, 8].

At a mechanistic level, increased central gain, a homeostatic response whereby the auditory neurons exhibit an increased response to a given input, could explain hyperacusis [9]. Functional neuroimaging studies have provided support for this theory, demonstrating that individuals with hyperacusis show increased sound-evoked activity in the auditory pathway [10–12]. Moreover, hyperacusis may also involve non-auditory brain regions, such as the orbitofrontal cortex, the anterior cingulate cortex, and the supplementary motor area [13, 14]. In particular, in a recent whole-brain voxel-based morphometry (VBM) study, we observed that in participants with tinnitus and hearing loss, concurrent hyperacusis was associated with a smaller gray matter volume in the right supplementary motor area (SMA) [14]. Since the SMA is a brain region involved in action initiation, this finding suggests that these changes in the gray matter there might be related to a motor hyper-reactivity to sounds. In support of this hypothesis, a resting state (electroencephalogram) EEG by Song et al. demonstrated increased beta waves with a generator located in the SMA in individuals with hyperacusis [13].

In our previous VBM study, all participants had tinnitus and hearing loss [14]. This study design allowed us to identify gray matter differences that are specifically associated with hyperacusis, as the two groups were matched for tinnitus and hearing loss. Hyperacusis is, however, also diagnosed in individuals with clinically normal hearing thresholds. In this

population, it is thought that hidden hearing loss, with various possible underlying etiologies, could induce hyperacusis and tinnitus [15]. In a recent meta-analysis, we established that the presence or absence of hearing loss can significantly influence gray matter volumes in participants with tinnitus [16]. By including a group with tinnitus and clinically normal hearing thresholds, the current study aimed to clarify whether smaller SMA gray matter volumes are attributable to hyperacusis alone or if they are influenced by the presence or absence of hearing loss.

In this retrospective case-control study, therefore, we examined whether SMA gray matter volumes were smaller in hyperacusis adults with tinnitus and clinically normal hearing. If the SMA gray matter volume differences associated with hyperacusis are independent of hearing status, then individuals with clinically normal hearing and hyperacusis should show the same trend as their counterparts with hearing loss. To test this hypothesis, we conducted 2×2 between-group ANOVAs (hyperacusis $\times$ hearing loss) within independently defined volumes of interest in the SMA. With this approach, we aim to examine the potential interactions between the effects of hyperacusis and hearing loss on gray matter volumes in the SMA region. Ultimately, we hope that these findings will offer insights into the structural mechanisms of hyperacusis, aiding the identification of structural biomarkers for more targeted and effective interventions for individuals suffering from hyperacusis, ultimately improving their quality of life.

2. MATERIALS AND METHODS

Data for this study were gathered from four prior neuroimaging studies conducted between 2004 and 2019 at the Department of Otorhinolaryngology of the University Medical Centre Groningen (UMCG) [17–20]. These studies were conducted in accordance with the Declaration of Helsinki and were approved by the UMCG's medical ethics committee (METc), which additionally granted approval for data reanalysis (METc number: 2020/347). All participants provided written informed consent.

2.1. Participants

A total of 101 participants with tinnitus and either clinically normal hearing (the normal hearing with tinnitus or NHT group, $n = 35$) or bilateral sensorineural hearing loss (the hearing loss with tinnitus or HLT group, $n = 66$) were included. Pure tone air-conduction audiometry was utilized

to assess hearing thresholds across octave frequencies ranging from 0.25 to 8 kHz. Hearing loss was defined as an average hearing threshold (or pure tone average-PTA) of ≥ 25 dB HL across all tested frequencies (0.25, 0.5, 1, 2, 4, and 8 kHz) for both ears. Hyperacusis was evaluated using the 14-item Hyperacusis Questionnaire (HQ) [21]. Additionally, the tinnitus burden was quantified using the 25-item Tinnitus Handicap Inventory (THI) [22]. Anxiety and depression levels were determined using the 14-item Hospital Anxiety and Depression Scale (HADS) [23]. Handedness was assessed with the 10-item Edinburgh Handedness Inventory (EHI) [24].

Similar to our previous study [14], a cut-off score of ≥ 22 on the Hyperacusis Questionnaire was used to distinguish the presence or absence of hyperacusis [25].

2.2. MRI data acquisition

For each participant, high-resolution 3-dimensional T1-weighted anatomical brain images were obtained using a 3-Tesla Philips Intera scanner (Philips Medical System, Best, The Netherlands) equipped with either an 8-channel [17–19] or 32-channel [20] phase-array head coil (SENSE). Fast-field echo images were acquired, with a reconstructed voxel size of $1 \times 1 \times 1$ mm, using the acquisition parameters outlined in Table S1 in the Supplementary Materials.

2.3. MRI Data Preprocessing

T1-weighted anatomical brain images were preprocessed using the CAT12.7 toolbox (v.1653, Structural Brain Mapping Group, University of Jena, Germany, <http://dbm.neuro.uni-jena.de/cat12/>, accessed on 11 July 2024) within SPM12 (v.7487, Wellcome Centre for Human Neuroimaging, University College London, <https://www.fil.ion.ucl.ac.uk/spm/>, accessed on 11 July 2024), running on MATLAB R2020a (The MathWorks, Natick, MA, USA). Firstly, the brain images underwent correction for bias, noise, and global intensity variations. Subsequently, the brain images were normalized to the Montreal Neurological Institute (MNI152) template, with voxel dimensions resampled to $1.5 \times 1.5 \times 1.5$ mm. During normalization, the brain image was segmented into gray matter, white matter, and cerebrospinal fluid. The segmented gray matter images were then modulated using the Jacobian determinant [26, 27]. Finally, recommended quality control measures were used to assess the quality of the resulting normalized and modulated gray matter images. More details can be found in our previous study [14].

2.4. Volume-of-interest selection for the supplementary motor area

Similar to our previous study [14], we selected the volume of interest (VOI) corresponding to the supplementary motor area (SMA), as defined in a previous study, using both anatomical as well as functional MRI data [28]. This meta-analysis utilized both task-related activation coordinates and anatomical boundaries to delineate the motor cortex areas. This SMA VOI is freely accessible as the Human Motor Area Template (HMAT) atlas from <http://lrnlab.org/> (accessed on 11 July 2024). More details can be found in Supplementary Figure S1 in the Supplementary Materials.

In order to deepen our investigation into SMA gray matter in hyperacusis, we also investigated VOIs corresponding to the anterior and posterior subdivisions of the SMA (SMA_A6m and SMA_A4ll respectively), as defined by the Human Brainnetome atlas based on the structural and functional connectome architecture [29]. These SMA VOIs are freely accessible as the Human Brainnetome (HBN) atlas from <https://atlas.brainnetome.org/bnatlas.html> (accessed on 11 July 2024). More details can be found in Supplementary Figure S1.

2.5. Gray matter extraction from volume-of-interest areas

First, the selected VOIs were resampled to $1.5 \times 1.5 \times 1.5$ mm voxel dimensions to align with the voxel dimensions of normalized and modulated gray matter images. Next, gray matter volumes were extracted from each selected VOI for every participant using the “get_totals” MatLab script. This MatLab script is freely available for download from http://www0.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m/ (accessed on 18 July 2024). Subsequently, these extracted gray matter volumes were subjected to further analyses using SPSS (v.29, IBM Corp., Armonk, New York, NY, USA).

2.6. Statistical analyses

2.6.1. Demographic, audiometric, and questionnaire data

The statistical analyses of demographic, audiometric, and questionnaire data were performed using SPSS29. To assess the between-group differences for continuous variables, a nonparametric independent sample one-way ANOVA (Kruskal–Wallis H test) was used, followed by Bonferroni-corrected post hoc t-test analysis. For categorical variables, the chi-square test was used to examine between-group differences. A statistical threshold of $p \leq 0.05$ was used for all analyses.

2.6.2. Gray matter volume of the supplementary motor area in association with hyperacusis

2.6.2.1. Effects of hyperacusis and hearing loss on SMA gray matter volume

We performed 2×2 between-group ANOVAs to investigate the main effects of hyperacusis and hearing loss, as well as the effect of their interaction on the gray matter volumes of the selected SMA VOIs. Age, handedness scores, and TIV were included as covariates. The statistical threshold for these analyses was set at $p \leq 0.05$. We tested for group differences in the HMAT left and right SMA VOI. To extend our analysis, we repeated the analysis in the posterior and anterior portions of the left and right SMA (see Section 2.4, Supplementary Figure S1).

