



UNIVERSITY OF
LIVERPOOL

An Investigation of the Neural Substrates of Tinnitus
Perception Using Advanced Magnetic Resonance
Imaging Techniques

Thesis submitted with the requirements of the University of Liverpool
for the degree of Doctor in Philosophy

BY

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June 2016

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Declaration

The work presented in this thesis is the result of my own work. The material contained in this thesis has not been presented, nor is currently being presented, either wholly or in part, for any other degree or qualification.

A handwritten signature in black ink, appearing to read 'Fahad H. Alhazmi', written in a cursive style.

Fahad H, Alhazmi

Acknowledgments

The work presented in this thesis would not have been possible without my close association with many people. I take this opportunity to extend my sincere gratitude and appreciation to all those who made this PhD thesis possible.

I would like to express my special appreciation and thanks to my PhD supervisors Dr. Vanessa Sluming and Prof. Graham J Kemp for supporting and encouraging me during this project journey. Special thanks to my mentor advisor Prof John Quinn for his advises and supports throughout my PhD journey. Also, I would like to thank Dr Ian Mackenzie and Mr Tony Kay for their supports in patients' recruitment from Aintree University Hospital NHS Foundation Trust. My special words of thanks should also go to Dr Jamaan Alghamdi for his continuous support and cooperation.

A huge thank to Miss Valerie Adams, Mr Bill Bimson and Mrs Sue Monaghan for their support, help and advice in MARIARC. I'm very grateful for all my friends and colleagues I have made throughout my PhD journey. My deepest gratitude goes to all of my study participants for their generosity of time and patience.

I would like to express my gratitude to Taibah University and the Ministry of Education in Saudi Arabia for the scholarship that allowed me to undertake this research project in the United Kingdom. Also, I would like to thank the Royal Cultural Bureau of Saudi Arabia in London for their supporting during my study journey in the United Kingdom.

A special thank to my family. Words cannot express how grateful I am to my parents, Hassan Alhazmi and Nora Alhazmi; my beloved wife, Haifaa Alhazmi; my lovely daughter, Ladun Alhazmi; my brothers, Dr. Abdullah Alhazmi, Eng. Abdualazizi Alhazmi, Eng. Ahmed Alhazmi; and my sisters, Dr. Sarah Alhazmi, Dr. Rawan Alhazmi. You all kept me going, and this thesis would not have been possible without you.

Fahad H, Alhazmi

Liverpool, June 2016

*I dedicate this thesis to
my family, my wife and my daughter
for their constant support and unconditional love.
I love you all dearly.*

Publications

Paper

- **Alhazmi F**, Kay T, Mackenzie I, Kemp G J and Sluming V (2016) *An Investigation of the Impact of Tinnitus Perception on the Quality of Life*. J Phonet Audiol 2: 1000113. doi:[10.4172/jpay.1000113](https://doi.org/10.4172/jpay.1000113)

Conference Proceedings

- **Alhazmi, F.**, Alghamdi, J., Mackenzie, I., Kay, T., Kemp, G J., & Sluming, V. A. (2016). Cortical Morphometry and Microstructure Integrity in Tinnitus: A Combined of VBM and TBSS Study. Poster session presented at the meeting of Scientific Meeting of the Organisation for Human Brain Mapping. Geneva Switzerland.
- **Alhazmi, Fahad**, Mackenzie, Ian , Kay, Tony , Kemp, Graham J and Sluming, Vanessa . *Neural Correlates of White Matter Integrity in Tinnitus: Tract-Based Spatial Statistics*. (2016) In: 10th International Tinnitus Research Initiative, 16-18 March 2016, University of Nottingham, UK.
- **Fahad Alhazmi** · Jamaan Alghamdi · Ian Machenzie · Graham J Kemp · Vanessa Sluming (2015). *The Influence of hearing acuity on brain sub-cortical structures and function*. European Society for Magnetic Resonance in Medicine and Biology, Edinburgh/UK; 10/2015
- **Fahad Alhazmi** · Jamaan Alghamdi · Laura Parkes · Batil Alonazi · Ian Mackenzie · Tony Kay · Graham J Kemp · Vanessa Sluming (2015). *ASL Perfusion MRI in Tinnitus*. British Tinnitus Association Annual Conference, Manchester, UK; 09/2015
- Batil Alonazi · Jamaan Alghamdi · **Fahad Alhazmi** · Simon Keller · Tony Marson · Vanessa Sluming (2015) *Resting-state functional connectivity alterations in patients with Newly Diagnosed Epilepsy*. The Organization for Human Brain Mapping (OHBM), 21st Annual Meeting of the Organization for Human Brain Mapping 14 Jun 2015 to 18 Jun 2015 Honolulu, Hawaii, US; 06/2015
- **Alhazmi, Fahad**, Alghamdi, Jamaan, Mackenzie, Ian, Kemp, Graham J and Sluming, Vanessa (2015) *Neuroanatomical alterations of mild to moderate hearing loss revealed by grey matter morphometry and diffusion tensor imaging*. In: BNA Festival of Neuroscience 2015, 12-15 April 2015, Edinburgh.
- **Alhazmi, Fahad**, Alghamdi, Jamaan, Mackenzie, Ian, Kemp, Graham J and Sluming, Vanessa (2015) *Functional Connectivity fMRI Changes in Mild to Moderate Hearing Loss: Independent Components Analysis Study*. In: European Conference on Clinical Neuroimaging, 23-24 March 2015, Consiglio Nazionale delle Ricerche, Piazzale Aldo Moro,7 - 00185, Roma, Italy.
- **Alhazmi, Fahad**, Alghamdi, Jamaan, Mackenzie, Ian, Kemp, Graham J and Sluming, Vanessa (2015). *Cortical Thickness and Sulcal Depth in Mild-to-Moderate Hearing Loss*. In: European Congress of Radiology, 4-8 March 2015, Austria International Centre, Vienna, Austria.
- **Alhazmi, F** and Kemp, G J and Sluming, V (2014). *Grey Matter Morphometry and Cortical Thickness Alteration in the Auditory Cortex: Differences Associated with Normal Variations of Hearing Acuity*. In: International Conference on Auditory Cortex, Germany. (In Press)
- **Alhazmi, F** and Alonazi, B and Alghamdi, J and Kemp, G J and Mackenzie, I and Sluming, V (2014). *White Matter Integrity Changes in Age-Related Hearing Loss: Tract Based Spatial Statistics Study*. In: Inner Ear Biology Workshop, University of Sheffield.

Abstract

Aims and objectives: The overall aim of this thesis is to investigate the neural substrates of tinnitus perception using advanced magnetic resonance imaging (MRI) techniques. The main objectives of this thesis dissertation are to (1) identify the impact of tinnitus perception on the quality of life, (2) investigate the morphological alterations in cortical and subcortical brain structures in tinnitus, (3) explore the auditory perception in tinnitus, (4) identify the perfusion pattern changes in tinnitus, (5) investigate the effect of tinnitus perception on brain functional connectivity, (6) explore the microstructure alterations in white matter structures in tinnitus and (7) investigate the relationship between tinnitus handicap scores and brain structure/function.

Materials and methods: A total of 34 individuals with tinnitus, 20 healthy controls with mild to moderate hearing loss (MH), and 20 healthy controls with normal hearing (NH) participated in the work presented in this thesis. Pure tone air conduction audiometry was performed to assess the hearing level. Behavior assessments were undertaken of handedness, anxiety and depression, and tinnitus severity. Different MR images were acquired: T1-weighted images, T2-weighted images, functional images (resting-state and task-based fMRI), arterial spin labelling images (ASL) and diffusion tensor imaging (DTI). Different MRI analysis techniques were applied including: voxel and surface based morphometry (VBM and SBM), shape appearance differences, independent component analysis (ICA), and tract-based spatial statistics (TBSS).

Results: In the behaviour study, we found that anxiety and depression, and tinnitus characteristics (i.e. laterality and onset) play an important role in the tinnitus severity. In the structural MRI study, we observed a reduction of grey matter (GM) volume and cortical thickness in frontal orbital cortex, insula, and fusiform and inferior temporal gyri in tinnitus patients relative to healthy controls. It was found that cingulate thickness, hippocampus volume and the shape of putamen and amygdala are involved in tinnitus severity. In the auditory perception study, auditory cortex was found hyperactive during acoustic stimuli in tinnitus patients compared to normal controls. In the perfusion study, a significant hypoperfusion was found in the tinnitus group compared to controls in left insula, orbital frontal and visual cortex. Also, the perfusion pattern of auditory and parahippocampus cortices were found to be involved in tinnitus severity, while cingulate cortex was found to be involved in tinnitus patients with anxiety and depression. In the functional connectivity study, seven functional RSNs were found to be significantly different or correlated to tinnitus perception: default mode network (DMN), dorsal attention network (DAN), ventral attention network (VAN), visual network (VN), auditory network (AN), sensorimotor network (SMN) and salience network (SN). In the structural connectivity study, the tinnitus group showed a significant reduction of white matter (WM) integrity at several WM tracts, and a significant increase of mean diffusivity (MD) at right inferior-frontal occipital fasciculus compared to healthy controls. The integrity of WM in corpus callosum and cingulum were found to be involved in tinnitus laterality, and anxiety and depression respectively.

Conclusion: This research extends the literature demonstrating the differences of brain structure and function in tinnitus patients. Findings clearly show behavioral and brain structural and functional associations to tinnitus perception. These results indicate that abnormal processing of auditory, limbic and attention brain networks may cause persistent tinnitus noise.

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Abbreviations

- AC Auditory cortex
- ACC Anterior cingulate cortex
- ADC Apparent diffusion coefficient
- Alns Anterior insula
- AMG Amygdala
- AN Auditory network
- ASL Arterial spin labelling
- ATR Anterior thalamic radiation
- BA Boardman Area
- CA Cornu ammonic
- CC Corpus callosum
- CN Cochlear nucleus
- CNS Central nervous system
- CSF Cerebrospinal fluid
- DAN Dorsal attention network
- DMN default mood network
- dmPFC Dorsomedial prefrontal cortex
- DTI Diffusion tensor Imaging
- EEG Electroencephalogram
- EHI Edinburgh Handedness Inventory
- FA Fractional anisotropy

- FAF Frontal arcuate fasciculus
- fMRI Functional magnetic resonance imaging
- FOV Field of the view
- GM Grey matter
- H Hypothalamus
- HADS Hospital anxiety and depression scale.
- HIPPO Hippocampus
- HL Hearing loss,
- IC inferior colliculus
- ICA independent components analysis
- IFG Inferior frontal gyrus,
- IFOF Inferior frontal occipital fasciculus
- IHC Inner hair cells
- ILF Inferior longitudinal fasciculus
- ITG Inferior temporal gyrus,
- LL Lateral lemniscus,
- MD Mean diffusivity
- MEG Megnetoencephalography
- MFG Middle frontal gyrus
- MGB Medial geniculate body
- MGN Medial geniculate nucleus
- mHG Medial heschl's gyrus

- MRI Magnetic resonance imaging
- MTG Middle temporal gyrus
- NAc: nucleus accumbence
- NHC Normal hearing controls
- NRES National Research Ethics Services
- OFC Orbital frontal cortex
- OHC Outer hair cells
- OL Occipital lobe
- PAC Primary auditory cortex
- PAF Parietal arcuate fasciculus
- PCC Posterior cingulate cortex
- PCG Precentral gyrus
- PET Positron emission tomography
- PFC prefrontal cortex
- PHIPP Parahippocampus
- PIS Participants Information Sheet
- PTSD Post-traumatic stress disorder
- ROIs Regions of interests
- RSN Resting state networks
- SAC Secondary auditory cortex
- SBM Surface based morphometry
- ScACC Subcallosal anterior cingulate cortex

- SFG Superior frontal gyrus
- SLF Superior longitudinal fasciculus
- SMG Supramarginal gyrus
- SOAE Spontaneous oto-acoustic emissions
- SOC Superior olivary complex
- SPECT Single photon emission computer tomography
- STG Superior temporal gyrus
- TBSS Tract-based spatial statistics
- TFI Tinnitus functional index
- THI Tinnitus Handicap Inventory
- TIN Tinnitus patients
- TM Tympanic membrane
- TR Repetition time
- TE Echo time
- TRT Tinnitus retraining treatment
- VAS Visual analogue scale
- VBM Voxel-based morphometry
- VN Visual network
- VSN Ventral stream network
- vmPFC Ventromedial prefrontal cortex
- WM White matter

Chapter 1: Introduction

1.1. General introduction

Tinnitus is a phantom sound described as a ringing in the ears, and can have a negative impact on the quality of life for millions of people around the world (Roberts et al., 2010). Tinnitus is derived from the Latin verb *tinnire*, which means to ring. In individuals with severe tinnitus, all aspects of their life may be affected, including the inability to do intellectual work, and the disease may have a negative effect on their health in general (Alsanosi, 2011). There are some factors that may cause tinnitus perception and influence the degree to which the person suffers from it include loud noise exposure, head injury, certain medications, and depression (Baguley et al., 2013). Tinnitus is usually accompanied by hearing loss (Savastano, 2008) and hyperacusis (Gu et al., 2010, Rauschecker et al., 2015). Hyperacusis is defined as the lowered loudness discomfort levels (LDL) associated with an abnormal annoyance to sounds (Coelho et al., 2007)

Although much research has been undertaken to understand the pathophysiological mechanism of tinnitus, no effective treatments are currently available for tinnitus patients (Baguley et al., 2013, Noble and Tyler, 2007, Tunkel et al., 2014). In the past, surgical interventions were found successful in some cases (Roland et al., 1995); however, surgery does not provide valid and reliable results for all tinnitus patients (House and Brackmann, 1981, De Ridder et al., 2007, Moller et al., 1993, De Ridder et al., 2010, Bell et al., 2016). Other available tinnitus treatments including hearing aids, wide-band sound therapy, brain stimulation, laser treatments, and

counseling, however, these treatments have been tried with somewhat inconsistent results (Baguley et al., 2013).

Despite the fact that tinnitus has been traditionally considered as diseases of the cochlea, there is evidence from many animal and human neuroimaging studies that plastic changes in the central nervous system are involved in causing many forms of tinnitus (Elgoyhen et al., 2015, Møller, 2011b). Expression of neural plasticity might cause some symptoms of sensory system disorders by changing neural processing and rerouting of information (Møller, 2011b). There are several observations that show tinnitus has neural correlates in the brain, regardless of peripheral damage that might trigger it (Adjamian et al., 2014). Tinnitus status was found to persist, and may become worse after the transection of the eighth cranial nerve that destroys the inputs from cochlear to the brain (House and Brackmann, 1981, Baguley et al., 2013). Not all tinnitus patients suffer from hearing loss, which is considered to be one of the challenges to tinnitus generation models that are based on a compromised cochlear function that causes hyperactivity in brain structures (Schaette and McAlpine, 2011). In addition, tinnitus loudness measures obtained psychophysically are not strongly associated with tinnitus-related distress (Hiller and Goebel, 2006). Therefore, research interest in tinnitus has moved from studies of cochlear pathology to those of brain plasticity, as there is not sufficient evidence to support the former concept.

The participation of the CNS in tinnitus perception has been investigated widely using different neuroimaging techniques including an electroencephalogram (EEG)

(De Ridder and Vanneste, 2014), magnetoencephalography (MEG) (Hoke et al., 1998), single-photon emission computed tomography (SPECT) (Shulman et al., 1995), positron emission tomography (PET) (Arnold et al., 1996) and magnetic resonance imaging (MRI) (Aldhafeeri et al., 2012a, Maudoux et al., 2012). Based on previous neuroimaging studies, tinnitus pathology was found to be involved in both auditory and non-auditory brain areas: the auditory cortex (AC), the anterior and posterior cingulate cortex, the prefrontal cortex (PFC), the insula, the orbitofrontal cortex (OFC), and the precuneus and parahippocampus (Vanneste and De Ridder, 2012).

Identifying neural correlations in tinnitus subgroups (i.e. tinnitus descriptions, laterality, and severity) is very important for therapeutic purposes, as different treatments may be needed for different tinnitus subgroups (Tyler et al., 2008). Since subjective tinnitus is a subjective phenomenon, medical practitioners rely on the patients' own experiences to describe the problem. Tinnitus descriptions are categorized into tonal (which includes hissing, musical tone, ringing, whistling), noise (which includes buzzing, roaring, rushing), crickets (which includes clicking, chirping), and others (Tyler et al., 2008). In terms of tinnitus laterality, individuals with tinnitus experience tinnitus bilaterally (both ears) or unilaterally (right or left ear). Nearly 5% of tinnitus sufferers report troublesome and annoying tinnitus perception that affects their ability to sleep, and roughly 0.5-1.0% of adult tinnitus sufferers report very severe tinnitus that has a significant negative impact on the quality of their life (Davis, 1989).

1.2. Aim of the study

The main aim of this thesis was to investigate the effect of tinnitus perception on underlying neural processes, cortical structural integrity, and the auditory resting state network using advanced magnetic resonance imaging (MRI) techniques.

1.3. Problems and hypotheses

Tinnitus is a phantom auditory perception that is only described by the patient. There is no objective sign of tinnitus can be diagnosed. A lack of a proven pathophysiological mechanism of tinnitus, and the perception of tinnitus in patients with significant tinnitus has a strong connection with the emotional system (Jastreboff and Hazell, 1993).

Tinnitus may be caused by structural and functional alterations to central auditory pathway processing (Rauschecker et al., 2010, Noreña and Farley, 2013). Investigation of the neurophysiological changes in tinnitus patients could lead to understanding the pathophysiological mechanism (Vanneste and De Ridder, 2012). However, the pathophysiological mechanism of tinnitus is not fully understood (Baguley et al., 2013). Evidence suggests that there are some auditory and non-auditory brain areas that might be involved in tinnitus perception. However, the present understanding of the complex pathophysiology of tinnitus has not led to the discovery of an effective treatment for all tinnitus cases (Baguley et al., 2013). The research questions, hypothesizes and methodologies undertaken in this thesis is summarized in table 1.1.