2.6.2.2. Differentiating the effects of the attentional and social subscales of the hyperacusis questionnaire on SMA gray matter volumes

Responses to the Hyperacusis Questionnaire can be classified into two component groups: attentional and social [30]. To assess whether smaller SMA gray matter volumes were (more) influenced by the scores for either the attentional or social component of the HQ, Spearman's rank correlation coefficients between the SMA gray matter volumes (HMAT and HBN VOIs) and scores on the attentional and social subscales of the HQ were computed across all participants. The statistical threshold for these analyses was set at $p \leq 0.05$.

2.6.2.3. Effects of anxiety, depression, or tinnitus burden

To assess the impact of anxiety, depression, and tinnitus burden on SMA gray matter volumes (HMAT and HBN VOIs), Spearman's rank correlation coefficients between the gray matter volumes of the selected SMA VOIs and either the HADS (anxiety and depression) or the THI (tinnitus burden) scores were computed across all participants. The statistical threshold for these analyses was set at $p \leq 0.05$.

3. RESULTS

3.1. Demographic, audiometric, and questionnaire data

Participants were classified in the hyperacusis groups if they scored above 22 on the Hyperacusis Questionnaire [14,21,25]. Accordingly, in the NHT group, 22 out of 35 participants (63%) were classified as having hyperacusis (NHT with hyperacusis: mean age $\pm$ standard deviation = 44.1 ± 12.2 years, 12 females). The remaining 13 participants who

scored below the cut-off score were assigned to the control group (NHT without hyperacusis: mean age $\pm$ standard deviation = 45.5 ± 11.2 years, 5 females). In the HLT group, 25 out of 66 participants (38%) scored above the cut-off score for hyperacusis (HLT with hyperacusis: mean age $\pm$ standard deviation = 59.5 ± 7.9 years, 10 females). The remaining 41 participants who scored below the cut-off score were assigned to the control group (HLT with hyperacusis: mean age $\pm$ standard deviation = 58.3 ± 10.5 years, 9 females). Participant inclusion criteria for the four groups are shown in Figure 1. The proportion of individuals suffering from hyperacusis was significantly higher in the group with clinically normal hearing thresholds, compared to the group with hearing loss [$X^2(1) = 5.7$, $p = 0.017$].

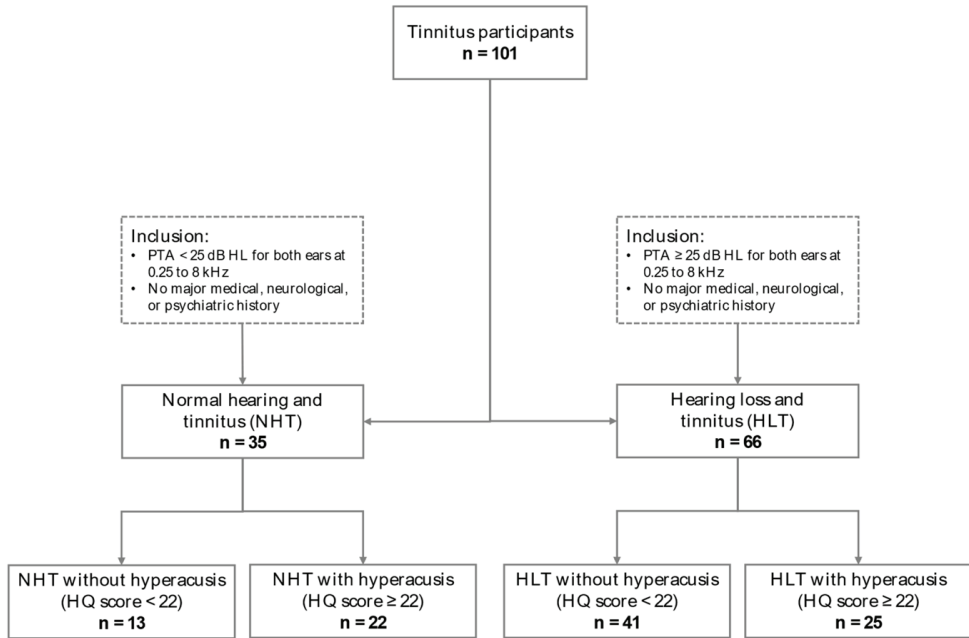


Figure 1. Flowchart of participant inclusion criteria for the four participant groups. For all participants, pure tone air-conduction audiometry was utilized to assess hearing thresholds across octave frequencies ranging from 0.25 to 8 kHz. Hearing loss was defined as an average hearing threshold (or pure tone average-PTA) of ≥ 25 dB HL across all tested frequencies (0.25, 0.5, 1, 2, 4, and 8 kHz) for both ears. A cut-off score of ≥ 22 on the Hyperacusis Questionnaire (HQ) was used to distinguish the presence or absence of hyperacusis.

These four groups showed significant differences across several variables. The statistical analyses revealed significant between-group differences in age [$X^2(3) = 30.0$, $p < 0.001$], hearing thresholds [PTA 0.25

to 8 kHz: $X^2(3) = 65.8$, $p < 0.001$], HADS scores [HADS-anxiety: $X^2(3) = 11.0$, $p < 0.001$; HADS-depression: $X^2(3) = 8.4$, $p < 0.001$]; and THI scores [$X^2(3) = 13.0$, $p < 0.001$]. Participants with hearing loss (the HLT group either with or without hyperacusis) were, on average, older than those with clinically normal hearing (the NHT group either with or without hyperacusis). Mean hearing thresholds for both ears at octave frequencies ranging from 0.25 to 8 kHz per group are shown in Figure 2. Summarized data for the four groups are shown in Table 1 (more details are provided in Supplementary Table S2 in the Supplementary Materials).

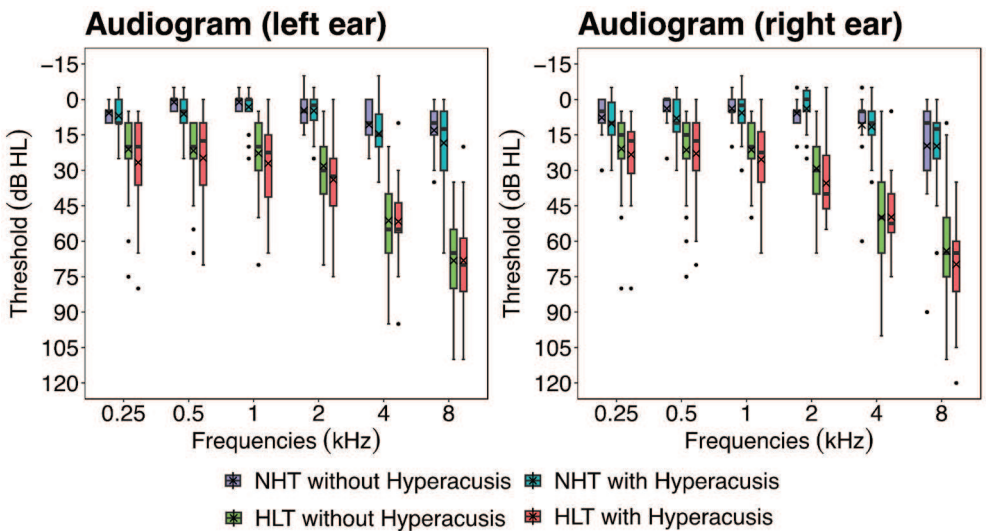


Figure 2. Box plots showing the hearing thresholds for the four participant groups. Participants with hearing loss (the HLT groups) had worse hearing thresholds than those with clinically normal hearing (the NHT groups) [$X^2(3) = 30.0$, $p < 0.001$]. The crosses added on the box plots represent the mean. HLT participant group with tinnitus and bilateral sensorineural hearing loss (either with or without hyperacusis), NHT participant group with tinnitus and clinically normal hearing (either with or without hyperacusis).

Table 1. Overview of demographic, audiometric, and questionnaires data for the four participant groups.