The effect of tinnitus perception on the quality of life

Tinnitus patients frequently define the disorder as life-changing (Baguley et al., 2013) and display negative effects generally associated with chronic pain (Moller, 2007) including sleep interference, cognitive difficulties, poor concentration at work and home, and negative emotional reactions such as fear, anxiety and depression (Tyler and Baker, 1983). Although many efforts have been made to provide a cure for tinnitus, no treatments have shown a complete relief for tinnitus patients (Noble and Tyler, 2007). The inability to find an effective treatment might be due to the lack of standardized outcome measures that would improve the effect of tinnitus treatments in different tinnitus groups (Meikle et al., 2012, Newman et al., 1996). Because of the nature of subjective tinnitus, self-report questionnaires have been developed to scale its negative impact and measure its severity. Chapter 5 examines the effect of tinnitus perception on quality of life.

- Research question 1: Does tinnitus perception have a negative impact of the quality of life?

In this thesis, I have administrated the following questionnaires: the tinnitus handicap inventory (THI), the tinnitus functional index (TFI), the Edinburgh Handedness Inventory (EHI), and the Hospital Anxiety and Depression Score (HADS). These surveys were used in conjunction with participants' audiogram results to assess the associations between four different factors (handedness, hearing loss, anxiety, and depression) and tinnitus severity. Also, I assessed the association between certain tinnitus characteristics such as tinnitus laterality, duration, and tinnitus severity. The following hypothesis was considered:

- Tinnitus perception has a negative impact of the quality of life.

Morphological alterations in cortical and subcortical structures in tinnitus patients

Subjective tinnitus is an idiopathic condition as the pathophysiological mechanism is unknown (Shulman and Goldstein, 2009). One hypothesis states that tinnitus is a disorder involving a distributed network of the peripheral and central nervous systems (Adjajian et al., 2014). Chapter 3 describes the wide range of morphological findings that have been previously identified in tinnitus patients. Neuroimaging results were found to be inconsistent, possibly due to the differences of sample sizes, hearing loss range, participants' age and gender, tinnitus severity, image acquisitions, and data analysis methods. These factors could have had a major effect on the results. Chapter 6 attempted to investigate whether tinnitus patients exhibit central morphological alterations as compared to normal healthy controls, particularly in cortical and subcortical structures.

- Research question 2: Is the morphology of cortical and subcortical structures of the brain affected in tinnitus patients?

I applied three novel techniques that have never previously employed together in a study of chronic tinnitus patients, namely, voxel-based morphometry, surface-based morphometry, and vertex-based morphometry, all using a high-resolution T1-weighted anatomical MR scan. The following hypothesis was investigated:

- Tinnitus patients will demonstrate morphological abnormalities of cortical and subcortical structures relative to healthy controls.

Auditory perception in tinnitus patients during tonal stimuli

The function of the central auditory system in tinnitus patients might work differently when compared to normal healthy subjects, which leads to the hypothesis that the central auditory system plays a key role in tinnitus perception (Lanting et al., 2008). Animal and human studies revealed that noise-induced tinnitus showed an increase in spontaneous neural activity at several levels of the auditory pathway (Norena and Eggermont, 2003a, Lanting et al., 2008). In this thesis, Chapter 7 attempted to investigate the response of the auditory cortex to acoustic stimuli that was measured using functional magnetic resonance imaging (fMRI).

- Research question 3: Is the central processing of auditory cortex affected in tinnitus patients?

An acoustic stimulus with different frequencies was applied during an fMRI scan in order to activate the auditory cortex, and then show how the auditory cortex responded to sound. The following hypothesis was investigated:

- Tinnitus patients will demonstrate hyperactivity of auditory cortex compared to normal controls.

Cerebral blood perfusion alterations in tinnitus patients

Neuroimaging studies have shown that tinnitus is associated with increased activity in the central auditory system (Cacace et al., 1999, Cacace, 2003, Kaltenbach, 2000). Brain perfusion aims to understand the pattern of blood perfusion, and identifies the abnormal function in specific brain areas (Mahmoudian et al., 2012). In this

thesis, Chapter 8 attempted to investigate cerebral blood pattern (CBF) in tinnitus patients.

- Research question 4: Is the pattern of the brain blood perfusion altered in tinnitus patients?

I applied a novel MRI technique, namely, arterial spin labeling (ASL) to investigate the brain blood perfusion pattern in tinnitus patients. This technique had not ever previously been applied (non-invasive) in tinnitus research. The following hypothesis was considered:

- The pattern of brain blood perfusion is altered in tinnitus patients.

Resting state functional connectivity alterations in tinnitus patients

Few neuroimaging studies have assessed cerebral functional connectivity in tinnitus patients. Electroencephalography (EEG) and magnetencephalography (MEG) studies have shown an alteration of functional connectivity in tinnitus patients (Vanneste et al., 2011b). The use of these modalities provided high temporal resolution with a poor anatomical resolution that making the interpretation of the results very difficult (Maudoux et al., 2012). In this thesis, Chapter 9 attempted to investigate functional connectivity in tinnitus patients using high magnetic field 3T, which has a better spatial resolution (fMRI) than EEG and MEG.

- Research question 5: Does tinnitus perception affect resting state functional connectivity?

I applied a novel whole brain functional connectivity analysis technique that called independent component analysis (ICA). This technique aims to investigate whether tinnitus patients demonstrate alterations in functional connectivity between brain network components at rest. The following hypothesis was investigated:

- Functional connectivity between some brain networks will be altered in tinnitus patients relative to healthy controls.

Microstructural alterations in white matter of tinnitus patients

Neuroimaging studies have revealed that white matter integrity correlates with tinnitus perception with a variety of results (Aldhafeeri et al., 2012a, Benson et al., 2014, Crippa et al., 2010, Husain et al., 2011a). This might be due to the differences in sample size, hearing loss levels, participants' characteristics, and image acquisition and analysis. Alterations of white matter integrity could be related to the functional abnormalities of auditory and limbic structures. In this thesis, Chapter 10 attempted to investigate the effect of tinnitus perception on white matter integrity in a group of patients with a wide range of tinnitus severity.

- Research question 6: Is the integrity of white matter microstructure altered in tinnitus patients?

In the present study, diffusion tensor imaging (DTI) was analyzed using tract-based spatial statistics (TBSS), which is a whole brain analysis that aims to evaluate white matter alterations possibly related to tinnitus perception. The following hypothesis was considered:

Chapter 1: Introduction

- Tinnitus patients will show a reduction of white matter integrity in the brain structures that are associated with auditory and limbic processes.

Table 1.1: Summary of the research questions, methodologies and hypothesizes

Chapter	Research questions	Methodologies	Hypothesises
5	Does tinnitus perception have a negative impact of the quality of life?	Audiogram, Questionnaires: EHI, THI, TFI, HADS.	Tinnitus perception has a negative impact of the quality of life
6	Is the morphology of cortical and subcortical structures of the brain affected in tinnitus patients?	VBM, SBM and vertex based morphometry	Tinnitus patients will demonstrate morphological abnormalities of cortical and subcortical structures relative to healthy controls.
7	Is the central processing of auditory cortex affected in tinnitus patients?	fMRI task-based using pure tone stimuli	Tinnitus patients will demonstrate hyperactivity of auditory cortex compared to normal controls
8	Is the pattern of the brain blood perfusion altered in tinnitus patients?	ASL perfusion MRI	The pattern of brain blood perfusion is altered in tinnitus patients
9	Does tinnitus perception affect resting state functional connectivity?	fMRI resting state (ICA)	Functional connectivity between some brain networks will be altered in tinnitus patients relative to healthy controls
10	Is the integrity of white matter microstructure altered in tinnitus patients?	DTI-TBSS	Tinnitus patients will show a reduction of white matter microstructure integrity in brain structures that are associated with auditory and limbic processes

1.4. Summary

In order to expand upon the existing knowledge of the neural correlates of tinnitus perception, this thesis utilized six studies including a variety of behavior and neuroimaging techniques. For the behavioral portion of the study, different questionnaires were administered to investigate the effect of tinnitus perception on quality of life. For neuroimaging studies, structural and functional data were analysed using novel techniques to enhance our understanding of the pathophysiological mechanism of tinnitus.

Chapter 2: The Human Brain and Auditory Pathway

2.1 Introduction

This chapter will review the anatomy and physiology of the auditory system. It provides a brief introduction of the human brain, the main structure and function of auditory pathway and the limbic system that are more likely relevant to tinnitus.

2.2 The Human Brain

The human brain weight is between 1200 and 1400 g, which made up of about 100 billion neurons (Hartmann et al., 1994). Central nervous system is responsible to process information from all around in the body. It consists of the brain (which includes brainstem, medulla, pons, cerebrum, midbrain, diencephalon and cerebral hemispheres) and spinal cord. The human brain consists of three main brain tissue types: grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The GM is the darker side of the brain, which contains the neuron cell bodies and dendrites, and involves in information processing, while the WM is the fiber bundle that contains glial cells and myelinated axons, and involves in information transmission (Miller, 2011). The CSF is the body fluid that is colorless, and links the brain with the spinal cord (Miller, 2011).

2.2.1 Grey matter cerebral hemisphere

The largest division of the brain is cerebral, which is divided into two hemispheres, and each of them divided into four lobes that are frontal, parietal, occipital and temporal lobes (figure 2.1). Brain lobes can be differentiated by some of the cerebral features with some landmarks. Gyri is one of these features that seen in the brain as

an elevated ridge 'winding' around the brain. Gyrus is divided by a small groove that is called sulci. Central sulcus is one example of sulci that divides the frontal lobe from the parietal lobe, whereas lateral sulcus separates the temporal lobe from the parietal lobe (figure 2.2). Another landmark divides brain regions and lobes are fissures that classified into three deep grooves: longitudinal fissure (divides the two hemispheres), transverse fissure (separates the cerebrum from the cerebellum) and sylvian/lateral fissure (separates the temporal lobe from the frontal and parietal lobes) (Miller, 2011).

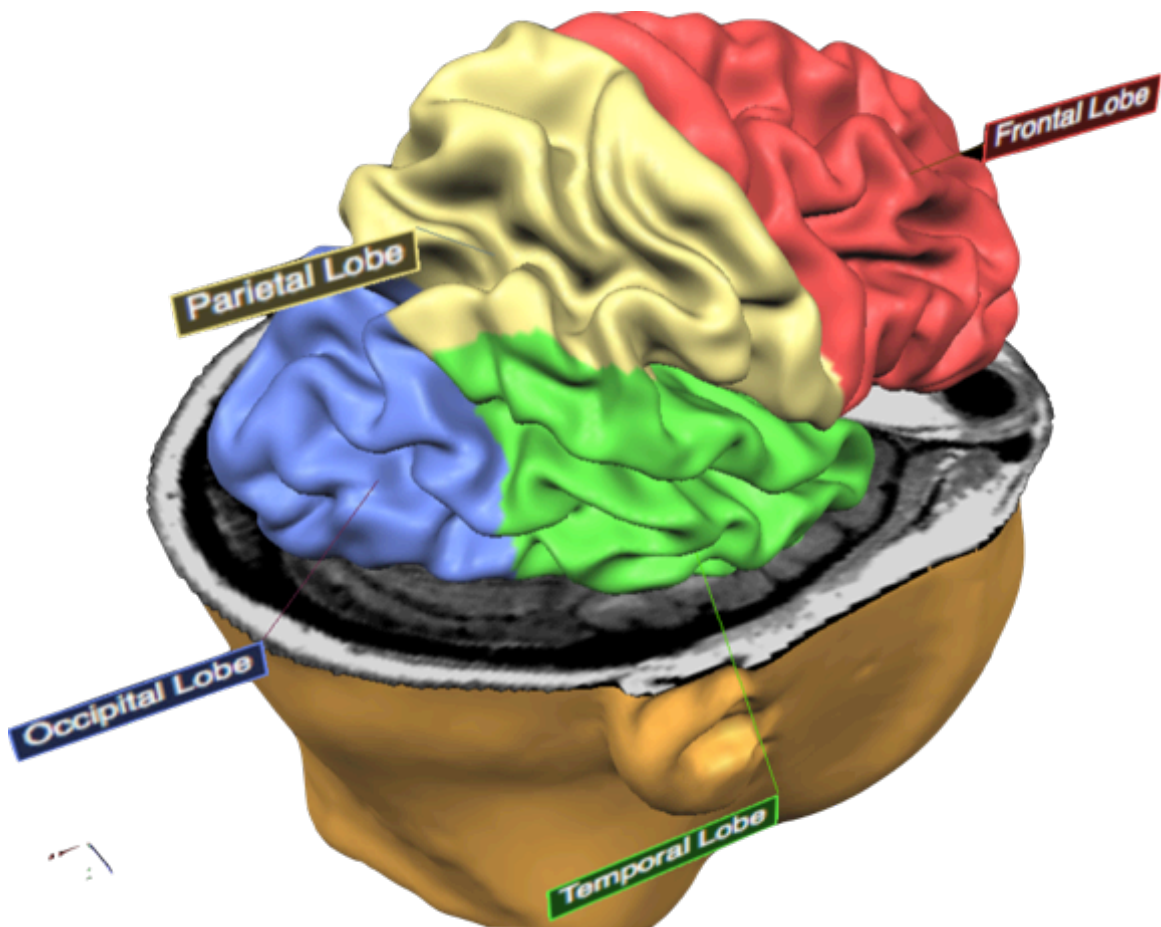


Figure 2.1: Surface view of the main brain lobes: frontal (red), temporal (green), parietal (yellow) and occipital (blue) lobes. Subject NH01, 3D mesh reconstructed for illustration purpose using BrainVoyger OX 2.8

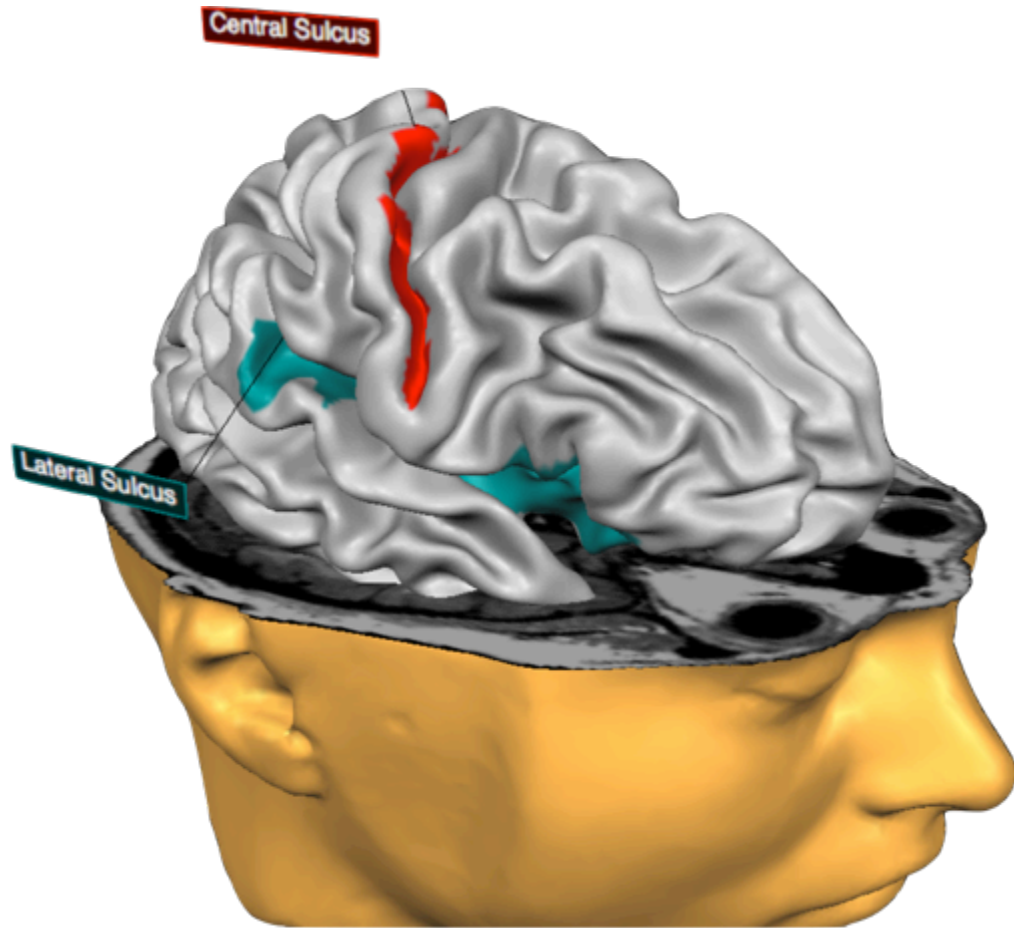


Figure 2.2: Surface view of the central (red) and lateral sulci (green). (Subject NH01, 3D mesh reconstructed for illustration purpose using BrainVoyager OX 2.8)

There are some cytoarchitecture features in each layer of human cerebral that can characterize brain areas from each other's regarding to theirs' cell type, density and folding. The local brain tissue volume can be estimated, which reflects the tissue density (Hartmann et al., 1994). Cortical thickness measures the distance between outer and inner surfaces, which can determine changes across the axes of the cortical columns, and measure these changes in mm (Fischl and Dale, 2000). There are variations of cortical thickness between brain areas, which ranges from 4.5 mm in the precentral gyrus to 1.5 mm in the calcarine sulcus. For instance, within the

temporal lobe, cortical thickness is a relatively thicker cortex in superior temporal gyrus compared to middle and inferior temporal gyri (Fischl and Dale, 2000).

2.2.2 White matter fibres of the cerebral hemisphere

White matter fibres of the cerebral hemisphere are allocated underneath the cortical surface, which have their origins and terminals within the cortex. These white matter fibres are categorised into three main classifications regarding to their locations: association, commissural and projection fibres (figure 2.3) (Miller, 2011).