Group	Normal Hearing and Tinnitus (NHT)		Hearing Loss and Tinnitus (HLT)		Statistic
	Without Hyperacusis	With Hyperacusis	Without Hyperacusis	With Hyperacusis	
Demographic					
n	13	22	41	25	–
Mean Age (years)	45.5 ± 11.2 (29 – 62) ^{c,d}	44.1 ± 12.2 (19 – 59) ^{c,d}	58.3 ± 10.5 (27 – 76) ^{a,b}	59.5 ± 7.9 (41 – 73) ^{a,b}	$X^2(3) = 30.0$, $p < 0.001$
Sex (male female)	8 5	10 12	32 9	15 10	$X^2(3) = 7.0$, $p = 0.071$
Audiometric (for both ears 0.25 to 8 kHz)					
Mean PTA (dB HL)	7.3 ± 5.4 ^{c,d}	9.4 ± 7.3 ^{c,d}	35.0 ± 8.6 ^{a,b}	38.2 ± 12.3 ^{a,b}	$X^2(3) = 65.8$, $p < 0.001$
Questionnaires					
HQ	16.5 ± 3.6 (10 – 21) ^{b,d}	27.1 ± 4.1 (22 – 35) ^{a,c}	13.2 ± 5.4 (0 – 21) ^{b,d}	26.2 ± 3.7 (22 – 33) ^{a,c}	$X^2(3) = 76.0$, $p < 0.001$
HQ-Attentional †	9.2 ± 2.3 ^{b,d}	13.5 ± 2.6 ^{a,c}	6.5 ± 2.7 ^{b,d}	13.5 ± 2.6 ^{a,c}	$X^2(3) = 57.5$, $p < 0.001$
HQ-Social †	1.8 ± 1.0 ^{b,d}	5.9 ± 2.7 ^{a,c}	2.6 ± 2.4 ^{b,d}	5.2 ± 2.0 ^{a,c}	$X^2(3) = 31.3$, $p < 0.001$
HADS-Anxiety	6.8 ± 4.2 (0 – 16)	6.5 ± 3.4 (2 – 15) ^c	3.7 ± 3.0 (0 – 11) ^b	5.9 ± 4.6 (0 – 16)	$X^2(3) = 11.0$, $p = 0.011$
HADS-Depression	5.0 ± 3.8 (0 – 14)	4.9 ± 3.7 (0 – 15)	3.2 ± 3.1 (0 – 10) ^d	6.2 ± 4.7 (0 – 16) ^c	$X^2(3) = 8.4$, $p = 0.038$
THI	33.7 ± 20.4 (4 – 66)	44.4 ± 20.5 (10 – 88) ^c	27.4 ± 19.3 (4 – 80) ^{b,d}	41.8 ± 20.8 (6 – 82) ^c	$X^2(3) = 13.0$, $p = 0.005$

Mean ± Standard Deviation (Range). dB HL decibel hearing loss; HADS hospital anxiety depression scale; HQ hyperacusis questionnaires; PTA pure tone average (025 to 8 kHz); THI tinnitus handicap inventory. ^aThe group differed significantly ($p \leq 0.05$) from the NHT group without hyperacusis; ^bThe group differed significantly ($p \leq 0.05$) from the NHT group with hyperacusis; ^cThe group differed significantly ($p \leq 0.05$) from the HLT group without hyperacusis; ^dThe group differed significantly ($p \leq 0.05$) from the HLT group with hyperacusis. [†]The attentional and social subscales corresponding to the HQ were defined according to a previous study [27].

3.2. Association between hyperacusis and SMA gray matter volumes

3.2.1. Human Motor Area Template (HMAT) atlas analysis

Within the right HMAT SMA VOIs, the 2×2 between-group ANOVAs

revealed a significant main effect of hyperacusis on gray matter volumes [$F(1,94) = 21.0$, $p < 0.001$], with participants scoring above the threshold for hyperacusis and displaying lower SMA gray matter volumes relative to the controls (Figure 3). There was also a trend toward a negative main effect of hyperacusis in the left hemisphere [$F(1,94) = 3.7$, $p = 0.058$] (Figure 3). However, we found no significant main effect of hearing loss [SMA left: $F(1,94) = 1.0$, $p = 0.329$; SMA right: $F(1,94) = 0.8$, $p = 0.774$], nor any interaction between the effects of hyperacusis and hearing loss [SMA left: $F(1,94) = 1.1$, $p = 0.298$; SMA right: $F(1,94) = 2.1$, $p = 0.152$].

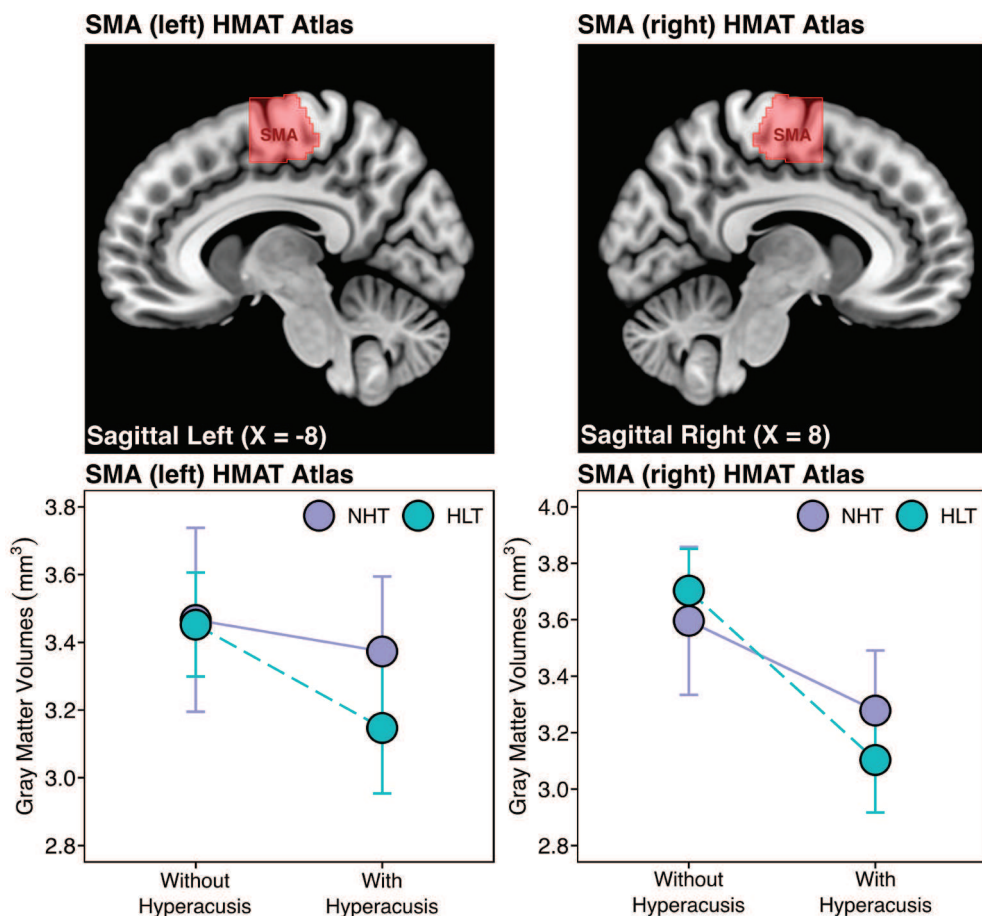


Figure 3. The SMA VOIs were derived from Human Motor Area Template (HMT) atlas (upper panels). The 2 x 2 between-groups ANOVAs (corrected for age, handedness scores, and total intracranial volume) revealed that hyperacusis has a significant negative main effect on the right supplementary motor area (SMA) gray matter volumes [SMA right: $F(1,94) = 21.0$, $p < 0.001$]. There was no significant main effect of hearing loss nor any interaction between the effects of hyperacusis and hearing loss on the bilateral SMA gray matter volumes. The presence of hyperacusis was defined by a cut-off score of ≥ 22 on the 14-item HQ. The graphs show the mean and 95% confidence interval (CI).

3.2.2. Human Brainnetome (HBN) atlas analysis

To further examine the effect of hyperacusis on SMA gray matter volumes, we repeated the analysis using the Human Brainnetome (HBN) atlas, which distinguishes between the anterior and the posterior SMA. For the anterior SMA (SMA_A6m) VOIs, the 2×2 between-group ANOVAs revealed a significant negative main effect of hyperacusis on the right anterior SMA gray matter volumes [$F(1,94) = 13.7$, $p < 0.001$], but no significant main effect on the left anterior SMA VOI [$F(1,94) = 1.7$, $p = 0.200$] (Figure 4). There was no significant main effect of hearing loss [SMA_A6m left: $F(1,94) = 0.2$, $p = 0.637$; SMA_A6m right: $F(1,94) = 0.2$, $p = 0.675$], nor any interaction between the effects of hyperacusis and hearing loss [SMA_A6m left: $F(1,94) = 0.4$, $p = 0.521$; SMA_A6m right: $F(1,94) = 1.6$, $p = 0.203$] on the anterior (A6m) SMA gray matter volumes.

For the posterior SMA (SMA_A4ll) VOIs, the 2×2 between-group ANOVAs revealed a significant negative main effect of hyperacusis on the posterior SMA gray matter volumes in both hemispheres [SMA_A4ll left: $F(1,94) = 10.5$, $p < 0.001$; SMA_A4ll right: $F(1,94) = 14.1$, $p < 0.001$] (Figure 4). Again, no significant main effect of hearing loss [SMA_A4ll left: $F(1,94) = 1.7$, $p = 0.193$; SMA_A4ll right: $F(1,94) = 1.2$, $p = 0.269$] and no significant interaction between the effects of hyperacusis and hearing loss [SMA_A4ll left: $F(1,94) = 0.5$, $p = 0.478$; SMA_A4ll right: $F(1,94) = 1.1$, $p = 0.323$] were observed on the posterior (A4ll) SMA gray matter volumes.

3.2.3. Smaller gray matter volumes in hyperacusis are associated with the attentional subscale of the hyperacusis questionnaire

As expected, participants with hyperacusis scored higher on both the attentional and social subscales of the HQ, compared to participants without hyperacusis (Table 1). Interestingly, significant associations were only observed between the SMA gray matter volumes (HMAT and HBN VOIs) and the attentional subscale of the HQ (Table 2).

3.2.4. Anxiety, depression, or tinnitus burden scores do not drive smaller SMA gray matter volumes in hyperacusis

Significant between-group differences were found in the HADS anxiety and depression and the THI tinnitus burden scores (Table 1). However, we found no significant relationship between the SMA gray matter volumes (HMAT and HBN VOIs) and either the HADS anxiety and depression scores or the THI tinnitus burden score (all $p \geq 0.284$; more details are provided in Supplementary Table S3 in the Supplementary Materials).

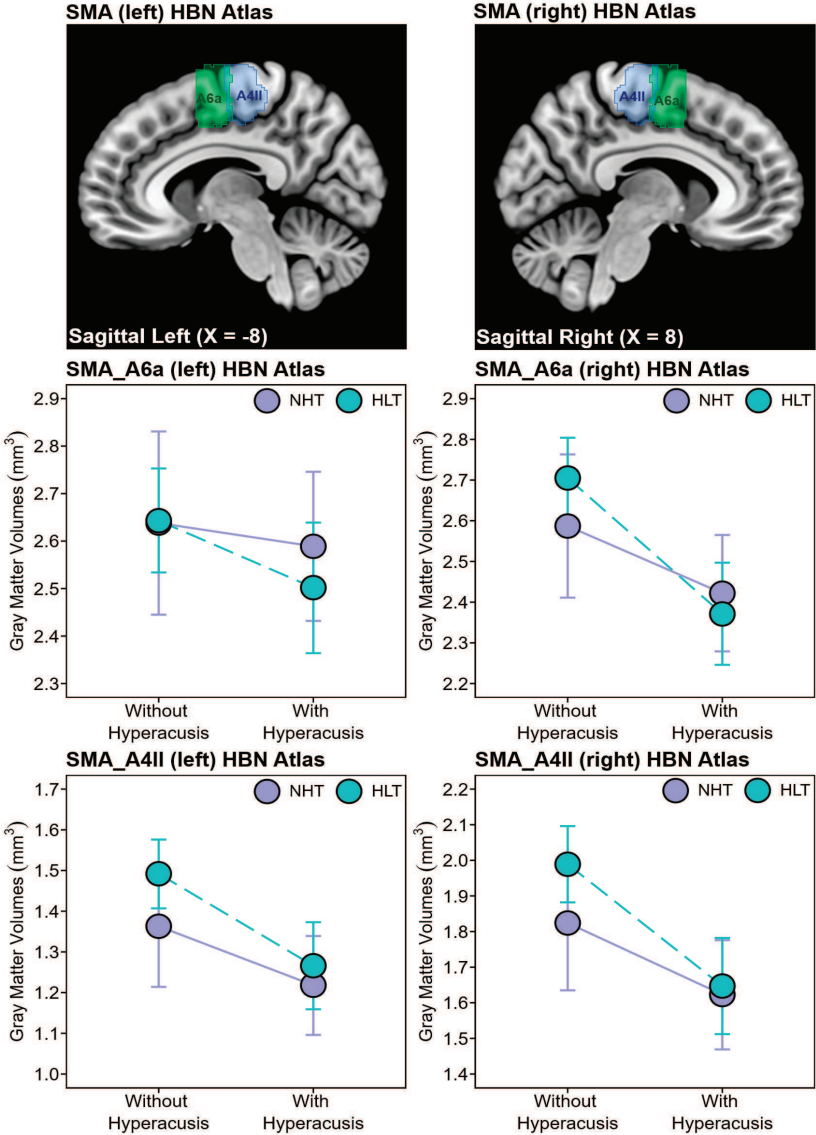


Figure 4. The SMA VOIs were derived from the Human Brainnetome (HBN) atlas (upper panels). The 2 x 2 between-groups ANOVAs (corrected for age, handedness scores, and total intracranial volume) revealed that hyperacusis has a significant negative main effect on the right anterior (A6a) SMA gray matter volumes [SMA_A6a right: $F(1,94) = 21.0$, $p < 0.001$] and the bilateral posterior (A4II) SMA gray matter volumes [SMA_A4II left: $F(1,94) = 10.5$, $p < 0.001$; SMA_A4II right: $F(1,94) = 14.1$, $p < 0.001$]. There was no significant main effect of hearing loss nor any interaction between the effects of hyperacusis and hearing loss on the bilateral anterior/posterior SMA gray matter volumes. The presence of hyperacusis was defined by a cut-off score of ≥ 22 on the 14-item HQ. The graphs show the mean and 95% confidence interval (CI).

Table 2. Results of Spearman’s Rank correlation coefficients between the SMA gray matter volumes (HMAT and HBN VOIs) and either the attentional or social subscales scores of the HQ across all participants. The SMA VOIs are shown in Supplementary Figure S1. The statistical threshold for Spearman’s Rank correlation coefficients was set at $p \leq 0.05$.

	HQ-Attentional Score	HQ-Social Score
HMAT VOIs		
SMA left	$r_s(101) = -0.26, p = 0.010$	$r_s(101) = 0.10, p = 0.327$
SMA right	$r_s(101) = -0.33, p < 0.001$	$r_s(101) = -0.11, p = 0.273$
HBN VOIs		
SMA_A6m left	$r_s(101) = -0.17, p = 0.089$	$r_s(101) = 0.13, p = 0.210$
SMA_A6m right	$r_s(101) = -0.30, p = 0.001$	$r_s(101) = -0.06, p = 0.569$
SMA_A4ll left	$r_s(101) = -0.32, p = 0.001$	$r_s(101) = -0.11, P = 0.259$
SMA_A4ll right	$r_s(101) = -0.35, p < 0.001$	$r_s(101) = -0.14, P = 0.165$

HBN Human Brainnetome atlas; HMAT Human Motor Area Template atlas; HQ Hyperacusis Questionnaires; SMA supplementary motor area; SMA_A6ma anterior subdivision supplementary motor area; SMA_A4ll posterior subdivision supplementary motor area; VOIs volume-of-interests.

4. DISCUSSION

In this study, we investigated whether the previously observed association between hyperacusis and smaller gray matter volumes in the right supplementary motor area (SMA) in individuals with bilateral sensorineural hearing loss and tinnitus would also be found in individuals with tinnitus and clinically normal hearing. Our findings confirmed that smaller SMA gray matter volumes related to hyperacusis are also present in individuals with tinnitus and clinically normal hearing. We did not find any interaction between hearing loss and hyperacusis on the SMA gray matter volumes while controlling for age. In other words, the smaller SMA gray matter volumes were associated with hyperacusis and not hearing loss.