Association fibres interconnect cortical sites within one cerebral hemisphere, which include superior longitudinal fasciculus (links the frontal and occipital lobes), arcuate fasciculus (interconnect the frontal and temporal lobes), inferior longitudinal fasciculus (pass from the occipital to the temporal lobes), uncinate fasciculus (run between the anterior and inferior parts of the frontal lobe and temporal gyri) (Miller, 2011).

Commissural fibres are interhemispheric fibres that connect one hemisphere to the other. Corpus callosum connects the two cerebral hemispheres, which has four parts: rostrum, genu, body and splenium. Anterior commissure is another commissural fibres that pass transversely and connects inferior and middle temporal gyri and the olfactory regions of the two cerebral hemispheres. The third type of commissural fibre is hippocampal commissure that links the posterior columns of fornix on each hemisphere (Miller, 2011).

Projection fibres run between the cerebral cortex areas and subcortical structures such as thalamus and brainstem. The mediodorsal nucleus of the thalamus is connected to the prefrontal cortex via anterior limb part of projection fibres. The

posterior limb of projections fibres is thalamocortical projections that connect thalamus to motor cortex in the frontal lobe. Retrolenticular is the third part of projection fibres that connects medial and lateral geniculate nuclei of the thalamus to the auditory and visual cortices respectively (Miller, 2011).

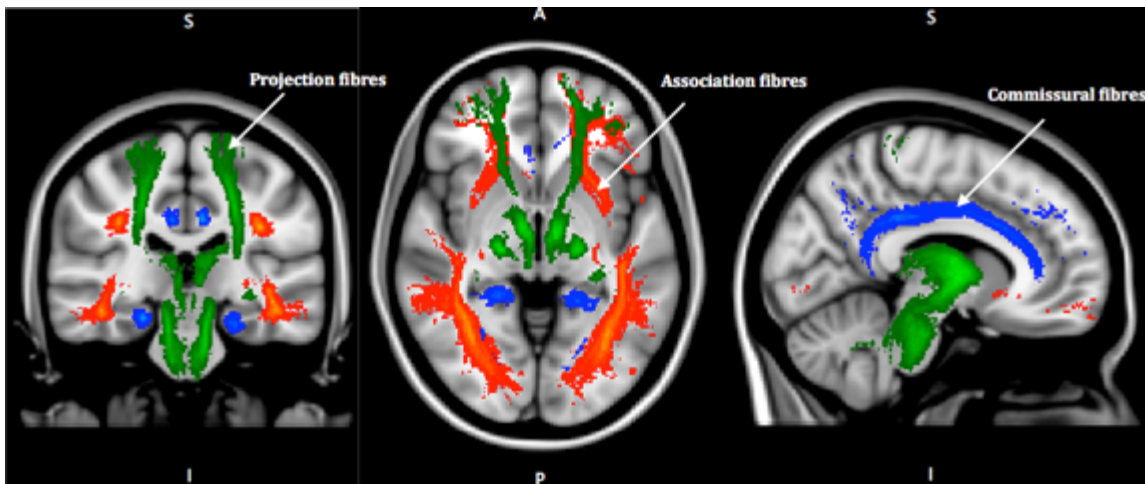


Figure 2.3: The main white matter fiber tracts: association (Red), commissural (Blue) and projection fibers (Green). Orthographic view for illustration purpose using FSLVIEW.

2.2.3 Brain development

Brain plasticity is the ability of the brain to reorganize neural pathways based on new experiences (Kolb and Gibb, 2011). These new events can change the normal brain to stimulate functional development or to alter injured brain that can be seen at different levels of analysis from behaviour to molecular levels (Bavelier et al., 2010).

Brain Lateralization refers to that some brain functions are more dominant in one hemisphere than another one. Corpus callosum is a large bundle of neural fibers (white matter) connecting the two hemispheres. Left hemisphere is more dominant for symbolic thinking, language, detail and literal meaning, while right hemisphere is more dominant for spatial perception, overall picture and context (Corballis, 2012).

Furthermore, right hemisphere controls left side of body and visual field, while left hemisphere controls right side of body and visual field (Kenneth, 2005).

Sensation and perception are complementary processes that allow us to experience the world. Sensation is a passive process that brings information from outside into the brain. It is a bottom up processing that refers to the process by which the central nervous system receives inputs such as touch, sight, sound and smell from the environment via sensory neurons. Perception is an active process that involves selecting organizing in interpreting information that has been brought to the brain by various senses. It is a top-down processing, which refers to the process by which the brain interprets and organizes sensory information (Coren et al., 2004).

2.3 The human auditory pathway

Acoustic journey travels from different systems and mediums in the human in order to process the auditory information. Hearing occurs in our brain as we hear from our ears, however, the nervous system is needed to clarify some acoustic features such as loudness, localization and meaning. The human auditory pathway is classified into two main systems: peripheral and central auditory systems (Adjamian et al., 2014).

2.3.1 Peripheral auditory system

The peripheral auditory system contains the ears, which is composed of three main parts: external, middle and inner parts that formed by different structures (figure 2.4). The external ear is formed by three structures that are auricle, external acoustic meatus and the layer of tympanic membrane (TM). It is narrow chamber in

the temporal bone that is linked with skin. The middle ear comprises of the tympanic cavity that communicates with air filled cavities, the auditory ossicles' bones (hammer, anvil and stapes) and the inner layer of the tympanic membrane (TM). The functions of middle ear are to transfer sound from air to fluids and protect ear from loud sounds. The third part of the ear is inner ear, which consists of the cochlea with the organ of corti that contains the auditory receptors (hair cells). These receptors are very sensitive to the frequency strength; high frequencies are allocated near of the base of cochlea, whereas low frequencies near the apex of the cochlea. The auditory nerve is the last terminal in the peripheral auditory system, which is a bundle of 25-30 thousand fibers that travels acoustic information from inner ear (cochlea) to the central auditory center (brain) (Warren, 2008).

Peripheral auditory processing is summarizing as following. Sound is filtered as it passes via the pinna and ear canal. This acoustic energy vibrates the tympanic membrane (TM) and then conveyed to mechanical energy by the amplification in the middle ear. The oval window is displaced by the stapes (middle ear bone), which leads hydrodynamic energy causes cochlear membranes to shear against hair cell bundles. An electrochemical signal is sent via auditory nerve to brain. (Warren, 2008)

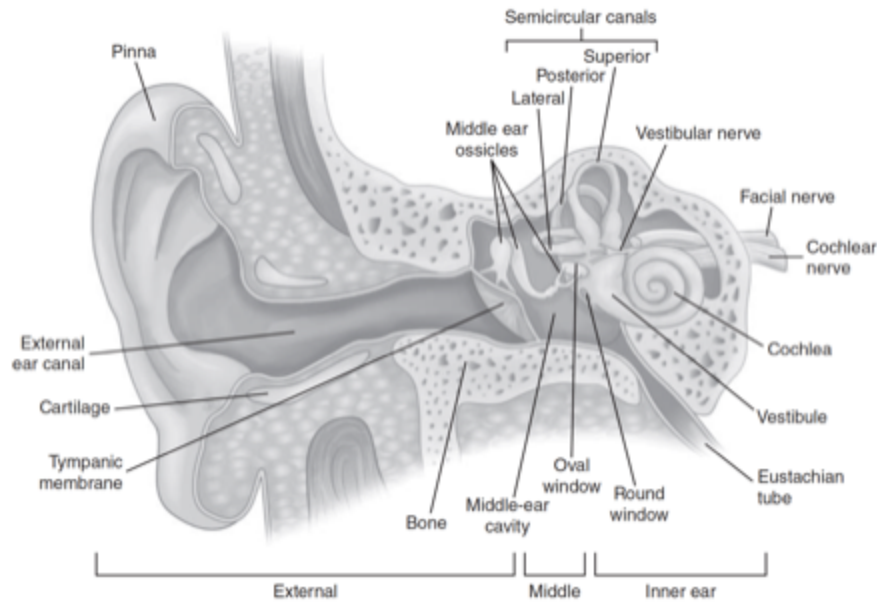


Figure 2.4: Anatomy of the ear that divides anatomically and functionally into three regions: external, middle and inner ear. Adopted with permission from (Stach, 2010).

2.3.2 Central auditory system

The central auditory system starts from the auditory receptors that are allocated in the inner ear (cochlea). These synapse on spiking neurons in the spiral ganglia, the axons of which form the auditory (8th cranial) nerve. These then lead to the cochlear nucleus (CN), then to the superior olive, then to the inferior colliculus (IC), then to the medial geniculate nucleus (MGN), and finally on to auditory cortex (AC) (figure 2.5) (Warren, 2008).

In the lower brain stem, auditory nerve fibers are terminated to form synapses with large groups of neurons in cochlear nuclei (CN). Each cochlear nuclei (right or left) receives fibers ipsilateral (the same side of the brain) from the same ear side. Then, most of fibers are projected directly to the mid-brain, especially in the contralateral (the opposite side of the brain) inferior colliculus (IC). The rest of fibers are sent to the superior olive in the pons in order to localize the sound source. Fibers are then projected from superior olive via lateral lemniscus to inferior colliculus, and then to

the medial geniculate nucleus (MGN) in the thalamus. The medial geniculate nucleus is the station where all ascending auditory signals are collected and then passed to bilateral auditory cortex (Kandel et al., 2000).

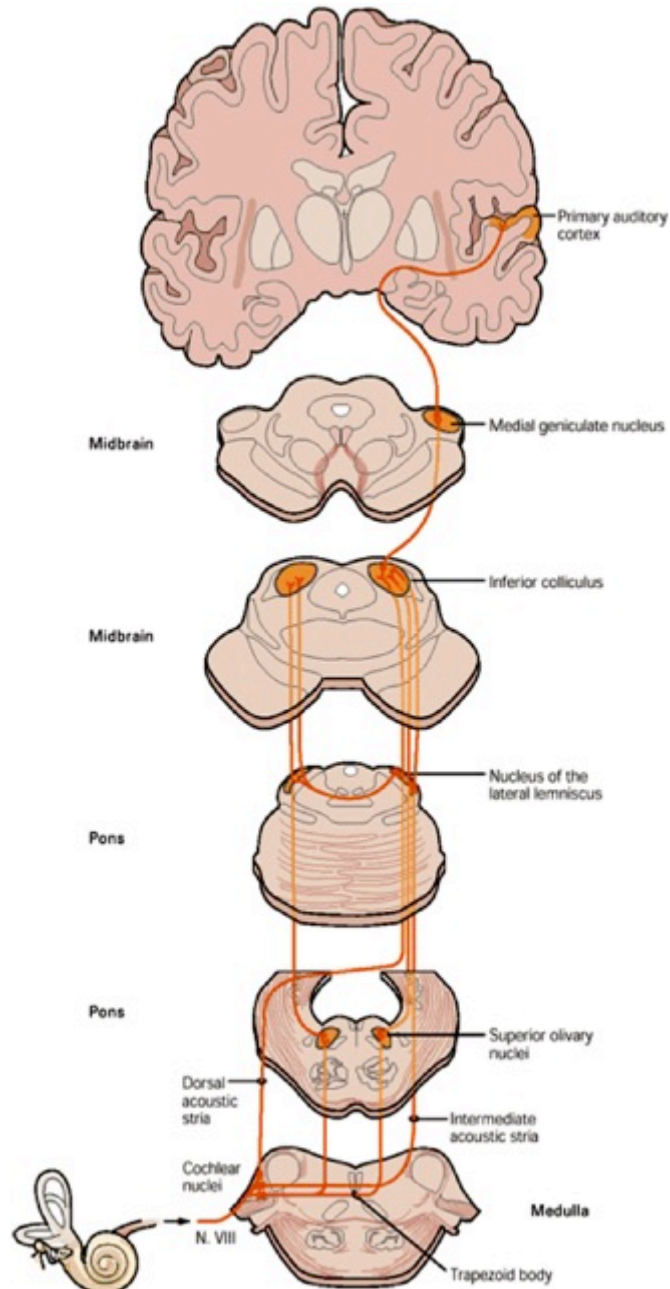


Figure 2.5: The central auditory pathways extend from the cochlear nucleus to the auditory cortex. Adapted from (Kandel et al., 2000)

2.3.3 Auditory cortex

Primary auditory cortex (PAC) is allocated on the superior temporal gyrus that is divided into two main parts: core auditory cortex (Brodmann's area 41) and association auditory cortex (area 42) (figure 2.6). PAC can be seen in both hemispheres, but they are not identical; left PAC is larger than the right PAC due to the grey matter volume is about 30-35% larger on the left hemisphere (Steinmetz, 1996). PAC is not an isolated structure of the brain, which interacts with other cortical, and neocortical structures.

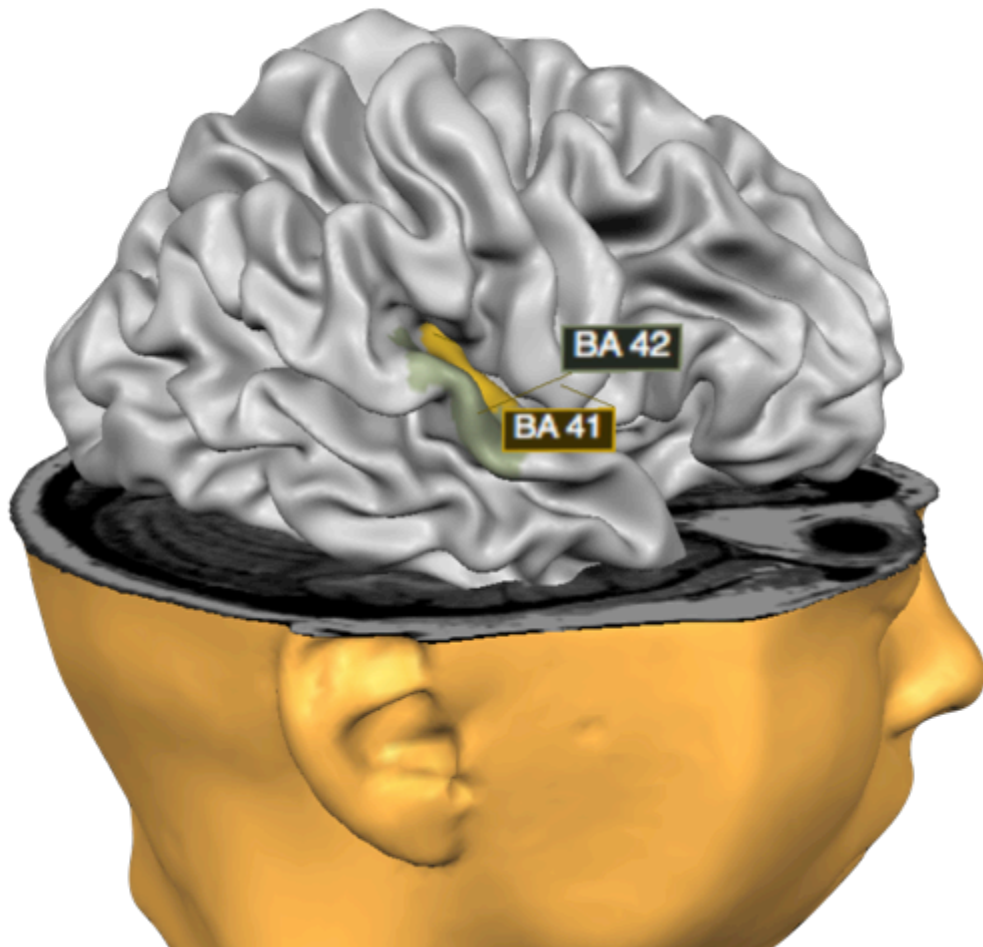


Figure 2.6: Surface view of the primary (BA 41) and associated (BA 42) auditory cortex. (Subject NH01 mesh reconstructed for illustration purpose using BrainVoyager OX 2.8).

Functional tonotopic organisation plays an important role in identifying the regions respond to speech frequencies. Basically, the auditory source arises in the cochlea and then maintained via auditory system. High frequency tones trigger mirror deep structure within PAC. Responding progress of the tone frequency is moved from lateral PAC to anterior and posterior medial PAC as the frequency increased (figure 2.7). Responding to certain frequencies is a specialization feature of the primary auditory cortex that specializes neurons located in the anterior region of the PAC to respond most to high frequencies sound, while those lay more posterior regions in the PAC respond to lower frequencies. A contralateral fashion is another feature of the PAC, where the right hemisphere corresponds with motor output and sensory input of the left and vice versa. This feature aims to represent the auditory projection or stimulation in both hemispheres (Chittka and Brockmann, 2005).

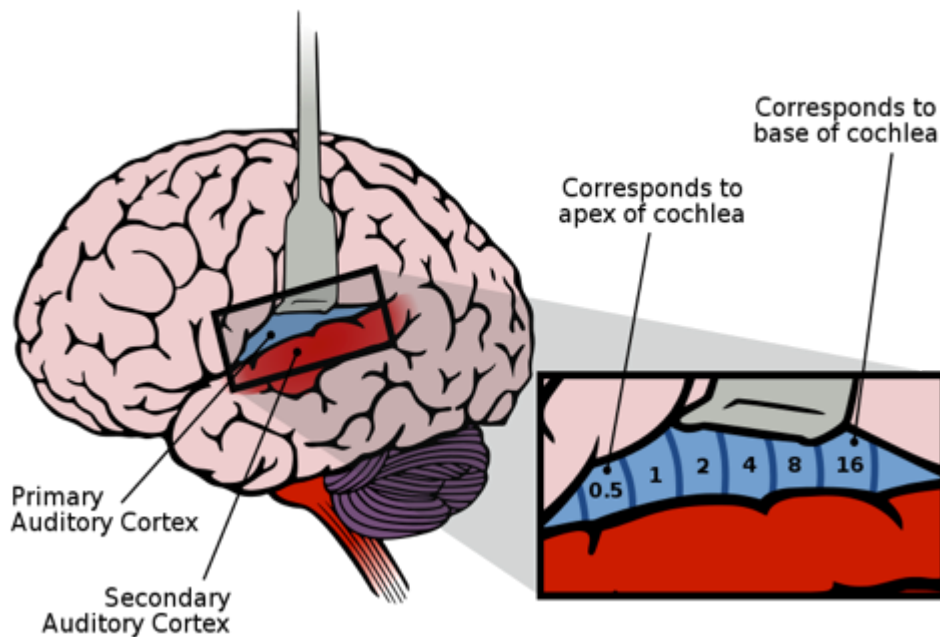


Figure 2.7: The primary auditory cortex includes a tonotopic map of the cochlear frequency spectrum. Adapted from (Chittka and Brockmann, 2005)

2.4 The limbic system

There is a growing body of evidence implicates the involvement of limbic system in tinnitus perception (Leaver et al., 2015). The word “limbic” refers to the brain emotional system. This brain part is involved with learning, memory and emotion processes, which was found affected in many neuropsychiatric diseases including schizophrenia (Tamminga et al., 1992), Alzheimer’s disease (Hopper and Vogel, 1976) and some forms of epilepsy (Boutros et al., 2008). The limbic system includes different brain regions: cingulate gyrus, hippocampal formation, thalamus and hypothalamus, nucleus accumbens, amygdala and prefrontal cortex (figure 2.8) (Adjamian et al., 2014).