The SMA functions in the fields of action preparation, initiation, and inhibition and is an integral part of the motor network [31, 32]. Neurons in this area fire prior to the onset of an action, and a subset of those neurons responds to specific input modalities (visual, tactile, or auditory) when matched to a motor response [33, 34]. Our previous work showed that the SMA is the only brain region to exhibit structural gray matter

differences between groups associated with hyperacusis in tinnitus, and SMA gray matter volumes could classify participants with or without hyperacusis with high accuracy [14]. The current work extends our previous findings and reveals that the reduction in the SMA gray matter volumes in individuals with hyperacusis is not the result of hearing loss and may, therefore, reflect specific changes in the motor response to the perceived loudness of sound. In this study, we also found that smaller SMA gray matter volumes were primarily associated with the attentional subcategory of hyperacusis rather than the social subscale, suggesting that hyperacusis is linked to increased alertness regarding sound. There may be a correspondence between this heightened alertness and a constant readiness to provide a motor response to sound.

Our findings are consistent with previous reports in both animal models and humans. A recent study in mice has connected increased gain along the auditory pathway to involuntary facial movements [35], which may be related to the structural changes reported here. Supporting evidence from animal studies shows that hyperacusis-induced animals respond faster to auditory stimuli, indicating a link between motor activity and hyperacusis [9, 36]. Additionally, a study in humans found that facial reaction and pupil dilatation to emotional sounds could distinguish between participants with or without hyperacusis and tinnitus who have clinically normal hearing thresholds [37]. Furthermore, an EEG study found increased functional activity in the SMA region in hyperacusis participants [13]. Similarly, participants with misophonia, a condition where very specific sounds (e.g., chewing or breathing) provoke strong emotional and physiological reactions, showed increased SMA activity when listening to triggering sounds relative to any other sound, as measured with an fMRI [38, 39]. In addition, participants with obsessive-compulsive disorder (OCD) may exhibit heightened sensitivity to sound, which can lead them to develop fixations on avoiding certain sounds [40, 41]. Interestingly, OCD has been closely linked with functional changes, including increased activation, in the SMA region [42, 43]. Together, these findings and our study suggest a close association between hyperacusis and the brain network that is involved in preparing and conducting motor movements.

This study has several limitations. First, this study included a limited number of participants with tinnitus and clinically normal hearing and a limited number of female participants. These factors may affect the comparison and generalizability of the findings. Future research should

aim to include larger and more sex-balanced groups. Second, the study utilized subjective hyperacusis questionnaires. Although these questionnaires are well-validated [25], future research should consider incorporating objective methods, such as psychoacoustic measurements [44], to precisely describe and quantify hyperacusis. Third, air-conduction tone audiometry was used to define hearing loss in this study. Future studies could also measure bone-conduction thresholds in order to explicitly differentiate between conductive and sensorineural hearing loss. Lastly, future research should also investigate the effects of the duration of hyperacusis, tinnitus, and hearing loss on changes in gray matter volumes. Only a few studies have previously examined the impact of tinnitus duration on gray matter volumes [45, 46]. To our knowledge, no study has yet explored changes in response to the duration of hyperacusis or hearing loss (although the onset of the problem is often difficult to identify). These limitations highlight areas where future research could improve the study design to enhance the validity of findings related to gray matter changes in hyperacusis.

5. CONCLUSIONS

Our study highlights the importance of understanding the structural mechanisms underlying hyperacusis, and particularly the role of the SMA in the attentional control of auditory-motor coordination. By identifying specific brain regions associated with hyperacusis, we can develop more targeted and effective interventions to improve the quality of life for those affected by this condition. Future research should continue to explore the complex interactions between hyperacusis, tinnitus, and hearing loss, as well as the potential for neuroplasticity-based therapies to mitigate the impact of hyperacusis on daily functioning.

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SUPPLEMENTARY MATERIALS

Supplementary Table S1. Acquisition parameters for T1-weighted anatomical brain imaging.

	Langers et al., 2012	Boyen et al., 2013	Amaral et al., 2015	Koops et al., 2020
SENSE Coil Type	8-channel head coil	8-channel head coil	8-channel head coil	32-channel head coil
Repetition Time (ms)	9	9	9	10.4
Echo Time (ms)	3.6	3.5	3.6	5.7
Field of View (mm)	192 x 176	232 x 256	232 x 256	256 x 224
Flip Angle (°)	8	8	8	8
Number of Slices	144	170	170	160
Slice Thickness (mm)	1	1	1	1
Slice Gap (mm)	0	0	0	0
Scan Time (sec)	160	251	251	614

Supplementary Table S2. Overview of demographic, audiometric, questionnaires, morphometric data for the four participant groups.

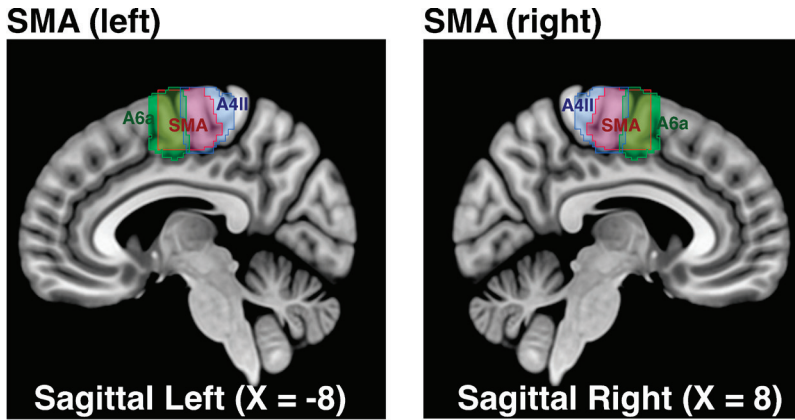
Group	Normal Hearing and Tinnitus (NHT)		Hearing Loss and Tinnitus (HLT)		Statistic
	Without Hyper-acusis	With Hyper-acusis	Without Hyper-acusis	With Hyper-acusis	
Demographic					
n	13	22	41	25	–
Mean Age (years)	45.5 ± 11.2 ^{c,d} (29 – 62)	44.1 ± 12.2 ^{c,d} (19 – 59)	58.3 ± 10.5 ^{a,b} (27 – 76)	59.5 ± 7.9 ^{a,b} (41 – 73)	$\chi^2(3) = 30.0$, $p < 0.001$
Sex (male female)	8 5	10 12	32 9	15 10	$\chi^2(3) = 7.0$, $p = 0.071$
Handedness (L R NP NA)	11 2 0 0	21 1 0 0	5 29 0 7	0 23 2 0	–
Audiometric (for both ears 0.25 to 8 kHz)					
Mean PTA (dB HL)	7.3 ± 5.4 ^{c,d}	9.4 ± 7.3 ^{c,d}	35.0 ± 8.6 ^{a,b}	38.2 ± 12.3 ^{a,b}	$\chi^2(3) = 65.8$, $p < 0.001$
Questionnaires					
HQ	16.5 ± 3.6 ^{b,d} (10 – 21)	27.1 ± 4.1 ^{a,c} (22 – 35)	13.2 ± 5.4 ^{b,d} (0 – 21)	26.2 ± 3.7 ^{a,c} (22 – 33)	$\chi^2(3) = 76.0$, $p < 0.001$
HQ-Attentional	5.3 ± 1.5 ^b	8.2 ± 1.8 ^{a,c}	3.7 ± 1.7 ^{b,d}	7.3 ± 1.4 ^b	$\chi^2(3) = 56.0$, $p < 0.001$
HQ-Social	5.5 ± 2.1 ^{b,d}	10.7 ± 3.4 ^{a,c}	5.4 ± 3.4 ^{b,d}	10.5 ± 2.4 ^{a,c}	$\chi^2(3) = 42.1$, $p < 0.001$
HQ-Emotional	5.7 ± 1.8 ^{b,d}	8.3 ± 2.1 ^{a,c}	4.1 ± 1.39 ^{b,d}	8.4 ± 2.1 ^{a,c}	$\chi^2(3) = 48.1$, $p < 0.001$
HADS-Anxiety	6.8 ± 4.2 (0 – 16)	6.5 ± 3.4 ^c (2 – 15)	3.7 ± 3.0 ^b (0 – 11)	5.9 ± 4.6 (0 – 16)	$\chi^2(3) = 11.0$, $p = 0.011$
HADS-Depres-sion	5.0 ± 3.8 (0 – 14)	4.9 ± 3.7 (0 – 15)	3.2 ± 3.1 ^d (0 – 10)	6.2 ± 4.7 ^c (0 – 16)	$\chi^2(3) = 8.4$, $p = 0.038$
THI	33.7 ± 20.4 (4 – 66)	44.4 ± 20.5 ^c (10 – 88)	27.4 ± 19.3 ^{b,d} (4 – 80)	41.8 ± 20.8 ^c (6 – 82)	$\chi^2(3) = 13.0$, $p = 0.005$
THI-Functional	18.9 ± 12.2	24.7 ± 10.4 ^c	15.6 ± 10.2 ^{b,d}	25.6 ± 11.8 ^c	$\chi^2(3) = 15.0$, $p = 0.002$
THI-Emotional	7.5 ± 6.8	11.3 ± 8.5	6.7 ± 6.8	9.6 ± 6.9	$\chi^2(3) = 6.6$, $p = 0.085$
THI-Catastrophic	7.2 ± 3.6	8.4 ± 5.1 ^c	5.2 ± 4.1 ^b	6.7 ± 3.9	$\chi^2(3) = 8.0$, $p = 0.045$
Morphometric					
TIV (cm ³)	1429.6 ± 159.9	1419.1 ± 147.1	1494.9 ± 139.1	1451.0 ± 96.3	$\chi^2(3) = 4.1$, $p = 0.248$

Mean ± Standard Deviation (Range). dB HL decibel hearing loss; HADS hospital anxiety depression scale; HLT participant group with tinnitus and bilateral sensorineural hearing loss (either with or without hyperacusis); HQ hyperacusis questionnaires; L left; NA not available; NHT participant group with tinnitus and clinically normal hearing (either with or without hyperacusis); NP no preference; PTA pure tone average (0.25 to 8 kHz); R right; THI tinnitus handicap inventory. ^aThe group differed significantly ($p \leq 0.05$) from the NHT group without hyperacusis; ^bThe group differed significantly ($p \leq 0.05$) from the NHT group with hyperacusis; ^cThe group differed significantly ($p \leq 0.05$) from the HLT group without hyperacusis; ^dThe group differed significantly ($p \leq 0.05$) from the HLT group with hyperacusis.

Supplementary Table S3. Results of Spearman’s Rank correlation coefficients between the SMA gray matter volumes (HMAT and HBN atlas VOIs) and either the HADS anxiety and depression scores or the THI tinnitus burden scores across all participants. The statistical threshold for Spearman’s Rank correlation coefficients was set at $p \leq 0.05$. The SMA VOIs (HMAT and HBN atlas VOIs) are shown in **Supplementary Figure S1**.

	HADS Anxiety Score	HADS Depression Score	THI Tinnitus Burden Score
HMAT Atlas VOIs			
SMA left	$r_s(100) = -0.05, p = 0.639$	$r_s(100) = -0.03, p = 0.722$	$r_s(99) = 0.09, p = 0.371$
SMA right	$r_s(100) = 0.04, p = 0.717$	$r_s(100) = 0.03, p = 0.805$	$r_s(99) = 0.04, p = 0.720$
HBN Atlas VOIs			
SMA_A6m left	$r_s(100) = -0.04, p = 0.686$	$r_s(100) = 0.01, p = 0.903$	$r_s(99) = 0.10, p = 0.303$
SMA_A6m right	$r_s(100) = -0.05, p = 0.612$	$r_s(100) = -0.01, p = 0.945$	$r_s(99) = 0.02, p = 0.875$
SMA_A4ll left	$r_s(100) = -0.04, p = 0.719$	$r_s(100) = -0.10, p = 0.284$	$r_s(99) = 0.05, p = 0.642$
SMA_A4ll right	$r_s(100) = 0.10, p = 0.338$	$r_s(100) = 0.03, p = 0.737$	$r_s(99) = 0.06, p = 0.564$

HADS Hospital Anxiety Depression Scale; HBN Human Brainnetome atlas; HMAT Human Motor Area Template atlas; SMA supplementary motor area; SMA_A6ma anterior subdivision of the supplementary motor area; SMA_A4ll posterior subdivision of the supplementary motor area; THI Tinnitus Handicap Inventory; VOIs volume-of-interests.



Supplementary Figure S1. We selected the volume-of-interests (VOIs) for the supplementary motor area (SMA) as defined in a previous study using both anatomical and functional MRI data. These SMA VOIs (highlighted in red) are freely accessible as the Human Motor Area Template (HMAT) atlas from <http://lrnlab.org/>. We also selected VOIs for the anterior and posterior subdivisions of the SMA, labelled as SMA_A6m (highlighted in green) and SMA_A4ll (highlighted in blue) respectively, as defined by the structural and functional connectome architecture. These SMA VOIs are freely accessible as the Human Brainnetome (HBN) atlas from <https://atlas.brainnetome.org/bnatlas.html>.

DATA AVAILABILITY STATEMENT

The datasets analyzed in the study are available from the corresponding author upon reasonable request. The datasets are not publicly available, due to privacy and ethical restrictions.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.



Chapter 6

General discussion

Makani P. (2026)

This thesis set out to investigate the structural neural correlates of tinnitus and hyperacusis by examining gray matter differences while carefully controlling for hearing status. Across four complementary studies [1–4], consistent patterns of gray matter differences in both auditory and non-auditory brain regions were identified, highlighting the significant influence of hearing status and suggesting the distinct structural neural mechanisms underlying tinnitus and hyperacusis. This discussion summarized the key findings, situated them within the existing literature, explored their clinical and research implications, and acknowledged both limitations and directions for future research.

1. SUMMARY OF KEY FINDINGS

In **Chapter 02**, a meta-analysis demonstrated that the presence or absence of hearing loss significantly influences reported structural gray matter differences in individuals with tinnitus. This finding suggests that variability in hearing status may account for some of the inconsistencies (groups not matched for age and hearing loss) observed in previous neuroimaging studies.

Chapter 03 extended these findings by examining four comparatively large groups: individuals with normal hearing and tinnitus, hearing loss and tinnitus, normal hearing without tinnitus, and hearing loss without tinnitus. The results directly confirmed the conclusions of Chapter 02, showing that tinnitus is associated with distinct patterns of gray matter alterations. Specifically, individuals with tinnitus and normal hearing exhibited smaller gray matter volumes compared to normal hearing without tinnitus, whereas those with tinnitus and hearing loss showed larger gray matter volumes to hearing loss without tinnitus. Both auditory (Heschl's gyrus and medial geniculate body) and non-auditory brain (ventromedial prefrontal cortex and the lingual gyrus) regions were identified, indicating that different neural mechanisms may underlie tinnitus depending on hearing status.

Chapters 04 and 05 focused on hyperacusis and revealed that, irrespective of hearing status, hyperacusis was associated with smaller gray matter volumes in the supplementary motor area. In addition, in a subset of participants where functional data were available, hyperacusis was associated with increased sound-evoked responses in the supplementary motor area. These findings suggest that structural and functional alterations in the supplementary motor area may represent a potential neural biomarker for hyperacusis.

2. INTERPRETATION OF FINDINGS

This thesis presented multiple lines of evidence indicating that hearing status significantly influences the structural brain alterations associated with tinnitus.

Consistent with previous research [5, 6], individuals with tinnitus and clinically normal hearing demonstrated reduced gray matter volumes in both the auditory cortex and the prefrontal cortex. These reductions likely reflect neural atrophy or deficient inhibitory mechanisms that contribute to the persistent perception of phantom sounds [7, 8]. Given the central role of the auditory cortex in sound processing, reduced gray matter in this region may indicate maladaptive neural activity resulting from abnormal auditory input. In addition, the prefrontal cortex is critical for top-down regulation and suppression of irrelevant sensory information, including internally generated auditory noise. Therefore, structural degradation within this region may impair the brain's ability to inhibit or modulate tinnitus perception [9, 10].

In contrast, individuals with tinnitus and hearing loss showed larger gray matter volumes in non-auditory brain regions, especially the lingual gyrus and precuneus. Previous studies [11, 12] have reported similar findings, suggesting that these changes may reflect the brain's attempt to adapt to reduced sound input from the ear. When hearing loss occurs, the brain may recruit other networks to compensate for missing auditory information and preserve a sense of continuous hearing. This adaptive change might also enhance internal attention and prediction processes, which could potentially sustain the ongoing perception of tinnitus.

Collectively, these findings highlight distinct neuroanatomical patterns of tinnitus that depend on hearing status. This distinction helps explain inconsistencies across previous neuroimaging studies by identifying hearing loss as a critical moderating variable. Importantly, the results emphasize that tinnitus is a heterogeneous condition involving multiple neural pathways, underscoring the need for hearing-status-specific approaches in future research development.

This thesis also presented strong evidence that the supplementary motor area plays a central role in hyperacusis, independent of hearing status.