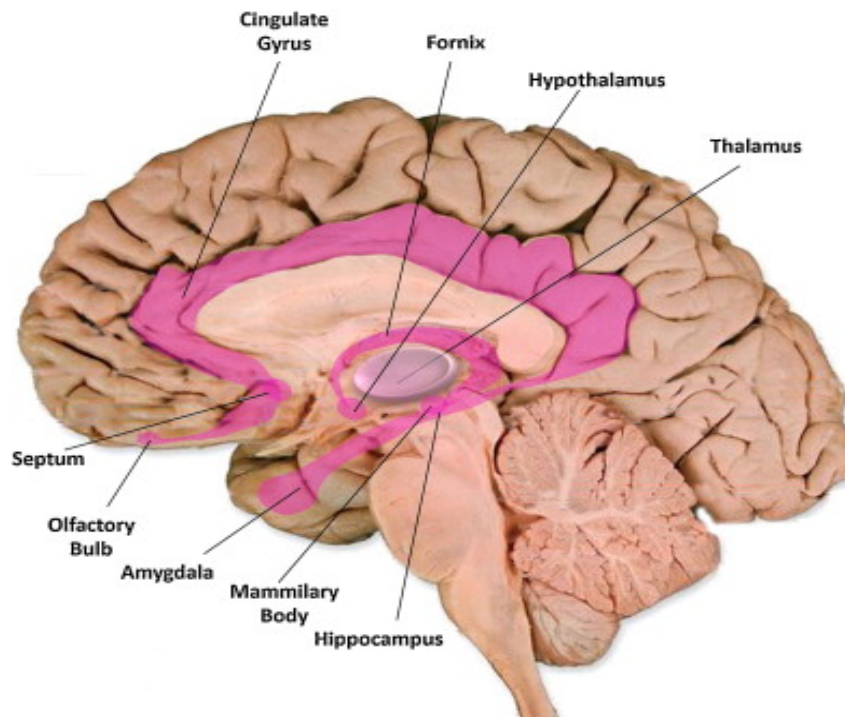


Figure 2.8 The limbic system structures. Adopted with permission from (Adjamian et al., 2014).

The cingulate gyrus is located above the corpus callosum that receives inputs from thalamus and then projects them to hippocampus through the cingulum. It is involved on focusing process (attention) in emotional events (Mendoza and Foundas, 2008). The anterior cingulate gyrus is activated in acute and chronic pain (Bliss et al., 2016), and may be responsible for tinnitus distress and loudness (Ueyama et al., 2013).

The hippocampal formation is a compound structure in the medial temporal lobe of the brain. It consists of the hippocampus, dentate gyrus (lies between hippocampus and parahippocampus), subiculum (at the base of hippocampus), entorhinal area (Brodmann's area 28), and induseum gresium (grey matter on the upper surface of the corpus callosum). The hippocampus consists of two main parts: dentate gyrus and cornu ammonic (CA). The latter is divided into four parts that are designed as CA1 to CA4. The hippocampus involves in short and long-term memory processing (Duvernoy et al., 2013). The hippocampus plays an important role in mood regulation (Campbell and MacQueen, 2004).

The thalamus is the brain's sensory switchboard that is suited on the top of the brainstem. The anterior thalamic nuclei are the parts of thalamus that involve in emotional process that receive projections from the fornix and then send them to the orbitofrontal and anterior cingulate cortex (Mendoza and Foundas, 2008). The main auditory-responsive portion of the thalamus is called the medial geniculate body (MGB), which is the information station for the neural representations of sounds being sent to the auditory cortices (Bartlett, 2013).

The hypothalamus is a neural structure lying below the thalamus that directs several maintenance activities such as eating, drinking and body temperature. Also, it is the integrative center of the endocrine and autonomic systems that is linked to the auditory system via inferior colliculus (IC) (Mazurek et al., 2010). It is linked to emotion that receives projection inputs from amygdala, and then sends them to autonomic nervous system (Mendoza and Foundas, 2008).

The amygdala is the window of limbic system, which has wide off connections with visual and auditory associations areas. In each hemisphere, there is almond-shaped neural cluster that is linked to emotion and fear. It receives projections from the hippocampal formation and then sends them hypothalamus (Phillips et al., 2003).

The nucleus accumbens (NAc) plays an important role in the limbic system, which is responsible for motivation, and allocated in the basal forebrain at the top of the brainstem and between the caudate and putamen (Miller, 2011).

2.5 Conclusion

In this chapter, I reviewed the anatomy and physiology of auditory pathway in the human that might be relevant to tinnitus perception. The auditory pathway consists of the peripheral auditory system where the sound is perceived, and the central auditory system where the sound is processed. The auditory system is connected to the limbic system that evaluates the acoustic sounds and controls the basic emotions such as fear, pleasant and anger.

Chapter 3: The Mechanism of Tinnitus: Perspectives from Human Brain Neuroimaging

3.1 Introduction

This chapter aims to give an overview of the tinnitus mechanism from the human brain neuroimaging point of view. The contribution of advance human neuroimaging to the current understanding of tinnitus mechanism will be highlighted. Human brain neuroimaging in tinnitus aims to demonstrate objectively the neural substrates of tinnitus in the central auditory system (Adjajian et al., 2009).

3.2 An overview of Tinnitus

3.2.1 Tinnitus definition

Tinnitus is an auditory phenomenon of a sound perception in the absence of external sound source (Jastreboff, 1990). The description of tinnitus is different between individuals as some may describes tinnitus sound as a tone, hissing, roaring and clicking (Møller, 2011b). Tinnitus sound can experience anyone for less than 10 seconds when moving from a very loud environment to quite zone, which in this case is considered as healthy and normal (Møller, 2011b). However, it can occur after exposing to a very loud noise source such as rock concert or gunfire which may experience tinnitus sound for few days that is called transient tinnitus (Eggermont, 2012). On the other hand, chronic tinnitus is considered when tinnitus onset is more than 6 months (Møller, 2011b).

The quality of life can be affected by having chronic tinnitus as work productivity

could be decreased, and tinnitus subjects may suffer from sleep deprivation (Alsanosi, 2011). Diagnosing tinnitus at an early stage may provide more effective treatment than later diagnosed (Møller, 2011b). So, tinnitus management is a very important concept in order to cope with tinnitus symptoms.

3.2.2 Tinnitus research interest

Number of tinnitus papers cited in PubMed has increased exponentially in the recent years that can be seen in figure 3.1. In 1950, 17 tinnitus scientific papers were published that was nearly doubled after three decade, which reached 29 papers per year. The tinnitus interest increased sharply from 183 publications in 2011 to 274 publication in 2012. This potential increased has been continued until now, which exceed 300 papers per year in 2015. This interest may reflect the high demand to understand the pathophysiology mechanism of tinnitus, which has not been defined yet.

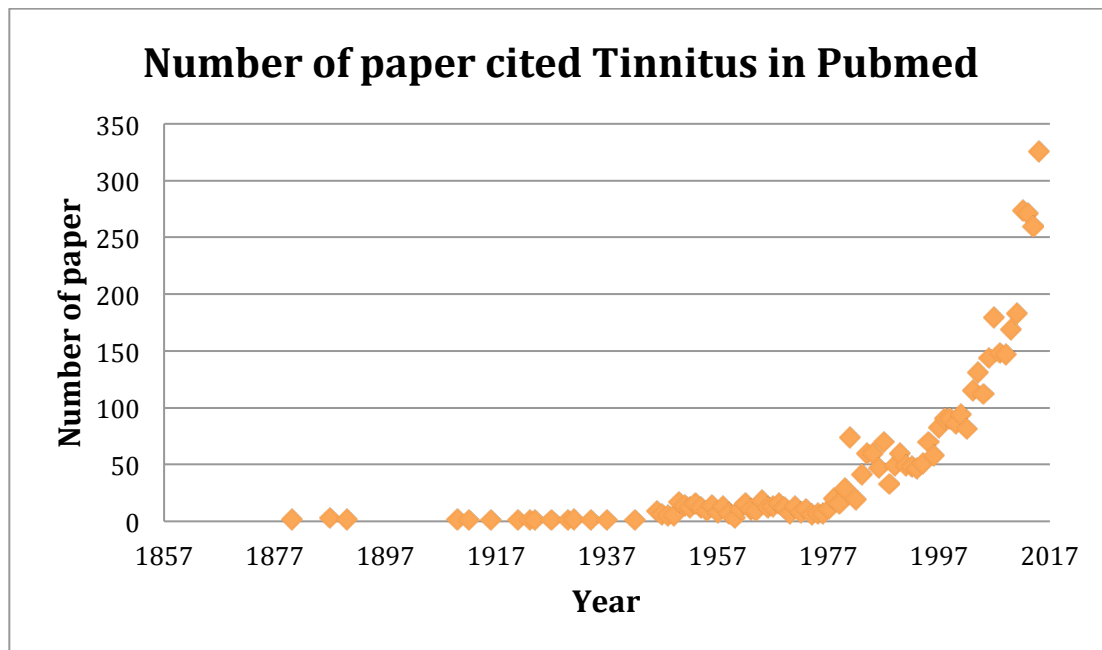


Figure 3.1 Number of paper cited 'Tinnitus' in Pubmed per year.

3.2.3 Different forms of tinnitus

There are two main forms of tinnitus that are objective and subjective. Objective tinnitus is less common could be caused by disturbance of blood flow or muscle contractions, which can be heard by the examiner or the observer, whereas subjective tinnitus more common that can only be heard or described by the tinnitus patient because of no visible signs of disease (Baguley et al., 2013).

Due to the lack of objective signs of subjective tinnitus, there are some features of tinnitus can be examined to diagnosis tinnitus. The intensity of tinnitus can be measured using visual analog scale (VAS) that can estimate the intensity of tinnitus from 0 to 10 (Adamchic et al., 2012). The description of tinnitus is different between individuals as some may describes tinnitus sounds as a tone, hissing, roaring and clicking (Møller, 2011b). Tinnitus severity or annoying could be classified into a grey scale, for instance, from slight to catastrophic using wide range of tinnitus inventories such as Tinnitus Handicap Inventory (THI) (Newman et al., 1996). Tinnitus laterality is another feature can be examined to identify the location of tinnitus sound that can be unilateral (right of left ear), bilateral (both are) or in the head (Baguley et al., 2013). The ability to modulate tinnitus intensity or severity is another feature. It was found that some tinnitus patients could modulate tinnitus intensity via somatosensory systems such as eye movement (Coad et al., 2001), manipulating the jaw (Pinchoff et al., 1998) and neck regions pressure (Abel and Levine, 2004).

3.2.4 Aetiology and Epidemiology of Tinnitus

Tinnitus symptoms are considered as symptoms with multiple aetiologies including physiological, pathophysiological, pathological and pseudotinnitus aetiologies (McCombe et al., 2001a).

Tinnitus is widespread and affects 16-19% of the adult population in the U.S. who may have experience of tinnitus for at least more than five minutes (Coles, 1984), 8% of them are seeking assistance as they may have moderate or severe tinnitus (Coles and Hallam, 1987). Other studies have been conducted in other countries such as England, Sweden, and Italy that demonstrate the prevalence tinnitus are 9.6% (Davis, 1989), 14.2% (Axelsson and Ringdahl, 1989) and 14.5% (Quaranta et al., 1996) respectively.

Tinnitus prevalence is affected by some factors that were demonstrated in the literature such as aging, exposure to loud noise, gender, ethnicity and association with other diseases. These variables were demonstrated clearly in the National Centre for Health Statistics survey (1996). It has been taken into account in this survey the following factors aging, race and education achieved level.

Aging is considered as a vital factor of neurodegeneration process as the structure and function of neurons changed, which might be a consequence of neurodegeneration process. Tinnitus can happen to adults and children as well (Eggermont, 2012). The prevalence of tinnitus was found to be increased with age until the age of 60-69 year (Shargorodsky et al., 2010). Another study found that the prevalence of tinnitus is 4.7% in the age of 20-29 year olds compared with 12.1% in the 60-69 year olds (Heller, 2003). Hence, the correlation between tinnitus and age

is very clear and positive until a specific stage, which may illustrate the influence of aging on the prevalence of tinnitus.

Exposure to a very loud noise is considered as another important factor of the prevalence of tinnitus in younger age. The leisure time history (social life) and work environment may play an important role in increasing the prevalence of tinnitus (Shargorodsky et al., 2010). It has been stated that working in a very loud noisy environment such as the military, music and heavy industries may be strongly causing tinnitus and hearing loss because the inner and outer hair cell and acoustic nerve function are affected chronically (Shargorodsky et al., 2010). Furthermore, it has been found that a strong correlation between acoustic trauma and tinnitus, with 90% of acute acoustic trauma patients reporting tinnitus symptoms (Mrena et al., 2004).

Another factor is correlated to the prevalence of tinnitus is mental health, which includes many disorders such as anxiety and depression. Participants with a history of these disorders are more likely to report tinnitus symptoms than those are not affected by these disorders (Shargorodsky et al., 2010). Once the general mental health getting worse, the quality of life will be affected that can be seen in sleep deprivation, low work productivity and lifestyle detriment (Stephens and Hallam, 1985). Despite the fact that the origin of these correlations has not been proven yet, the correlation between general mental health and tinnitus is positive and important that can increase the prevalence of tinnitus (Chandra et al., 2009).

The association between ethnicity and tinnitus has been investigated widely. It has been found that some ethnical groups are more or less likely to report tinnitus than

other groups. For instance, the prevalence of tinnitus was compared between Caucasian and African Americans, and found that the former (9%) was more likely to report tinnitus than the latter (5.5%) (Adams et al., 1999). Another study was conducted to investigate the effect of ethnical factor in the prevalence of tinnitus, which showed Hispanic, and non-Hispanic blacks had lower prevalence of tinnitus than non-Hispanic whites (Shargorodsky et al., 2010). Hence, ethnicity may play a major role in the prevalence of tinnitus, which needs to be considered carefully from the genetic point of view.

Although, the previous mentioned factors play an important role in the prevalence of tinnitus, gender factor does not seem playing the same role as some studies found that men are more likely to tinnitus than women, while others found it versa. A Canadian study showed that there is not a significant different between men and women in reporting tinnitus, which found 6,6% in versus 5,6% respectively (Heller, 2003). Another study was conducted in U.S. population reported that women had more prevalence of tinnitus than men by 52% and 48% respectively. From these studies, gender factor might not be considered as a factor of prevalence tinnitus. Interestingly, it is found that there is a limiting correlation between tinnitus and health factors such as smoking status, cardiovascular disease, hypertension, dyslipidemia and diabetes mellitus (Shargorodsky et al., 2010).

Tinnitus can occur with other diseases such as hearing loss in particular. It found that the majority of tinnitus cases (80%) is accompanied with hearing loss (Elgoyhen et al., 2015). For instance, a tinnitus subject who is suffering from a unilateral noise-trauma induced hearing loss at a specific 4 kHz would perceive

tinnitus as 4 kHz tone on the same of the hearing loss (Elgoyhen et al., 2015).

Another disorder associated with the prevalence of tinnitus is Ménière's disease that considers tinnitus as symptoms of this disease (Møller, 2011b). Not only are hearing disorders association with tinnitus, but it was also reported the correlation between Down's syndrome and perception of loudness which found the abnormal perception to the auditory stimuli (Khalifa et al., 2004). Furthermore, it was reported that some cardiovascular diseases such as hypertension is associated with the incidence of tinnitus (Podoshin et al., 1997). These associations may be the key to understand the pathophysiological mechanism of tinnitus.

3.3 Tinnitus models

3.3.1 The neurophysiological model of tinnitus

3.3.1.1 *Development of the neurophysiological model of tinnitus*

Despite the fact that tinnitus has been known for long time ago, there is no cure to treat tinnitus completely (Jastreboff and Hazell., 2004). Many ways of treatment have been tried to treat tinnitus symptoms with the advance of medical science and technology, however, most of these treatments were just to eliminate the tinnitus symptoms and enhance tinnitus patient's quality of life (Baguley et al., 2013). The failure of discovering treatment for tinnitus encourages health professionals to open different fields' door in attempt to find a clue that would help to discover a cure of tinnitus. Different specialists have seen tinnitus subject: otolaryngologist, audiologists, psychologists, neurologists, psychiatrists, neurosurgeon, dentist, pharmacologists and neuroscientists who see this subject from their point of views

(Møller, 2011b).

The neurophysiological model of tinnitus was created and established by P.J. Jastreboff in the middle of 1980s, which has been widely accepted and used by clinicians and researchers (Jastreboff and Hazell., 2004). It includes three systems that could be involved in tinnitus perceptions: the auditory perceptual, emotional and reactive systems.

The ability to cope with tinnitus has been considered as a crucial point, which could not be measured objectively (Møller, 2011b). It was stated that less than a quarter of people who have tinnitus are suffering from it and seeking medical assistance (Jastreboff and Hazell., 2004). This emerges a question "why do some people of tinnitus suffer of this experience while others not?". Furthermore, no significant differences were found of the psychoacoustical properties of tinnitus (e.g., loudness pitch and masking level) between people who can cope with tinnitus and those suffering from tinnitus (Stephens and Hallam, 1985). Also, no correlation was found between tinnitus severity and psychoacoustic characterization of tinnitus (Hazell et al., 1985).

Another observation was highlighted, which supports the idea of the correlation between tinnitus severity and psychoacoustic properties, which indicated that tinnitus is not represented in the cochlea by mechanical vibration, whereas neuronal activity is reflected within the auditory pathway (Jastreboff and Hazell., 2004). Some cortical systems in the brain are responsible of tinnitus severity such as limbic, somatosensory, and visual and attention systems that are connected with

auditory system via anatomical or functional connections (Jastreboff and Hazell, 2004). Furthermore, the majority of tinnitus patients have some problematic experiences with the functions of these brain systems that connected with auditory system such as sleeping, attention and concentration (Jastreboff and Hazell, 2004). It was found that chronic tinnitus patients have more sleeping difficulties and in contrast to healthy controls (Crönlein et al., 2016). Also, significant changes in tinnitus level occur as a result of sleep deprivation (Alster et al., 1993). In term of attention deficit, reaction-time assessments showed that tinnitus patients have attention deficits relative to controls (Dornhoffer et al., 2006). Ongoing perception of tinnitus has the potential to impair the ability to concentrate (Møller, 2011b)

In order to understand the tinnitus mechanism, it is essential to understand some mechanisms that related to tinnitus subject. As the incoming sound or signal perceived in the brain, this will evoke a variety of reactions and activate various structures, which involve the auditory and non-auditory systems. The involvement of non-auditory systems in tinnitus mechanism was considered as a crucial point in this subject, which leads to create the neurophysiological model of tinnitus (Jastreboff and Hazell, 2004).

The neurophysiological model of tinnitus was introduced with two important questions; 1)- which non-auditory systems are responsible for tinnitus mechanism? 2)- what are the mechanisms that involved in tinnitus perception? (Jastreboff and Hazell, 2004). In order to have answers for these two questions, it is essential to identify the problems enclosed with tinnitus such as attention, sleep, performing

activities in quite environment, anxiety and depression, which are allocated under two non-auditory systems: limbic and autonomic nervous system. The limbic system controls emotions and is strongly connected with all sensory systems. On the other hand, the autonomic nervous system controls all the autonomic body functions such as breathing, the heartbeat and digestive processes that normally connected to the limbic system. Brain areas controlling heart rate was found variable in tinnitus and tinnitus-related distress (Vanneste and De Ridder, 2013). The limbic and autonomic nervous systems are normally activated by positive (pleasant) and negative (unpleasant) emotions that can disturb the function of sleeping, attention, concentration and performing activities (Jastreboff and Hazell, 2004).

3.3.1.2 Mechanism of tinnitus signal generation

Human brain plays an important role to adjust high levels of spontaneous activities within the auditory pathways at the same time. It was indicated that we are not reacting to the absolute stimulus strength rather than its relative strength compared to the background (Jastreboff and Hazell., 2004). Therefore, loudness perception of all sounds increases in a reduced sound environment due to the increased gain or amplification at all levels of the auditory pathway. For instance, entering a sound proof room may evoke tinnitus.

Exposing to external sound leads to increase the neuronal activity and become more regular. Baguley (2002) reviewed the possible mechanism of tinnitus, which was classified into three main possible mechanisms models: cochlear, non-cochlear and analogies with pain models (Baguley, 2002).

The dysfunction of cochlear was considered in tinnitus generation. In normal healthy conditions, cochlear may produce low intensity tonal or narrow band when there is no external acoustic source. Spontaneous oto-acoustic emissions (SOAE) measurements were found variables in 38-60% of normal hearing adults (Wilson and Sutton, 1981). The incidence of tinnitus originating as an SOAE was found in few tinnitus cases ranging between 2-4.5% (Penner, 1990, Baskill JB, 1992, MJ, 2000). Another possibility of the cochlear involvement in tinnitus generation is the discordant damage of inner hair cells (IHC) and outer hair cells (OHC) (Jastreboff and Hazell., 2004). In addition, cochlear neurochemistry was found distributed due to adults human with distressing tinnitus may have experiences of agitation, stress and anxiety, (Baguley, 2002).

There has been a shift toward retro-cochlear and central mechanisms to understand tinnitus generation. The neurophysiological model considers the role for 'signal recognition and classification circuits' in chronic tinnitus (Jastreboff, 1990). It illustrates the involvement of the auditory perceptual, emotional and reactive systems in tinnitus perception. Also, it was assumed that tinnitus perception is associated with spontaneous over-activity of the cochlear nerve (Evans EF, 1981).

Another possibility of non-cochlear tinnitus mechanism proposed that tinnitus might result from the imposition of a temporal pattern upon stochastic cochlear nerve activity (Eggermont, 2000). It was suggested that the efferent system might play a role on the perceived intensity of tinnitus, which is associated with annoyance that based on the observation that stressful situations may exacerbate tinnitus, and

that techniques such as biofeedback may reduce tinnitus (Eggermont, 1984).

Somatic modulation was noted to modulate tinnitus by somatosensory inputs (Levine, 1999). Face stroking and head movement were found to play a role to change the intensity and pitch of tinnitus (Levine, 1999).

The associations between phantom pain and tinnitus have been found (House and Brackmann, 1981). The analogy between tinnitus and chronic pain was considered in terms of peripheral generation and of central persistence once the acute injury has resolved (Baguley, 2002). Also, it was revealed that the concept of cortical re-organization caused by phantom limb pain could be found in the auditory brain regions following auditory deprivation (Salvi RJ, 2000). It was proposed that spontaneous activity in auditory brain areas might be perceived as tinnitus (Norena and Eggermont, 2003a).

3.3.1.3 The components of the neurophysiological model

The main systems involved in this model are auditory, limbic and autonomic nervous systems. The auditory system generates the tinnitus sound signal that is received by the limbic and autonomic nervous systems causing annoyance and distress.

According to this model, the tinnitus signal is generated in the peripheral auditory system, which is detected and processed by the auditory subconscious centers, and then finally interpreted at the highest level of central auditory system (auditory cortices) (figure 3.2). It was proposed that this tinnitus signal might be constrained

within the auditory system in tinnitus patients who can cope with tinnitus symptoms. However, if this activity spreads to the limbic and autonomic nervous systems, it may cause several negative reactions, which leads to decrease the quality of life (Møller, 2011b). It was hypothesized that prefrontal cortex may be the brain structure that integrates sensory and emotional aspects of tinnitus, which could be involved in the emotional and autonomic reaction to tinnitus (Jastreboff, 1990)

Tinnitus Retraining Therapy (TRT) is based on this neurophysiological model of tinnitus. It aims to adjust the reactions evoked by tinnitus and its perception. This could be achieved by mimicking the activation of the limbic and autonomic nervous, such as preventing the signal from reaching higher cortical areas involved in signal awareness (Jastreboff and Hazell., 2004).

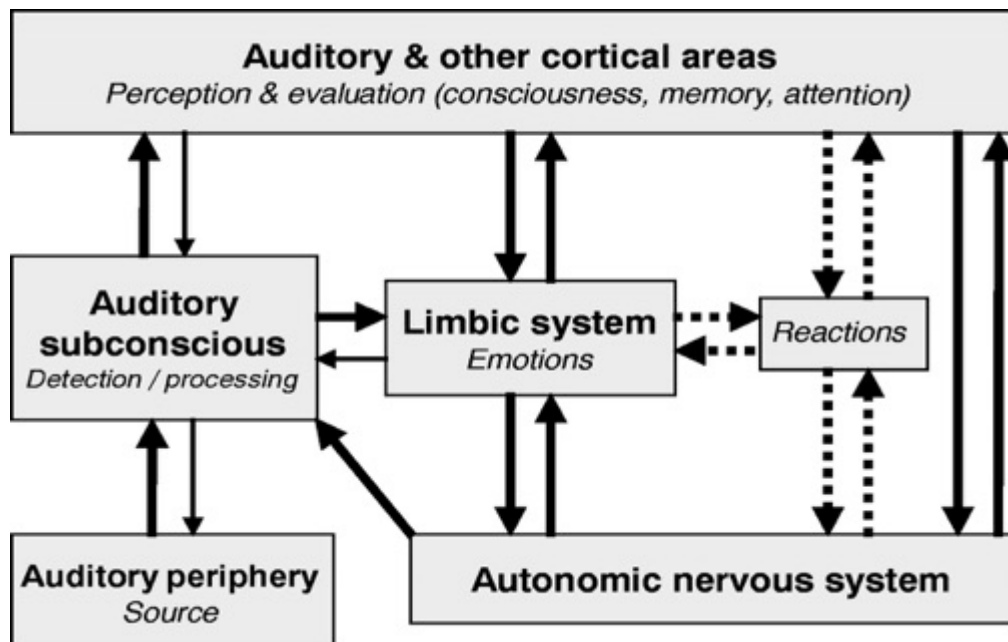


Figure 3.2: The diagram of neurophysiological model of tinnitus. Adapted with permission from (Jastreboff and Hazell., 2004).

3.3.2 Inhibitory gating model of tinnitus

In previous assumptions of the tinnitus mechanism, it was assumed that tinnitus is caused by peripheral noise induced hearing loss following changes in the central auditory pathways, however, the location and nature of these changes are unknown (Jastreboff, 1990). In fact, functional neuroimaging studies showed the hyperactivity in tinnitus patients compared to normal controls in the auditory pathway (Arnold et al., 1996, Lanting et al., 2008, Melcher et al., 2009) and non-auditory brain regions such as prefrontal cortex, hippocampus and amygdala (Mirz et al., 2000b, Lockwood et al., 1998). In the inhibitory gating model of tinnitus, the origin of tinnitus signal could be in the auditory system, however, tinnitus noise is assumed not cancelled at the level of thalamus via limbic system that fails to block the tinnitus signal, which could cause chronic tinnitus (Rauschecker et al., 2010).

Under normal circumstances, limbic system can identify the unwanted noises and eliminated them from being perceived by feeding back to inhibitor at thalamus reticular nucleus (TRN) that subtracts the unwanted noises from the afferent auditory signal (Rauschecker et al., 2010). This showed the importance of noise cancellation system to prevent tinnitus signal from being perceived.

However, if the limbic and paralimbic brain regions are compromised, noise cancellation was found breaking down due to the inhibition of tinnitus signal is lost at the level of thalamus, and tinnitus signal is perceived in auditory cortex that might be permanently reorganized (Rauschecker et al., 2010). The noise cancellation system could be broken down due to the overload of chronic firing of nucleus accumbens (NAc) neurons in order to compensate tinnitus signals, and the

transmitter level may decline over time or with age in chronic tinnitus sufferers compared to unaffected individuals (Rauschecker et al., 2010) (figure 3.3).

Deep-brain stimulation of NAc has proven to be an effective treatment for obsessive-compulsive disorder and depression (Mayberg et al., 2005, McCracken and Grace, 2009). Tinnitus patients could simply have fluctuating levels of serotonin in NAc/Subcallosal network that was proposed previously in “serotonin hypothesis of tinnitus” (Dobie, 2003, Simpson and Davies, 2000).

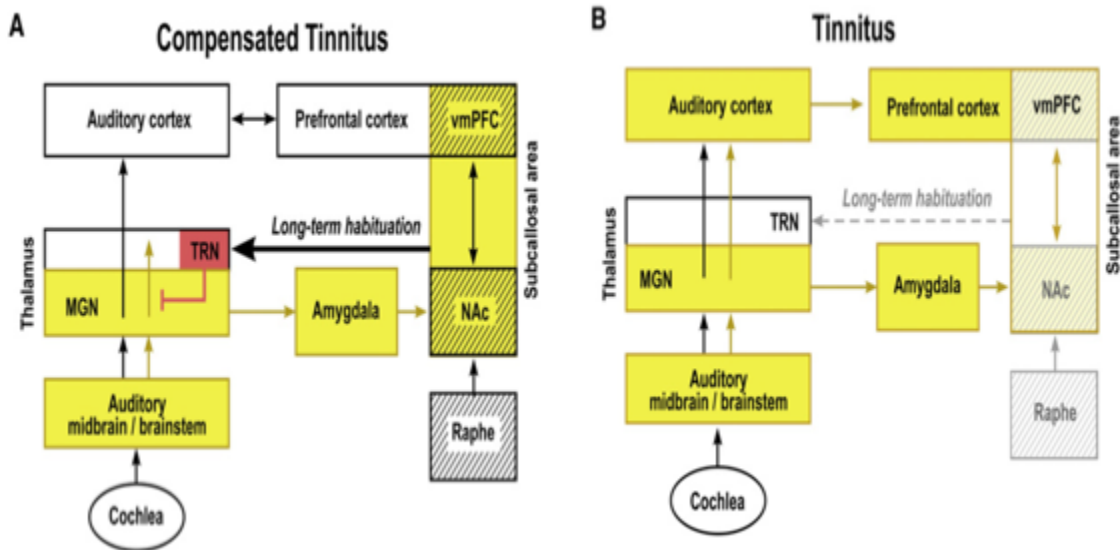


Figure 3.3: Tinnitus as the result of a Broken Neural “Noise Cancellation” Mechanism. Adapted with permission from (Rauschecker et al., 2010).

3.3.3 The integrative model of the auditory phantom perception

The integrative model of tinnitus is the most recent model of tinnitus, which combines the neurophysiological model and noise canceling process (De Ridder et al., 2014).

It is assumed that separable tinnitus characteristics such as loudness, sidedness, the sound descriptions (pure tone, noise), mood and distress may unify coherent

percept. It proposes a minimum set of brain regions called a ‘tinnitus core’ subnetwork, including auditory cortex, inferior parietal area, ventromedial prefrontal cortex and parahippocampus gyrus that are jointly activate to cause the tinnitus perception (Figure 3.4). Communication between these different networks is hypothesized to happen at hubs that can take a part in separable network at different frequencies (De Ridder et al., 2014).

The integrative model of tinnitus theorizes that tinnitus core involved neural correlation of auditory pitch awareness and memory that are connected with other networks through hubs, which cause bothersome effects, for instance, mood disorders and distress.

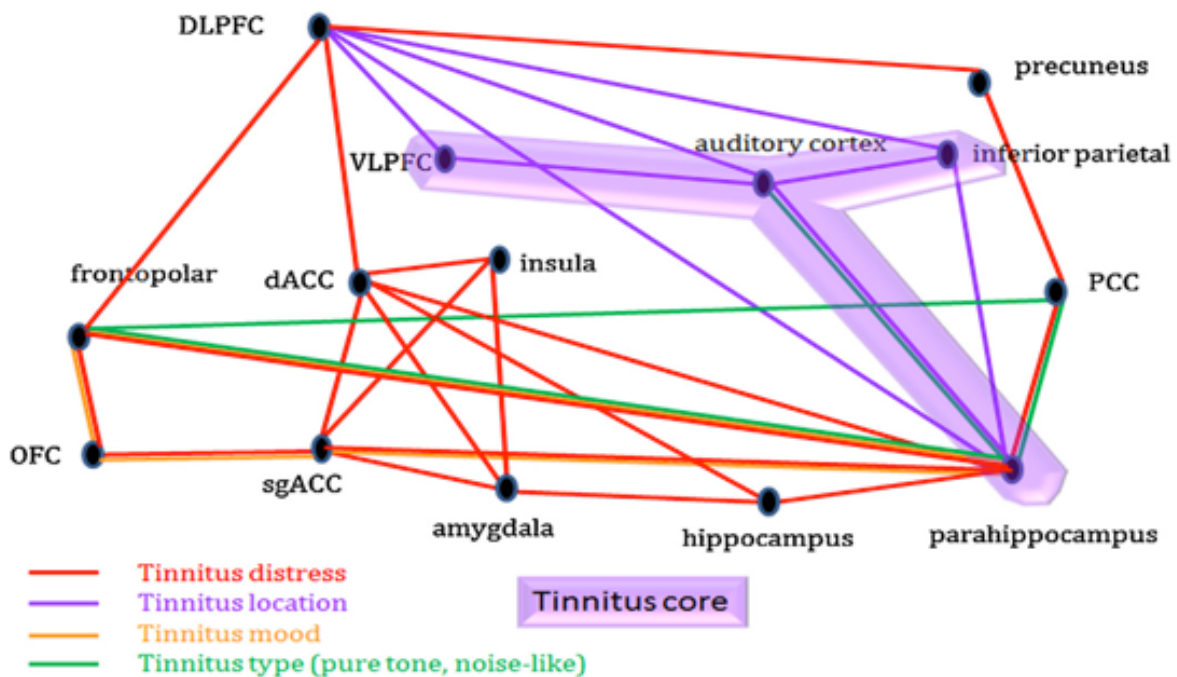


Figure 3.4: The integrative model of tinnitus. Adapted from (De Ridder et al., 2014)

3.4 Auditory deprivation and tinnitus

Deprivation of normal auditory stimulation can be found as an obstruction of ear canal, middle ear and cochlea disorders, which leads to cause two common forms of

hearing loss: age-related hearing loss and noise induced hearing loss. Deprivation of auditory input could lead to activate the neural plasticity, and make changes of the balance between inhibition and excitation. Noise induced hearing loss is one of the commonest cause of tinnitus. The reduced of hearing acuity caused by noised induced hearing loss may activate neural plasticity (Møller, 2011b).

Similarity between tinnitus and acoustic trauma was found in the increased of neural firing in the auditory brain regions. Increased neural firing in auditory brain regions was reported as one cause of tinnitus generation (Eggermont, 2012). Also, it was found that acoustic trauma could lead to increase neural firing in the auditory cortex (Norena and Eggermont, 2003b).