Individuals with tinnitus and hyperacusis showed reduced gray matter volumes in the supplementary motor area, implicating dysfunction in motor planning, response inhibition, and attentional control circuits. The supplementary motor area coordinates facial and hand movements, which may underlie the reflexive facial expressions and defensive hand, or arm movements frequently observed in response to loud or aversive sounds. Consistent with these findings, objective autonomic measures, such as pupil dilation and facial movement, have been shown to correlate with hyperacusis severity [13], indicating heightened emotional and autonomic reactivity to auditory stimuli. These results align with prior neuroimaging studies implicating motor and premotor regions in sound intolerance [14, 15, 16], supporting the notion that the supplementary motor area may serve as neural marker for hyperacusis.

3. CLINICAL AND RESEARCH IMPLICATIONS

The findings presented in this thesis demonstrate that structural brain alterations associated with tinnitus are substantially modulated by hearing status. This observation underscores the necessity of accounting for auditory function in both clinical and research contexts, and highlights the importance of individualized, mechanism-based therapeutic approaches. In individuals with normal audiometric hearing, tinnitus may result from cochlear synaptopathy, a form of “hidden hearing loss” characterized by degeneration of synapses between inner hair cells and auditory nerve fibers, which remains undetectable through conventional audiometry [17, 18]. This loss of afferent input can trigger maladaptive cortical reorganization, leading to auditory hyperactivity and reduced inhibitory control. For such cases, interventions aimed at reducing aberrant neural excitation and restoring inhibitory balance, including sound therapy, targeted neuromodulation, or pharmacological enhancement of GABAergic transmission, may yield the most favorable therapeutic outcomes.

Conversely, in tinnitus accompanied by measurable hearing loss, structural brain changes are typically secondary to reduced peripheral input and the ensuing neuroplastic adaptations within auditory and non-auditory networks [7, 19, 20]. In these cases, therapeutic strategies should prioritize facilitating adaptive plasticity through auditory rehabilitation and amplification via hearing aids or cochlear implants.

Beyond tinnitus, this thesis identified the supplementary motor area as a key neural substrate in hyperacusis, independent of peripheral hearing status. Consistent findings of reduced gray matter volumes within the supplementary motor area suggest its role in aberrant sensorimotor gating and attentional modulation of auditory input. These results highlight the supplementary motor area as a promising neural marker and candidate target for therapeutic intervention in hyperacusis. Neuromodulatory interventions, including transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and neurofeedback, may support top-down regulation of auditory hypersensitivity, thereby improving emotional and behavioral responses to sound [21].

4. LIMITATIONS AND FUTURE DIRECTIONS

Although the studies presented here offer valuable insights into structural gray matter differences associated with tinnitus and hyperacusis while carefully controlling for hearing status, several limitations must be acknowledged. First, variability in sample size (particularly the smaller sample size for the normal hearing with tinnitus group – Chapter 03, and the normal hearing with tinnitus and hyperacusis group – Chapter 05) may have influenced the findings, underscoring the need for future studies with larger and more homogeneous cohorts. Second, tinnitus and hyperacusis are heterogeneous in their clinical presentation. Future research should account for additional factors e.g., symptom duration, laterality, and psychological factors to further refine the understanding of these conditions. Third, although structural brain imaging provides critical information about brain morphology in tinnitus and hyperacusis, a more comprehensive understanding of the underlying neural mechanisms may require multimodal imaging, combining functional brain imaging and gamma-aminobutyric acid (GABA) brain imaging. Finally, longitudinal designs are essential, particularly large-scale population cohorts that can assess structural and functional brain differences associated with the onset of tinnitus and hyperacusis, as well as with their chronification.

5. CONCLUSION

In conclusion, the studies presented in this thesis highlighted the significant impact of hearing status on structural gray matter differences

associated with tinnitus. Distinct patterns of gray matter differences were observed in both auditory and non-auditory brain regions between individuals with tinnitus who had clinically normal hearing and those with hearing loss, when compared to their hearing-matched controls. These findings suggest that hearing status strongly influences structural gray matter differences. The studies also highlighted the involvement of both auditory and non-auditory brain regions in hyperacusis, with a significant gray matter reduction observed in the supplementary motor area, regardless of hearing status. This gray matter difference in the supplementary motor area was robust enough to classify the presence of hyperacusis in individuals with tinnitus. These findings suggest that the supplementary motor area may serve as a potential biomarker for hyperacusis. Overall, this thesis highlighted the complex nature of tinnitus and hyperacusis, pointing toward the need to carefully consider hearing status and the involvement of both auditory and non-auditory neural mechanisms in understanding these conditions.

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SUMMARY

Tinnitus is characterized by a persistent ringing or buzzing in the ears, occurring without any external sound stimuli. It significantly affects quality of life, leading to issues such as impaired concentration, sleep disturbances, and often results in psychological challenges like anxiety and depression. Tinnitus commonly co-occurs with other auditory conditions, particularly hearing loss and/or hyperacusis. About 90% of individuals with tinnitus have reported some degree of hearing loss, while up to 10 % of individuals with tinnitus have reported normal hearing. Additionally, hyperacusis, an increased sensitivity to normal sound levels, is prevalent in about 63% of individuals with tinnitus.

Neuroimaging offers a unique possibility to explore both structural and functional characteristics in the brain of individuals with tinnitus, hearing loss, and hyperacusis. While earlier studies, especially structural neuroimaging studies, have highlighted some brain alterations linked to tinnitus, the findings have been largely inconsistent and heterogeneous. This inconsistency has been attributed to factors such as small sample sizes and the heterogeneity within tinnitus populations, including variations in the presence or absence of hearing loss and/or hyperacusis.

This thesis aims to untangle the complex interaction between tinnitus, hearing loss, and hyperacusis using a larger and more representative sample, addressing factors that have been suggested to contribute to the inconsistent and heterogeneous findings reported in previous studies.

In **chapter 2**, a meta-analysis was conducted comparing four groups: 1) individuals with normal hearing and tinnitus, 2) individuals with normal hearing, 3) individuals with hearing loss and tinnitus, and 4) individuals with hearing loss. In the normal hearing groups, tinnitus was associated with smaller gray matter volumes in the inferior temporal gyrus, whereas among the hearing loss groups, tinnitus was linked to higher gray matter volumes in the lingual gyrus and the precuneus. Consequently, these findings provide an explanation for the heterogeneous results in earlier studies and led to conclude that the presence or absence of hearing loss plays a critical role in brain structural differences in individuals with tinnitus.

In **chapter 3**, a retrospective case-control study was conducted to investigate the interaction between tinnitus and hearing loss on structural

gray matter volumes comparing four groups: 1) individuals with normal hearing and tinnitus, 2) individuals with normal hearing, 3) individuals with hearing loss and tinnitus, and 4) individuals with hearing loss. A significant interaction was found between the effects of tinnitus and hearing loss on several gray matter brain structures. Consistent with the meta-analysis, the results indicated that in the normal hearing groups, tinnitus was associated with smaller gray matter volumes in Heschl's gyrus, the posterior insula, the ventromedial prefrontal cortex, the middle cingulate cortex, the supplementary motor area, and the inferior frontal gyrus. In sharp contrast, in the hearing loss groups, tinnitus was linked to higher gray matter volumes in Heschl's gyrus, the posterior insula, the lingual gyrus, the parahippocampal region, the ventromedial prefrontal cortex, and the inferior frontal gyrus. This study highlighted the distinction between the structural abnormalities associated with tinnitus in normal hearing and those linked to tinnitus in hearing loss, which led to conclude that there are potentially unique mechanisms of tinnitus in normal hearing compared to those in hearing loss.

In **chapter 4**, a retrospective case-control study was conducted to investigate gray matter differences associated with hyperacusis by comparing two groups of individuals with tinnitus and hearing loss: 1) those who scored above the threshold for hyperacusis based on the validated hyperacusis questionnaire, and 2) those who scored below the threshold for hyperacusis. The findings indicated that hyperacusis was associated with smaller gray matter volumes in the supplementary motor area (part of the motor region). Additionally, using the gray matter volumes of the supplementary motor area, individuals with hyperacusis were classified with an accuracy of 80% in area-under-the-curve analysis. **Chapter 5** describes similar results in subjects without hearing loss confirm a reduction of white matter in the supplementary motor cortex associated with hyperacusis. Previous studies by other research groups have found hyperacusis to be associated with central gain within the auditory pathway. The studies in Chapter 4 and 5 were the first to demonstrate that non-auditory regions, such as the motor region, were also impacted in hyperacusis. It led to conclude that differences in the motor region in hyperacusis might be associated with motor behavior, potentially related to hyperactive and avoidance behaviors in response to loud sound stimuli. It further led to the hypothesis that the supplementary motor area could serve as a structural biomarker to identify hyperacusis in individuals with tinnitus, regardless of hearing levels.