One of the proposed theories of the role of auditory deprivation is the change in balance between inhibition and excitation. In a single auditory nerve fiber, there are excitatory and inhibitory responses areas that interact between each other (Sachs and Kiang, 1968). Acoustic trauma was found to decrease evoked potential recorded from the auditory nerve and increased the neural firing in the inferior colliculus (Wang et al., 2002). This indicates that alterations of the balance between inhibition and excitation could cause hyperactivity in the auditory brain regions.

Neural plasticity is thought to be involved in tinnitus generation (Eggermont, 2015) and is considered as one of the maladaptive plasticity disorders (Møller, 2008). Deprivation of sensory stimulation is considered as one of the strongest premotor neural plasticity (Møller, 2006). The function of nervous system could be changed because of the effect of activation of neural plasticity that may happen for a short or

long period of time. It was shown that auditory deprivation in animals could cause cortical map modification and cortical plasticity that linked to the decrease of inhibition (Rajan, 2001). This indicated that deprivation of auditory stimulation could affect the function of auditory nervous system.

3.5 Neural plasticity and tinnitus

In the past, it was thought that each part of the brain is working in a single function and does not interact with other parts of the brain or has lost the ability and capacity for repair and regeneration. The brain has the ability to adapt with new development, experiences and environment by changing its organization and function of the nervous system to adapt to the new demands (Woolf and Salter, 2000).

Neuroplasticity was defined as “the ability of the nervous system to respond to intrinsic stimuli by reorganizing its structure, function and connection; can be described at many levels, from molecular to cellular to system to behavior; and can occur during development, in response to the environment, in support of learning, in response to disease, or in relation to therapy” (Cramer et al., 2011).

Neural plastic changes comprise changes in the efficacy of synapses, formation of new synapses and elimination of exciting synapses (Møller, 2008). These changes can be induced through two main mechanisms. Anatomical change is one of these mechanisms, where a new anatomical connection is formed through dendritic growth and sprouting, whereas the second mechanism of neural plasticity involves functional changes to nerves cells in the resting membrane potential (Nelson, 1999).

Neuroplasticity is a lifelong ability, where the brain can adapt and learn during development, through the normal lifespan, and in response to injury. It can be a consequence of a positive response (adaptive) that is associated with a gain in function such as learning new skills, or a negative response (maladaptive) of the body to certain pathology disorders.

Learning and memory processes are the most significant forms of adaptive neuroplasticity that have been shown in animals and human studies. The connection between neurons is rearranged when a new experience is acquired. It was found that environmental enrichment plays an important role to modify neural plasticity in the hippocampus in older mice (Kempermann et al., 2002). In human, it was found training induced structural changes in London taxi drivers who exhibit increased grey matter volume in the posterior hippocampus (Maguire et al., 2000).

Neural plasticity in the central nervous system has been found in many sensory system disorders such as phantom limb pain. Due to the sensorimotor cortex does no longer receive input from the missing body in the phantom limb pain; neural signals from other parts of the body may take over that area. Deprivation of sensory input could activate neural plasticity and leads to plasticity disorders. It was assumed that there are two main ways of neural plasticity in sensory system disorders by changing neural process and rerouting of information (Møller, 2011b).

Maladaptive neuroplasticity in the auditory system could be induced by some audiological disorders such as hearing loss and tinnitus due to an imbalance between excitation and inhibition (Saunders, 2007, Eggermont, 2015). Deprivation

of auditory input to the central auditory nervous system can activate neural plasticity, which may lead to cause tinnitus perception. The tonotopic maps of chronic tinnitus patients compared to controls in human core auditory cortex was found different (Mühlnickel et al., 1998), while another study found no significant difference (Langers et al., 2012). These inconsistent results may be because of the difference of medical imaging acquisition modalities as the former used MEG and EEG modalities to acquire tonotopic maps, while the latter used fMRI.

3.6 Similarities between tinnitus and pain

The analogue between tinnitus and intractable pain was firstly drew attention by Tonndorf who suggested that the cause of both of them being seeing in the deafferentation of nerve fibers (Tonndorf, 1987).

Tinnitus and central neuropathic pain have many forms that occur without any physical stimulation of sensory receptors (Møller, 2011b). Both of them are considered as neural plasticity disorders that may be caused by plastic changes of the nervous system. The absence of an external physical stimulus can be found in subjective tinnitus and central neuropathic pain that often referred to as 'phantom sensation' (Rauschecker et al., 2015). Also, the emotional component plays an important role in both of them to cope and suffer from their symptoms. The subjective natures of both of them make them difficult to be diagnosed and gain reliable data on epidemiology. The development of severe tinnitus was found similar to the development of chronic pain (Møller, 1997). Furthermore, tinnitus and chronic pain are often associated with hypersensitivity to sensory stimulation;

tinnitus is usually associated with hyperacusis, while chronic pain is often associated with hyperalgesia (Rauschecker et al., 2015).

The neuroanatomy of auditory and pain pathways has many similarities as they share the site of the anomalies in the nervous system. In the auditory pathway, there are two ascending pathways: classical and non-classical pathways. The neural pathway of chronic pain has similar structural pathways of auditory pathway as the lateral and medial tract of spinothalamic systems perform as classical and non-classical auditory pathway respectively. Furthermore, the brain structures involved in tinnitus and chronic pain are very similar as the ventromedial prefrontal cortex (vmPFC) and nucleus accumbence (NAc) are taking part in both sensory modalities to evaluate the relevance and affective value of sensory stimuli and controls information flow through descending pathways (Figure 3.5) (Rauschecker et al., 2015). However, there are some differences between tinnitus and chronic pain that should be taken into account in terms of its acute, physiological forms and pathologies (Rauschecker et al., 2015).

The exact mechanisms responsible for somatic modulation of tinnitus are unclear. Tinnitus might result from aberrant neuronal activity within the auditory pathway, which could mean that somatosensory stimuli are coming from head and neck muscle contractions, through a multisynaptic pathway, disinhibit the ipsilateral cochlear nucleus, producing an excitatory neuronal activity within the auditory pathway that results in tinnitus (Møller, 2011b).

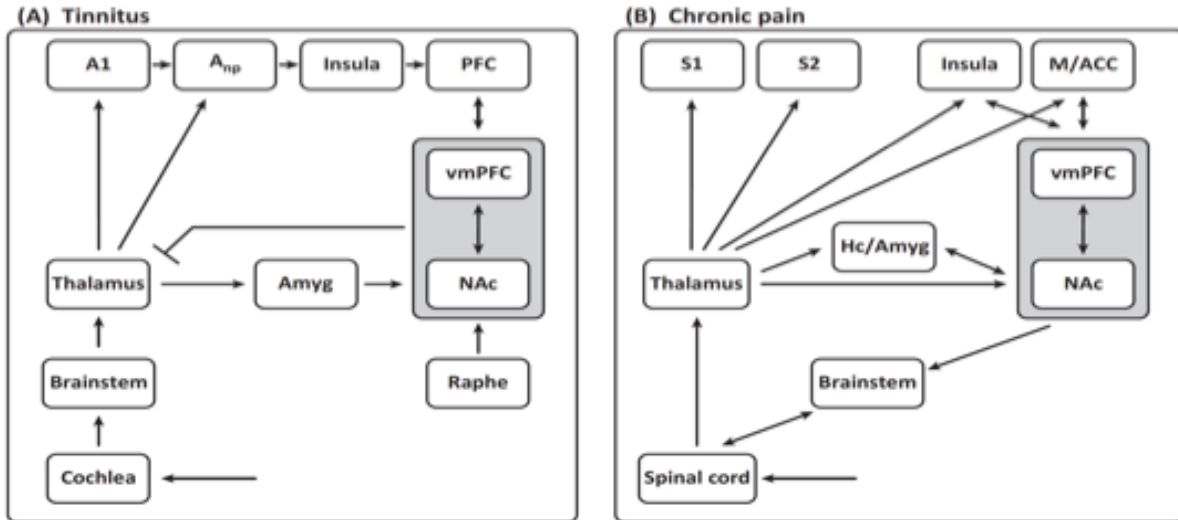


Figure 3.5: The block diagrams of brain structures involved in (A) tinnitus and (B) chronic pain. A1: Primary auditory cortex, A_{np}: Non-primary auditory cortex, PFC: Prefrontal cortex, vmPFC: ventromedial prefrontal cortex, NAc: Nucleus accumbence, Amyg: Amygdala, Hc: Hippocampus, S1: Primary somatosensory cortex, S2: Secondary Somatosensory cortex. Adapted with permission from (Rauschecker et al., 2015).

3.7 Association between tinnitus and post-traumatic stress disorder (PTSD)

Post-traumatic stress disorder (PTSD) is a psychological disorder developed after exposing to traumatic events such as stress, wars, natural disasters and sexual abuse. There are some symptoms of PTSD such as anxiety and depression that characterized by sleep disturbances, chronic depression and social avoidance (Creamer et al., 2004). The pathophysiology of PTSD was linked to the neurobiological mechanism of stress that affect the neural process of learning and memory networks (Francati et al., 2007).

The influence of PTSD on brain structure and function has been demonstrated. Cerebral blood flow was found increased in the limbic system (amygdala) in PTSD subjects compared to control group (Liberzon et al., 1999). Also, it was reported that hippocampus volume is significantly reduced in PTSD group compared to control group (Francati et al., 2007).

The analog of PTSD and tinnitus was reported as they share some symptoms such as anxiety and depression (Fagelson, 2007). Also, the medications described for both are similar (Andersson et al., 2005). Despite the fact that distinct perception events are involved in tinnitus and PTSD, it was reported that they might share the CNS mechanism in particular the limbic and auditory systems (Fagelson, 2007).

Amygdala as part of the limbic lobe, its function was found positively correlated in acute PTSD group with the severity of PTSD (Armony et al., 2005). According to the neurophysiological model of tinnitus, amygdala is involved in tinnitus perception (Jastreboff and Hazell, 2004). Prefrontal cortex (PFC) is another brain area of limbic system that was found involved in tinnitus perception and PTSD. The damage of ventromedial PFC could reduce the likelihood of developing PTSD (Koenigs and Grafman, 2009). Hyperactivity of prefrontal cortex was found in tinnitus subjects compared to controls (Mirz et al., 1999). The auditory behavior was found affected in both tinnitus and PTSD patients (Fagelson, 2007).

3.8 Structural and functional neuroimaging of tinnitus

3.8.1 Introduction

It was thought that tinnitus is an audiological problem due to tinnitus sufferers are experiencing the symptom on their ears. However, this idea has been changed recently due to some evidences support the involvement of central auditory system in tinnitus generation. For example, tinnitus perception was found still exists after surgical remove of the eighth cranial nerve (auditory nerve) in acoustic neuroma patients (House and Brackmann, 1981). Chronic tinnitus was found accompanied

with hearing loss in the majority of tinnitus sufferers; however, many people with hearing loss are not suffering from tinnitus (Konig et al., 2006). It was stated that tinnitus and hearing loss may only indirectly related (Jastreboff and Hazell., 2004). The majority of people with tinnitus can cope with tinnitus symptoms, while small portion of tinnitus sufferers are seeking for medical assistance (Baguley et al., 2013). Psychoacoustic characteristics of tinnitus, for instance, loudness and frequency may not reflect the level of annoying (Hazell et al., 1985). These observations have raised the question whether tinnitus is a neurological problem.

Tinnitus related neural substrates changes have been investigated widely using structural and functional neuroimaging techniques that have provided an insight to understand the pathophysiology mechanism of tinnitus. However, there are some tradeoffs need to be considered carefully such as spatial and temporal resolution, and ionizing and non-ionizing radiation. The aim of this section is to review the main advanced magnetic resonance imaging (MRI) techniques that have been applied in tinnitus research.

3.8.2 Tinnitus findings from structural neuroimaging studies

Structural neuroimaging aims to assess the brain structural differences in brain morphometry by showing the contrast between different brain tissues: grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Structural MRI high-resolution T1-weighted image has been widely used to investigate brain structural morphometry in people with tinnitus compared to normal healthy controls.

In the past, experienced observers assessed changes in brain structures or volume (observers-based morphometry) that is time-consuming and based on manual demarcation of brain region of interests (Adjamian et al., 2014). Nowadays, automated segmented tools are available to segment brain tissues using fast algorithms that do not require user interactions. There is variety-automated ways to assess anatomical alterations based on the differences of volume (voxel-based morphometry), surface (surfaced based morphometry) and shape (vertex based mprphometry). A summary of structural morphology studies in tinnitus is summarized in table 3.1.

3.8.2.1 *Voxel-based morphometry (VBM)*

Voxel-based morphometry (VBM) is the most common brain morphometric technique used in tinnitus neuroimaging research, which investigates voxel-wise differences in the local GM or WM volume. It does not require a prior hypothesis regarding to the location of possible differences in brain tissue. A number of brain regions have been reported involved in the pathophysiology mechanism of tinnitus.

The involvement of auditory and non-auditory brain areas was reported in different studies. A study has shown a significant reduction of auditory cortex volume in tinnitus subjects compared to controls (Schneider et al., 2009). On the other hand, other studies showed GM volume differences outside auditory cortex in thalamus (Muhlau et al., 2006) , hippocampus and inferior colliculus (IC) (Landgrebe et al., 2009), orbital frontal cortex (Mahoney et al., 2011), prefrontal vortex (Muhlau et al., 2006, Leaver et al., 2011, Leaver et al., 2012). This variability of results might be

because of the high inter-individual variability in the morphology of the auditory system (Møller, 2011b).

The changes of limbic structure in tinnitus individuals have been found in different structural neuroimaging studies. VBM analysis was applied to investigate the changes of GM volume in 28 tinnitus sufferers and 28 aged- and gender matched healthy controls, which found a significant GM volume reduction at vmPFC in tinnitus sufferers and GM volume increased at bilateral MGN (thalamus) (Muhlau et al., 2006). Moreover, the dysregulation of limbic network was reported in tinnitus as it was found a significant GM volume reduction in vmPFC in 11 tinnitus patients compared to gender and hearing loss matched 11 healthy controls (Leaver et al., 2011). These findings may reflect the important of limbic system to cancel tinnitus signal to be perceived in auditory cortex via thalamus that was proposed in inhibitor gating model in tinnitus (Rauschecker et al., 2010).

3.8.2.2 *Surface-based morphometry (SBM)*

Surface based morphometry is a morphometric measurement that investigates cortical surface differences between subjects in the cortical thickness, surface area and curvature. Cortical thickness analysis is a powerful tool that can assess changes across the axes of the cortical columns by measuring the distance between outer and inner grey matter surfaces. More than 99% of the surface is between 1- and 4.5-mm thick (Fischl and Dale, 2000). The thickness of cerebral cortex has received a great interest to investigate both normal brain development and a wide variety of neurodegenerative and psychiatric disorders (Fischl and Dale, 2000). In the past, a

trained anatomist can manually investigate cortical morphology, which is time consuming and only measure cortical volume, not cortical thickness that is measured on the sub-millimeter scale. Cortical thickness may be changed in response to exposing to repeated experiences.

Aldhafeeri et al (2012) used BrainVoyger software to investigate cortical thickness, which found a significant reduction of cortical thickness in prefrontal cortex, temporal lobe and limbic system in individual with tinnitus compared to normal healthy controls (Aldhafeeri et al., 2012a). Also, the same study found a significant negative correlation between hearing loss thresholds and cortical thickness measurement of primary auditory cortex in tinnitus group (Aldhafeeri et al., 2012a). Leaver et al (2012) found a significant reduction of ventromedial prefrontal cortex (vmPFC) in tinnitus patients compared to normal healthy group (Leaver et al., 2012). In the same study, the thickness of vmPFC showed a significant negative correlation with noise sensitivity score in tinnitus patients. A positive correlation was found between tinnitus distress and cortical thickness in anterior insula, between the percentage of time patients reported being aware of their tinnitus and superior temporal gyrus thickness, and between tinnitus duration and cortical thickness at precentral gyrus (PCG). In addition, anxiety and depression was found negatively correlated to cortical thickness at subcallosal anterior cingulate cortex. The most consistence changes of cortical morphology in tinnitus studies were found in auditory and prefrontal cortices.

Table 3.1: Summary of structural morphology studies in tinnitus.

Study	Subjects characteristics				Technique-Analysis methods	Findings TIN comparing to controls
	Number of Subjects	Age of Subjects	HL	Tinnitus severity		
Aldhafeeri et al 2012	14 NHC (5F) 14 TIN (6F)	46.5±8.76 49.5±8.28	None to mild to moderate (NHC & TI)	THI ≥38	SBM-ROI	CT reduction l IFG, l PCC, r MFG, r ACC, r CG, b STG, r MTG, r PAC CT neg corr with HLT r PAC
Muhlau et al 2006	28 NHC (15F) 28 TIN (15F)	39 (26-53) 40 (26-53))	None (TI)	Not given	VBM- whole brain, ROIs	GM reduction vmPFC; GM increases b MGN
Landgrebe et al 2009	18 NHC (13F) 28 TIN (13F)	31.20±9.50 32.20±9.40	None (NHC TI)	Not given	VBM- whole brain, ROIs	GM reduction r IC, l HIPP
Schneider et al 2009	16 NN (11F) 29 MN (12F) 35 NT (10F) 26 MT (10F)	40.80±3.10 37.70±1.90 49.30±1.90 39.40±2.50	None to mild to moderate All groups	Not given	SBM-ROI	GM reduction mHG GMV neg corr with HL
Leaver et al 2010	11 NHC (7F) 11 TIN (6F)	23 ± 3.3 44 ± 16	None to Mild to moderate (NHC & TI)	TCQ 2.45	VBM - whole brain	GM reduction vmPFC
Husain et al 2011	11 NHC (0F) 7 HLC (0F) 8 TIN (0F)	48.09±10.42 51.38±11.45 56.13±7.04	Mild to moderate (HLC and TI)	THI 17.25±5.01 (10-26)	Voxel-wise whole brain	GM volume increases SFG, dmFG, ACC, STG, MFG
Schecklmann et al 2012	44 TIN (14 F)	45.00±13.00	None to mild to moderate (TI)	TQ 35.00±16.00 (4-70)	VBM - whole brain	GM changes Frontal, cingulate, temporal and thalamic areas
Melcher et al 2013	24 NHC (12F) 24 TIN (12F)	45.80±7.60 46.90±8.30	None to mild to moderate (NHC & TI)	Not given	VBM- whole brain, ROIs	GM neg corr with HL vPCC, dmPFC and vmPFC.
Schecklmann et al 2013	335 TIN (88 F)	50.50±11.5	None to profound (TI)	TQ 41.50±17.00 (4-79)	VBM- whole brain, ROIs	GM neg correlation with tinnitus severity HG and insula
Boyen et al 2013	24 NHC (8F) 16 HLC (3F) 31 TIN (11F)	58.00±6.00 63.00±10.00 56.00±9.00	Mild to moderate to profound (HLC & TI)	THI 29.00±20.00 (4-72)	VBM- whole brain, ROIs	GM increases STG, MTG, SMG; GM reduction l SFG, r OL, b H
Leaver et al 2012	21 NHC (13F) 23 TI (11F)	49.0±2.60 47.4±2.90	None to profound (NHC & TI)	THI (0-90)	VBM- whole brain; SBM-ROIs	GM reduction dmPFC, vmPFC, SMG;CT reduction r vmPFC;Surface area reduction vmPFC, Curvature increases SMG, dmPFC CT neg corr with A/D scACC

Abbreviations: NHC *Normal hearing controls*, TIN *Tinnitus patients*, NN *Non tinnitus*, MN *Musician non-tinnitus*, NT *Non-musician tinnitus*, MT *Musician-tinnitus*, HLC *Hearing loss controls*, SD *Semantic dementia*, F *Female*, HL *Hearing loss*, THI *Tinnitus handicap inventories*, TQ *Tinnitus questionnaire*, SBM *Surface based morphometry*, VBM *Voxel based morphometry*, ROIs *Regions of interests*, PFC *prefrontal cortex*, vmPFC *Ventromedial prefrontal cortex*, dmPFC *Dorsomedial prefrontal cortex*, SMG *Supramarginal gyrus*, alns *Anterior insula*, PCG *Precentral gyrus*, STG *Superior temporal gyrus*, ACC *Anterior cingulate cortex*, PCC *Posterior cingulate cortex*, CC *Cingulate cortex*, CN *Cochlear nucleus*, SOC *Superior olivary complex*, IC *Inferior colliculus*, MGN *Medial geniculate nucleus*, Cere *Cerebrum*, OFC *Orbitofrontal cortex*, PAC *Primary auditory cortex*, SAC *Secondary auditory cortex*, mHG *Medial heschl's gyrus*, HIPP *Hippocampus*, PHIPP *Parahippocamus*, MTG *Middle temporal gyrus*, Aud *Auditory*, SFG *Superior frontal gyrus*, MFG *Middle frontal gyrus*, IFG *Inferior frontal gyrus*, MTG *middle temporal gyrus*, ITG *Inferior temporal gyrus*, OL *Occipital lobe*, H *Hypothalamus*, CG *Cingulate gyrus*, r *Right*, l *Left*, b *Bilateral*, GM *Grey matter*.

3.8.2.3 White matter integrity abnormalities and tinnitus

Diffusion tensor imaging (DTI) is a non-invasive MR imaging technique, which aims to assess the microstructure of WM in the brain (Le Bihan et al., 2001). DTI is sensitive to water diffusion that tracking the motion of water molecules along the white matter fibers, and giving measurements of the structural connectivity between cortical and subcortical brain regions.

Few studies have assessed white matter integrity in tinnitus patients using DTI. A significant reduction of integrity was found in tinnitus patients compared to controls in left frontal actuate fasciculus and right parietal fasciculus (Lee et al., 2007), and prefrontal cortex, temporal lobe, thalamus and limbic system (Aldhafeeri et al., 2012a). However, structural connectivity was found increased in tinnitus patients compared to controls in the WM tracts connecting the auditory cortex with the amygdala, the inferior colliculus (IC) with the amygdala, and the auditory cortex with IC (Crippa et al., 2010). A significant negative correlation was found between hearing loss thresholds and WM integrity, and between tinnitus loudness and mean diffusivity (MD) values in auditory brain areas (Seydell-Greenwald et al., 2014). The most consistence changes of structural connectivity in tinnitus patients were found in the inferior frontal occipital fasciculus, superior longitudinal fasciculus, and inferior longitudinal fasciculus.

Despite the apparent WM integrity findings, there are some methodological considerations have been raised such as smoothing, registration or alignment and cross-tracts or tract junctions. The diffusion data does not require data smoothing that could eliminate one of the limitations of voxel-based morphometry (VBM) as this step may play an important role to change the results (Jones et al., 2005). Local misalignment between individuals may occur even after image registration (Bach et al., 2014). Therefore, visual inspection is very important to make sure the brain images are co-registered correctly. Interpretation of WM changes at crossing tracts or tract junction is complicated and difficult due to tensor (DTI) will have trouble distinguishing voxels with crossing fibers from isotropic region (Smith et al., 2006).

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Table 3.2: Summary of diffusion studies in tinnitus.

Study	Subjects characteristics				Analysis methods	Networks examined	Findings TIN comparing to controls
	Number of Subjects	Age of Subjects	HL	Tinnitus severity			
Aldhafeeri et al 2012	14 NHC (5F) 14 TIN (6F)	46.5±8.76 49.5±8.28	None NHC & TI	THI ≥38	Voxel-wise	Whole brain	FA reduction IFOF, CC, l SLF, l ILF FA increases r ILF
Benson et al 2014	13 NHC 13 TIN	58 (22-88) 54 (28-80)	None to mild to moderate NHC & TI	THI ≥35	Voxel-wise, Tractography	Whole brain and ROI: SLF, ATR, SCR, ILF, IFOF	FA reduction l SLF FA increases l ATR, l ILF, l SLF, r IFOF, r SLF
Greenwald et al 2014	18 NHC (9F) 14 TIN (10F)	44.71±11.42 46.50±13.08	Mild to moderate NHC None TI	Not given	Voxel-wise	Aud and Limbic	FA decreases and MD increases with age; FA decreases with HLNeg corr betw MD and Tinnitus loudness, FA increases and MD decreases AC, IC
Crippa et al 2010	15 NHC (3F) 10 TIN (1F)	46 ± 16 49 ± 12	Not given NHC None to mild to moderate	VAS 5.18 (1-10)	Tractography	AC-IC, AC-Am, IC-AM	Increased connectivity btw AC and AM
Husain et al 2011	11 NHC (0F) 7 HLC (0F) 8 TIN (0F)	48.09±10.42 51.38±11.45 56.13±7.04	Mild to moderate (HLC and TI)	THI 17.25±5.01 (10-26)	Voxel-wise whole brain & ROIs	ATR, ILF, IFOF, SLF	FA reduction r ATR, IFOF, ILF
Lee et al 2007	12 NHC (6F) 28 TIN (11F)	26.5 (22-34) 48.3 (22-70)	None NHC Mild to severe TI	Not reported	Voxel-wise (FA)	CC, FAF, PAF	FA reduction FAF and PAF
Gunbey et al 2015	20 NHC (11F) 18 TIHL (9F) 18 TI (10F)	49.2±8.50 52.6±8.25 49.4±7.20	Mild to moderate (TIHL)	THI 55.5±15.2 43.0±8.30	ROIs (FA, ADC)	AC, IC, LL, MGB, TRN, AMG, HIP, PHIP, PFC	FA decreases IC, MGB, TRN, AMG, HIP; ADC increases IC, MGB, TRN, AMG, PHIP; Neg corr btw (FA IC, LL, MGB and HL), (FA MGB, HIP and THI, VAS), (ADC PHIP, PFC and VAS)

Abbreviations: *FA* Fractional anisotropy, *MD* Mean diffusivity, *IFOF* Inferior frontal occipital fasciculus, *CC* Corpus callosum, *SLF* Superior longitudinal fasciculus, *ILF* Inferior longitudinal fasciculus, *ATR* Anterior thalamic radiation, *AC* Auditory cortex, *IC* Inferior colliculus, *WM* White matter, *AMG* Amygdala, *FAF* Frontal arcuate fasciculus, *PAF* Parietal arcuate fasciculus, *MGB* Medial geniculate body, *TRN* Thalamic reticular nucleus, *HIP* Hippocampus, *ADC* Apparent diffusion coefficient, *PHIP* Parahippocampus, *LL* Lateral lemniscus, *THI* Tinnitus handicap inventory, *VAS* Visual analogue scale

3.8.3 Tinnitus findings from functional neuroimaging studies

3.8.3.1 *Contributions of resting state fMRI*

Resting state functional connectivity (FC) aims to investigate the interregional correlation of brain activity during rest. Resting state connectivity is defined as “spontaneous fluctuations in brain activity that can be reliably organized into coherent networks” (Husain and Schmidt, 2014). Biswal was the first scientist who examined resting state connectivity by discovering the correlation between motor regions during rest that was very similar to brain activity during a task (Biswal et al., 1995). The potential of using resting state networks (RSN) has begun to grow in popularity to understand brain FC (Husain and Schmidt, 2014).

There are three popular FC analysis methods: independent components analysis (ICA), seed and graph analysis. ICA is whole brain analysis using the time courses of voxels to produce specific number of components that have some correlations of the time course of voxels and are spatial independent. Seed based analysis requires a prior hypothesis regarding the seed region that can be assessed by finding the correlation of time course of seed regions selected and the rest voxels of the brain. Graph connectivity analysis is based on selected brain regions in order to investigate the connection strength of brain networks nodes via edges.

From correlation to connection, different brain networks are shown statistical dependencies between distinct units. Default mode network (DMN) is unique brain regions that are active during rest and becomes less active during a task (Raichle, 2015). It has been found involved in episodic memory and self-referential

processing (Fox et al., 2009). DMN includes posterior cingulate cortex, precuneus, the medial prefrontal cortex (mPFC) and superior frontal gyrus (Mantini et al., 2007). The limbic network is involved in emotional processing, which includes the parahippocampus, the insula and the amygdala (Robinson et al., 2010). Two networks show the involvement in attention processing; namely dorsal (including the bilateral frontal eye fields and intraparietal sulcus) and ventral (including the ventral frontal cortex and temporoparietal junction) (Fox et al., 2005). The salience network is relating to cognition and emotion processing, which comprises of anterior insula and the dorsal anterior cingulate cortex (Menon and Uddin, 2010). The visual network includes the occipital cortex and temporal-occipital regions, which involved in visual processing (Mantini et al., 2007).

FC changes have been reported in tinnitus population (Kim et al., 2012, Maudoux et al., 2012, Burton et al., 2012, Wineland et al., 2012, Schmidt et al., 2013, Davies et al., 2014, Lv et al., 2014). FC in auditory cortex was found significantly increased with amygdala and dorsal medial prefrontal cortex (Kim et al., 2012), parahippocampus gyrus (Maudoux et al., 2012, Schmidt et al., 2013), and visual cortex (Burton et al., 2012). A significant reduction of functional connectivity was found in default mood network (DMN) in tinnitus patients (Schmidt et al., 2013). The most consistence changes of functional connectivity in tinnitus patients were found in auditory cortex (AC), parahippocampus and inferior frontal gyrus (IFG).

Table 3.3: Summary of resting state functional MRI studies in tinnitus.

Study	Subjects characteristics				Analysis methods	Networks examined	Findings TIN comparing to controls
	Number of Subjects	Age of Subjects	HL	Tinnitus severity			
Kim et al 2012	6 NHC (2F) 4 TIN (1F)	45±2.76 45±3.92	Not given NHC None TI	Not given	Group ICA, Seed to voxel	Aud	r AC increases with l AC; AC increases with amy and dmPFC
Maudoux et al 2012a	15 NHC (6F) 13 TIN (6F)	51±13 52±11	Not given NHC Mild to severe	THI 43.5 (16-84)	Connectivity graph	Aud	AC increases with l phipp
Maudoux et al 2012a	15 NHC (6F) 13 TIN (6F)	51±13 52±11	Not given NHC Mild to severe	THI 43.5 (16-84)	Between group ICA	Aud	AC increases with stem, bg, cereb, phipp, rpf, pari, sm AC decreases with lpfc, Ifus, occip
Burton et al 2012	17 NHC (10F) 17 TIN (6F)	50.6±2.5 53.5±3.6	Non to severe	THI 53.5 (38-76)	Seed to seed, Seed to voxel	Aud, vis, Som, DAN, VAN, ECA	AC decreases with VC, VC decreases with tpj, IFG, ins Occip decreases with ins, IFG
Wineland et al 2012	23 NHC (11F) 18 TIN (6F)	46 (39-54) 54 (52-57)	Not given NHC None to severe	THI 9.67 (0-24)	Seed to seed, Seed to voxel	DAN, VAN, Cog, Aud, vis, Som, DMN	NO significant differences
Schmidt et al 2013	15 NHC (6F) 13 HLC (6F) 12 TIN (3F)	52.9±8.64 57.6±9.39 55.0±6.97	Mild to moderate (HLC and TI)	THI 8.33 (0-22)	Seed to voxel	DMN, DAN, Aud	AC & fef increases with phipp Rs-fc decreases with DMN ips decreases with r smg
Han et al 2014	42 NHC (39) 42 TIN (39)	37.0±10.0 37.2±10.2	None NHC & TI	THI 51.5±21.0	ALFF	Whole-brain	ALFF increases b PC, b IFG; ALFF decreases OL; THI Posi Corr PC & b IFG
Davis et al 2014	11 NHC (3F) 12 TIN (5F)	68.5 65.8	Mild to severe (NHC and TI)	THQ 43.7 (18-68)	Between group ICA, partial correlation	Aud	NO significant differences

Abbreviation: *NHC* Normal hearing controls, *TIN* Tinnitus patients, *HLC* Hearing loss controls, *F* Female, *HL* Hearing loss, *THI* Tinnitus handicap inventories, *THQ* Tinnitus handicap questionnaire, *ROIs* Regions of interests, *ICA* Independent component Analysis, *ALFF* Amplitude of low frequency fluctuation, *Aud* Auditory, *ECA* Executive control of attention, *Vis* Visual, *Som* Somatosensory, *DAN* Dorsal attention network, *VAN* Ventral attention network, *DMN* Default mode network, *PFC* prefrontal cortex, *vmPFC* Ventromedial prefrontal cortex, *dmPFC* Dorsomedial prefrontal cortex, *SMG* Supramarginal gyrus, *alns* anterior insula, *PCG* Precentral gyrus, *STG* Superior temporal gyrus, *ACC* Anterior cingulate cortex, *PCC* Posterior cingulate cortex, *CC* Cingulate cortex, *CN* Cochlear nucleus, *SOC* Superior olivary complex, *IC* Inferior colliculus, *MGN* Medial geniculate nucleus, *Cere* Cerebrum, *OFC* Orbitalfrontal cortex, *PAC* Primary auditory cortex, *SAC* Secondary auditory cortex, *mHG* Medial heschl's gyrus, *HIPP* Hippocampus, *PHIPP* Parahippocampus, *MTG* Middle temporal gyrus, *Aud* Auditory, *SFG* Superior frontal gyrus, *MFG* Middle frontal gyrus, *IFG* Inferior frontal gyrus, *MTG* middle temporal gyrus, *ITG* Inferior temporal gyrus, *OL* Occipital lobe, *H* Hypothalamus, *CG* Cingulate gyrus, *r* Right, *l* Left, *b* Bilateral, *GM* Grey matter.

3.8.3.2 *Interpreting stimulus-evoked fMRI in tinnitus research*

Early neuroimaging study of tinnitus patients were based on the comparison of neuroanatomical changes between subjects with tinnitus and normal controls. However, neuroanatomical findings are not sufficient to understand how the brain responds to certain conditions that relating tinnitus perception such as fear, anger and unpleasant.

Functional MRI is a neuroimaging method to map neural activity in the human brain, which relies on the differences in magnetic features between oxygenated and deoxygenated blood (Logothetis and Pfeuffer, 2004). Task-based fMRI aims to compare blood oxygenation level dependent (BOLD) during a task and a baseline state, and the results are showed as the subtraction between these two status. Different tasks were performed during fMRI scan in order to assess different cognition processes such as emotion (McRae et al., 2012), attention (Culham et al., 2001), language (Binder et al., 1997), visual (Calhoun et al., 2001) and auditory (Ait Bentaleb et al., 2002).

There are two common fMRI designs were used widely in neuroimaging research that are block design and event-related design (Amaro and Barker, 2006). Block design examines responses to series of similar stimuli, whereas event related design examines responses to each single stimulus. Blocked design has the advantages to increase statistical power (Friston et al., 1999) fMRI BOLD signal in response to a stimulus relative to baseline (Buxton et al., 1998). The main advantage of event-related design is the ability to detect transient variations in the hemodynamic

responses (Buxton et al., 2004).

There are number of tinnitus studies addressing brain function regarding to attention (Amaral and Langers, 2015, Husain et al., 2011b, Seydell-Greenwald et al., 2012a), auditory perception (Lanting et al., 2008, Melcher et al., 2000) and emotion (Golm et al., 2013, Carpenter-Thompson et al., 2015). Such neuroimaging studies have shown the dysfunction of brain activity in auditory and non-auditory regions.

Hyperactivity of auditory pathway was found involved in tinnitus patents in different studies. For instance, inferior colliculus (IC) shows increased activation to sound stimuli in tinnitus patients compared to controls (Melcher et al., 2009). This finding is consistence with animal studies that demonstrating IC hyperactivity in animal models of tinnitus (Bauer et al., 2008). Another study shows that auditory cortex is hyperactive related to tinnitus (Gu et al., 2010).