While the findings presented in this thesis advance understanding of the complex interaction between tinnitus, hearing loss, and hyperacusis, particularly with respect to underlying structural mechanisms, further research is required to strengthen their validity and clinical relevance. A promising direction for future studies is the use of pre- and post-treatment measurements in individuals undergoing targeted interventions, such as cognitive behavioral therapy for tinnitus or audiological treatment for hyperacusis. Combining structural and functional neuroimaging with behavioral and physiological measures pre- and post-treatment may also help identify objective biomarkers associated with symptom change, thereby improving mechanistic insight, and supporting the development of reliable biomarkers for tinnitus and hyperacusis. Finally, longitudinal designs are essential, particularly large-scale population cohorts that can assess structural and functional brain differences associated with the onset of tinnitus and hyperacusis, as well as with their chronification.

NEDERLANDSE SAMENVATTING

Tinnitus wordt meestal gekenmerkt door een aanhoudend suizen of zoe-men in de oren, dat optreedt zonder enige externe geluidsprikkel. Het heeft een aanzienlijke invloed op de kwaliteit van leven en leidt tot problemen zoals verminderde concentratie, slaapstoornissen en heeft vaak ook psychologische gevolgen zoals angst en depressie. Tinnitus komt vaak samen voor met andere auditieve aandoeningen, met name gehoorverlies en/of hyperacusis. Ongeveer 90% van de personen met tinnitus rapporteert een zekere mate van gehoorverlies, terwijl tot 10% van de personen met tinnitus een normaal gehoor rapporteert. Daarnaast komt hyperacusis, een verhoogde gevoeligheid voor normale geluidsniveaus, voor bij ongeveer 63% van de personen met tinnitus.

Neuroimaging biedt een unieke mogelijkheid om zowel structurele als functionele eigenschappen in de hersenen van personen met tinnitus, gehoorverlies en hyperacusis te onderzoeken. Hoewel eerdere studies, met name structurele neuroimagingstudies, enkele hersenveranderingen in verband met tinnitus hebben aangetoond, waren de bevindingen grotendeels inconsistent en heterogeen. Deze inconsistentie kan worden toegeschreven aan factoren zoals kleine steekproefgroottes en de heterogeniteit binnen tinnituspopulaties, waaronder variaties in de aanwezigheid of afwezigheid van gehoorverlies en/of hyperacusis.

Dit proefschrift heeft tot doel de complexe interactie tussen tinnitus, gehoorverlies en hyperacusis te ontrafelen door gebruik te maken van een grotere en meer representatieve steekproef, en daarbij rekening te houden met factoren die mogelijk bijdragen aan de inconsistente en heterogene bevindingen die in eerdere studies zijn gerapporteerd.

In **hoofdstuk 2** werd een meta-analyse uitgevoerd waarin vier groepen werden vergeleken: 1) personen met normaal gehoor en tinnitus, 2) personen met normaal gehoor zonder tinnitus, 3) personen met gehoorverlies en tinnitus, en 4) personen met gehoorverlies zonder tinnitus. In de groepen met normaal gehoor werd tinnitus geassocieerd met kleinere grijze-stofvolumes in de inferieure temporale gyrus, terwijl tinnitus in de groepen met gehoorverlies werd gekoppeld aan grotere grijze-stofvolumes in de linguale gyrus en de precuneus. Deze bevindingen bieden daardoor een verklaring voor de heterogene resultaten in eerdere studies en leidden tot de conclusie dat de aanwezigheid of afwezigheid van

gehoorverlies een cruciale rol speelt in structurele hersenverschillen bij personen met tinnitus.

In **hoofdstuk 3** werd een retrospectieve case-controlstudie uitgevoerd om de interactie tussen tinnitus en gehoorverlies op structurele grijze-stofvolumes te onderzoeken door vier groepen te vergelijken: 1) personen met normaal gehoor en tinnitus, 2) personen met normaal gehoor zonder tinnitus, 3) personen met gehoorverlies en tinnitus, en 4) personen met gehoorverlies zonder tinnitus. Er werd een significante interactie gevonden tussen de effecten van tinnitus en gehoorverlies op verschillende grijze-stofstructuren in de hersenen. In overeenstemming met de meta-analyse gaven de resultaten aan dat in de groepen met normaal gehoor tinnitus was geassocieerd met kleinere grijze-stofvolumes in de gyrus van Heschl, de posterieure insula, de ventromediale prefrontale cortex, de middelste cingulate cortex, het supplementaire motorische gebied en de inferieure frontale gyrus. Daarentegen werd tinnitus in de groepen met gehoorverlies in verband gebracht met grotere grijze-stofvolumes in de gyrus van Heschl, de posterieure insula, de linguale gyrus, de parahippocampale regio, de ventromediale prefrontale cortex en de inferieure frontale gyrus. Deze studie benadrukte het onderscheid tussen de structurele afwijkingen die geassocieerd zijn met tinnitus bij normaal gehoor en die welke verband houden met tinnitus bij gehoorverlies, wat leidde tot de conclusie dat er mogelijk unieke mechanismen van tinnitus bestaan bij normaal gehoor in vergelijking met tinnitus bij gehoorverlies.

In hoofdstuk 4 werd een retrospectieve case-controlstudie uitgevoerd om grijze-stofverschillen te onderzoeken die geassocieerd zijn met hyperacusis door twee groepen personen met tinnitus en gehoorverlies te vergelijken: 1) personen die boven de drempel scoorden voor hyperacusis op basis van de gevalideerde hyperacusisvragenlijst, en 2) personen die onder de drempel scoorden voor hyperacusis. De bevindingen gaven aan dat hyperacusis werd geassocieerd met kleinere grijze-stofvolumes in het supplementaire motorische gebied (onderdeel van de motorische regio). Daarnaast werden, op basis van de grijze-stofvolumes van het supplementaire motorische gebied, personen met hyperacusis geclassificeerd met een nauwkeurigheid van 80% in een area-under-the-curve-analyse. **Hoofdstuk 5** beschrijft vergelijkbare resultaten bij proefpersonen zonder gehoorverlies en bevestigt een vermindering van witte stof in de supplementaire motorische cortex die geassocieerd is met hyperacusis. Eerdere studies van andere onderzoeksgroepen hebben aangetoond dat hyperacusis geassocieerd is met centrale versterking binnen de auditieve

baan. De studies in hoofdstuk 4 en 5 waren de eerste die aantoonen dat niet-auditieve regio's, zoals de motorische regio, eveneens betrokken zijn bij hyperacusis. Dit leidde tot de hypothese dat verschillen in de motorische regio bij hyperacusis mogelijk samenhangen met motorisch gedrag, potentieel gerelateerd aan hyperactief en vermijdend gedrag als reactie op luide geluidsprikkels. Tevens werd geconcludeerd dat het supplementaire motorische gebied kan dienen als een structurele biomarker om hyperacusis te identificeren bij personen met tinnitus, ongeacht eventueel gehoorverlies.

Hoewel de bevindingen in dit proefschrift het begrip van de complexe interactie tussen tinnitus, gehoorverlies en hyperacusis vergroten, met name met betrekking tot de onderliggende structurele mechanismen, is verder onderzoek nodig om hun validiteit en klinische relevantie te versterken. Een veelbelovende richting voor toekomstig onderzoek is het gebruik van voor- en nametingen bij personen die gerichte interventies ondergaan, zoals cognitieve gedragstherapie voor tinnitus of audiologische behandeling voor hyperacusis. Het combineren van structurele en functionele neuroimaging met gedrags- en fysiologische metingen vóór en na behandeling kan bovendien helpen bij het identificeren van objectieve biomarkers die samenhangen met symptoomverandering, waardoor het mechanistisch inzicht wordt verbeterd en de ontwikkeling van betrouwbare

biomarkers voor tinnitus en hyperacusis wordt ondersteund. Ten slotte zijn longitudinale onderzoeksopzetten essentieel, met name grootschalige populatiecohorten die structurele en functionele verschillen in de hersenen kunnen beoordelen die samenhangen met het ontstaan van tinnitus en hyperacusis, evenals met de chroniciteit ervan.

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