The dysfunction of limbic system was found involved in tinnitus participants in different studies. Hyperactivity in tinnitus patients was found in nucleus accumbens (NA) when tinnitus patients presented sound stimuli that matched their perceived tinnitus frequency (Leaver et al., 2011). Furthermore, patients with chronic tinnitus showed stronger activation to tinnitus-related sentences in comparison to neural sentences than controls in different limbic/emotion processing brain regions: cingulate cortex, insula and frontal areas (Golm et al., 2013). Also, the same study has compared brain function between highly and low distressed tinnitus patients, which found that the former showed a stronger activation in the left middle frontal gyrus (Golm et al., 2013). Another study shows the dysfunction of ventral prefrontal

cortex in tinnitus patients compared to healthy controls (Seydell-Greenwald et al., 2012a). The most consistence changes of brain function in tinnitus patients were found in auditory cortex (AC) and inferior colliculus (IC).

3.9 Conclusion

Tinnitus is not a single disorder and the symptoms differ extensively. The tinnitus causes are wide variants between tinnitus patients. Despite the fact that different theories have been proposed to explain the possible reasons to generate tinnitus sounds, no cure has been discovered yet for tinnitus that might reflect the lack of understanding the pathophysiology mechanism of tinnitus. Auditory deprivation can have a strong effect to cause tinnitus perception. Tinnitus and chronic pain have some similarities such as forms, severity and neural plasticity.

Structural and functional neuroimaging have provided windows to the brain, which allow detection the tinnitus-related in the brain. The most consistence changes of brain structure and function in tinnitus patients were found in auditory and prefrontal cortices, inferior colliculus, parahippocampus, inferior frontal gyrus, inferior frontal occipital, superior longitudinal, and inferior longitudinal fasciculus.

One of the challenges to understand tinnitus mechanism is whether the structural atrophy and dysfunction of auditory and limbic system is related to the origin of tinnitus perception, or they are consequences of chronic tinnitus perception. The interaction between auditory and limbic system might be the key to understand the pathophysiology of tinnitus mechanism (Leaver et al., 2011).

Chapter 4: Participants, Materials and Methods

4.1 Ethical consideration

This research project has been approved from the University of Liverpool Committee on Research Ethics (Reference: RETH000688) to recruit normal healthy controls, and from the National Research Ethics Services (NRES) Committee North West-Liverpool Central (Reference: 14/NW/1473) to recruit tinnitus patients (Appendix IV).

4.2 Inclusion and exclusion Criteria

Certain inclusion and exclusion criteria were set for this study (table 4.1) to recruit homogeneous groups of individuals who are in healthy conditions, have similar etiologies and matching in terms of age and genders. Exclusion criteria were as followings: objective tinnitus, sever health problems, sever hyperacusis, metallic implants or pacemakers, physiological and psychological disorders.

Table 4.1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Subjective tinnitus (Tinnitus group)	Objective tinnitus (All groups)
Tinnitus onset >6 months (Tinnitus group)	Sever hyperacusis (All groups)
Age range between 30-65 years (All groups)	Conductive hearing loss (All groups)
Hearing loss should be no worse than 40 dB HL at 2 kHz 60 dB HL at 4 kHz (All groups)	Sever health problems: neurological, psychological and physiological disorders (All groups)

4.3 Recruitment and participants

40 normal healthy controls (NHC) were recruited at the University of Liverpool and via the University's announcement website, and 34 chronic tinnitus patients (TIN) were recruited through the tinnitus network support meeting at the Aintree University Hospital in Liverpool (Appendix I). All participants were given the Participants Information Sheet (PIS) (Appendix II) that includes the study aims, a full explanation of the procedures involved in this study and contact information of the researchers involved in this study. All participants signed a consent form (Appendix III) in the first visit prior audiometry and MRI scan. This consent form was signed as an agreement that participants fully read and understand the information in PIS and are agreed to take part in the study.

4.4 Audiology test

Pure tone air conduction audiometry was performed to assess the hearing level of the participants in this study. Audiograms were measured with a calibrated diagnostic audiometer (Amplivox 2160, with Audiocups to reduce noise and permit accurate pure tone audiometry). The pure tones were presented at seven different octave frequencies (0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 kHz), and at different sound intensities that ranged from -10 to 120 dB HL. A consultant audiological physician performed the hearing assessment at the University of Liverpool site in a quiet office with ambient noise levels less than 35dB. Hearing function was defined as the mean hearing loss thresholds, averaged over the seven frequencies tested for both ears.

4.5 Behavior assessments:

Different behavior assessments were undertaken in this study, Handedness was assessed in this study using Edinburgh Handedness Inventory (EHI). In addition, anxiety and depression was assessed for all participants by completing Hospital Anxiety and Depression Scale (HADS). In order to assess the effect of tinnitus on participants' lifestyle, tinnitus participants were asked to completed two tinnitus inventors: Tinnitus Handicap Inventory (THI) and Tinnitus Functional Index (TFI). In addition, some tinnitus characteristics information such as tinnitus onset, localization and sound were obtained from tinnitus group (Appendix V).

4.5.1 Edinburgh Handedness Inventory (EHI)

The Edinburgh Handedness Inventory (EHI) is a measurement scale that is applied in order to identify hand dominant (Oldfield, 1971). It is a self-report inventory that consists of ten items questions to assess which hand is preferred to use in common daily activities: writing, drawing, throwing, cutting with scissors, holding a toothbrush, knife (without fork), holding a spoon, holding a broom (upper hand), striking match and opening a box (lid). Participants were asked to score their preferred hands in each task by assigning number of crosses (one or two) depending on how often they use hands on each task. Handedness index was calculated using this formula: $\text{Handedness index} = \frac{R-L}{R+L} * 100$, where R and L are the number of crosses choosing for the right and left hands, respectively. EHI results are ranged from -100 for strong left-handed to +100 for strong right-handed.

4.5.2 Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale (HADS) is a measurement scale that is used to identify the level of anxiety and depression that participants may experience (Zigmond and Snaith, 1983). It consists of a fourteen items scale divided equally between two subscales: anxiety and depression. Scores of 0-7 in respective subscales are considered as normal cases, with 8-10 are borderline, and 11 or over indicating probable anxiety and depression cases.

4.5.3 Newman Tinnitus Handicap Inventory (THI)

Tinnitus Handicap Inventory (THI) is a self-reported tinnitus questionnaire that assesses the effect of tinnitus perception on life (Newman et al., 1996). The Newman THI consists of 25 items that categorised into three main scales, namely, the functional, emotional and catastrophic subscales. First scale in the Newman THI is functional scale that includes 11 questions fit in to three main categories; mental, social and physical. The second scale in the Newman THI is the emotional scale that includes nine questions that quantify the impact of tinnitus of the emotion daily life, for example, whether tinnitus makes suffers angry or leads to have depression. Third scale in the Newman THI is the catastrophic scale, which investigates the severe reactions of sufferers to tinnitus. Despite the fact that Neman THI questionnaire is divided into three subscales, the total score is sufficient for tinnitus patients' evaluations (Jastreboff, 2011).

The Newman THI takes roughly 10 minutes to complete the form. It is advisable to complete this form in the clinic with a specialist person who may offer some simple explanations and encouragement. In each question in the Newman THI, Tinnitus

participants answered each question with one of the three following choices: 'yes' (4 points), 'sometimes' (2 points) and 'no' (0 point). Responses were summed to determine the tinnitus participant's score that should be in a range of (0-100).

The Newman THI results are distributed into five gradual stages: slightly (0-16), mild (17-36), moderate (36-56), sever (57-76) and catastrophic (77-100). Slightly stage is described as tinnitus sound can only heard in a quiet environment, very easily masked and no interference with sleep or daily activities. In the mild stage, tinnitus sound can be masked easily by environment sounds, easily forgotten by activities and may interference suddenly with sleep but not in daily activities. Third scale is moderate, which can be noticed in the presence of environmental background, and daily activities can be performed. Sever tinnitus is the forth scale, which tinnitus sound can be heard even it is masked which leads to disturb sleep pattern and interferences with daily activities. Catastrophic tinnitus is the most sever tinnitus scale, which heard always, disturbs sleep patterns and having difficulty with daily activities. The descriptions of Newman THI results were summarized in the table (4.2).

Although the Newman THI was developed in the USA, it could be used in the UK without any modification (McCombe et al., 2001b). This kind of evaluation is used in most of centres that involves tinnitus treatment (Jastreboff, 2011). It is found out that the total score of the Newman THI is changed during tinnitus retraining treatment (TRT), which supports the significant improvement occurring during TRT (Jastreboff, 2011). However, observing change of the Newman THI score requires long interval in order to obtain a significant changes.

Table 4.2: The scale and description of THI score

Grade	Score	Scale	Description
1	0-16	Slightly	Only heard in a quiet environment, very easily masked and no interference with sleep or daily activities
2	17-36	Mild	Masked easily by environment sounds, easily forgotten by activities and may interference suddenly with sleep but not in daily activities
3	37-56	Moderate	Noticed in the presence of environmental background, and daily activities can be performed
4	57-76	Sever	Can be heard even it is masked which leads to disturber sleep pattern and interferences with daily activities
5	77-100	Catastrophic	Heard always, disturbs sleep patterns and having difficulty with daily activities

4.5.4 Tinnitus Functional Index (TFI)

Tinnitus Functional Index (TFI) is a new self-reported tinnitus questionnaire aims to scale the severity and negative impacts of tinnitus, which would provide comprehensive coverage of multiple tinnitus severity domains (Meikle et al., 2012). It includes as well 25 items, which categorized into 8 subscale dominants of tinnitus severity (table 4.3): intrusive (I), sense of control (Sc), cognitive (C), sleep (SL), auditory (A), relaxation (R), quality of life (Q) and emotion (E). The conceptual content of each TFI subscale is illustrated in table 4.3. The overall TFI score is calculated by summing all valid answers, divided them by the number of questions, and multiply by 10. It ranges from 0 (lowest impact) to 100 (highest impact).

Table 4.3: The conceptual content of each TFI subscale.

Abbreviation	TFI subscale	Conceptual content	Items in subscale
I	Intrusive	Unpleasantness, intrusiveness, persistence	#1, #2, #3
SC	Sense of control	Reduced sense of control	#4, #5, #6
C	Cognitive	Cognitive interference	#7, #8, #9
SL	Sleep	Sleep disturbance	#10, #11, #12
A	Auditory	Auditory difficulties attributed to tinnitus	#13, #14, #15
R	Relaxation	Interference with relaxation	#16, #17, #18
Q	Quality of life	Quality of life reduced	#19, #20, #21, #22
E	Emotional	Emotional distress	#23, #24, #25

4.6 Demographics of Healthy Controls and Tinnitus Sufferers

Normal controls (40 subjects) were categorized into two groups: normal hearers (n=20 (14 female, 1 left handed), age= 43 ± 9 years, hearing loss= 9.5 ± 3 and HADS= 10.9 ± 2.7) and mild to moderate hearing loss subjects (n=20 (11 Female, 1 left handed), age= 47 ± 10 years, hearing loss= 16 ± 5 , and HADS= 11.6 ± 3) (Figures 4.1, 4.2, 4.3, 4.4 and table 4.4).

The demographic of tinnitus patients is as following: n=34 (14 female and 6 left handed), age 46 ± 8 years, hearing loss= 24 ± 16 , anxiety and depression score= 10 ± 6 , tinnitus duration= 14.2 ± 15 years, tinnitus laterality= 11 unilateral (7 left and 4 right) and 23 bilateral, and tinnitus severity (THI= 30 ± 24 , TFI= 38 ± 23) (Figures 4.1, 4.2, 4.3, 4.4 and table 4.5). 8 tinnitus patients were withdrawn from the MRI studies due to they were not compatible with MRI environment and did not meet the MRI inclusion criteria. Therefore, 26 tinnitus patients (8 Female, 5 left handed, age= 45 ± 12 years, hearing loss= 25 ± 14 dB, HADS= 10.2 ± 6.7 , tinnitus duration= 9 ± 8 years, tinnitus laterality= 10 unilateral. THI= 26 ± 19 and TFI= 36 ± 21) completed all the studies including behavioral, audiological and MR imaging studies.

Table 4.4: Normal healthy controls (NHC) details.

ID	Group	Age	Gender	Handedness	H.A.D.S	HLA (m \pm std)
NHC02	MH	51	Male	Right	9	18 \pm 10
NHC06	MH	64	Male	Right	9	12 \pm 9
NHC07	MH	54	Male	Right	17	23 \pm 18
NHC08	MH	31	Male	Right	7	10 \pm 8
NHC12	MH	50	Female	Right	8	18 \pm 9
NHC13	MH	60	Male	Right	8	15 \pm 7
NHC14	MH	47	Male	Right	10	30 \pm 23
NHC15	MH	35	Female	Right	18	11 \pm 6
NHC16	MH	55	Female	Right	12	24 \pm 25
NHC20	MH	47	Male	Right	8	14 \pm 8
NHC21	MH	57	Female	Left	12	15 \pm 11
NHC23	MH	40	Female	Right	18	12 \pm 9
NHC27	MH	44	Female	Right	14	14 \pm 9
NHC29	MH	52	Female	Right	7	13 \pm 7
NHC32	MH	48	Female	Right	9	20 \pm 10
NHC33	MH	37	Female	Right	11	11 \pm 9
NHC34	MH	48	Female	Right	10	11 \pm 6
NHC37	MH	51	Male	Right	14	16 \pm 9
NHC40	MH	30	Male	Right	18	21 \pm 4
NHC42	MH	65	Female	Right	13	15 \pm 6
NHC01	NH	30	Female	Right	14	7 \pm 4
NHC03	NH	34	Female	Right	9	5 \pm 3
NHC04	NH	50	Female	Right	16	11 \pm 5
NHC05	NH	35	Male	Right	9	7 \pm 3
NHC09	NH	32	Male	Right	12	9 \pm 4
NHC10	NH	41	Female	Right	9	11 \pm 4
NHC11	NH	32	Male	Right	9	13 \pm 7
NHC18	NH	30	Female	Right	12	9 \pm 5
NHC19	NH	41	Male	Right	10	10 \pm 5
NHC22	NH	45	Female	Right	8	7 \pm 5
NHC24	NH	34	Female	Right	6	8 \pm 4
NHC25	NH	41	Female	Left	13	6 \pm 6
NHC26	NH	53	Female	Right	14	9 \pm 4
NHC28	NH	51	Female	Right	11	9 \pm 6
NHC31	NH	42	Female	Right	7	17 \pm 4
NHC35	NH	48	Female	Right	14	12 \pm 6
NHC36	NH	41	Female	Right	11	12 \pm 2
NHC38	NH	63	Male	Right	9	9 \pm 5
NHC41	NH	30	Female	Right	11	6 \pm 5
NHC44	NH	30	Male	Right	11	7 \pm 6

MH=Mild to moderate hearing loss, NH=Normal hearing, HADS=Hospital Anxiety and Depression Scale, HLA= Hearing loss thresholds

Chapter 4: Participants, Materials and Methods

Table 4.5: Tinnitus patients' details.

ID	Group	Age	Gender	Handedness	HADS	HLA (m±std)	Onset	Laterality	THI	TFI
P01	TIN	39	Male	Right	0	17±7	8	Bilateral	6	22
P02	TIN	34	Female	Right	6	11±7	3	Bilateral	16	15
P03	TIN	32	Male	Right	17	30±22	1	Bilateral	18	22
P04	TIN	47	Male	Left	11	16±13	4	Left side	28	52
P05	TIN	45	Female	Right	14	26±26	10	Left side	40	53
P06	TIN	32	Female	Right	7	13±7	2	Bilateral	36	55
P07	TIN	65	Male	Right	9	49±7	5	Bilateral	16	32
P08	TIN	49	Female	Left	7	23±26	22	Left side	20	34
P09	TIN	37	Female	Right	4	16±11	20	Right side	20	23
P10	TIN	37	Male	Right	19	16±6	5	Bilateral	12	16
P11	TIN	45	Male	Left	11	20±13	1	Bilateral	10	14
P12	TIN	37	Male	Right	13	24±16	3	Left side	76	90
P13	TIN	57	Male	Right	9	40±25	57	Bilateral	20	24
P14	TIN	35	Male	Left	8	60±27	35	Bilateral	12	29
P15	TIN	42	Male	Right	17	18±10	20	Bilateral	26	25
P16	TIN	30	Male	Right	2	18±6	10	Bilateral	16	19
P17	TIN	45	Female	Right	14	5±5	4	Bilateral	12	22
P18	TIN	56	Male	Right	9	34±16	30	Right side	54	67
P19	TIN	60	Female	Right	2	38±15	10	Right side	26	32
P20	TIN	57	Male	Right	10	29±11	10	Left side	20	64
P21	TIN	33	Male	Right	3	8±4	17	Left side	10	13
P22	TIN	51	Male	Right	5	33±13	2	Bilateral	8	8
P23	TIN	65	Male	Left	15	36±26	10	Left side	44	52
P24	TIN	65	Male	Right	11	45±23	10	Bilateral	28	41
P25	TIN	33	Male	Right	33	0±5	15	Bilateral	80	72
P26	TIN	53	Female	Right	9	13±6	6	Bilateral	28	37
W01	TIN	46	Female	Right	3	64±19	47	Bilateral	8	16
W02	TIN	64	Male	Left	14	19±16	2	Bilateral	92	84
W03	TIN	60	Male	Right	1	34±8	10	Bilateral	8	19
W04	TIN	55	Female	Right	14	24±16	55	Right side	36	56
W05	TIN	53	Female	Right	18	13±6	40	Bilateral	24	22
W06	TIN	43	Female	Right	4	10±6	8	Bilateral	22	24
W07	TIN	32	Female	Right	10	70±18	2	Bilateral	56	50
W08	TIN	56	Female	Right	20	69±18	0.5	Left side	94	92

P= Tinnitus patients completed the study, W= Tinnitus patients withdrawn from the MRI study, TIN=Tinnitus, HADS=Hospital Anxiety and Depression Scale, HLA= Hearing loss thresholds, Onset=Tinnitus duration (years), Laterality=Tinnitus location, THI=Tinnitus Handicap Inventory, TFI=Tinnitus Function Index.

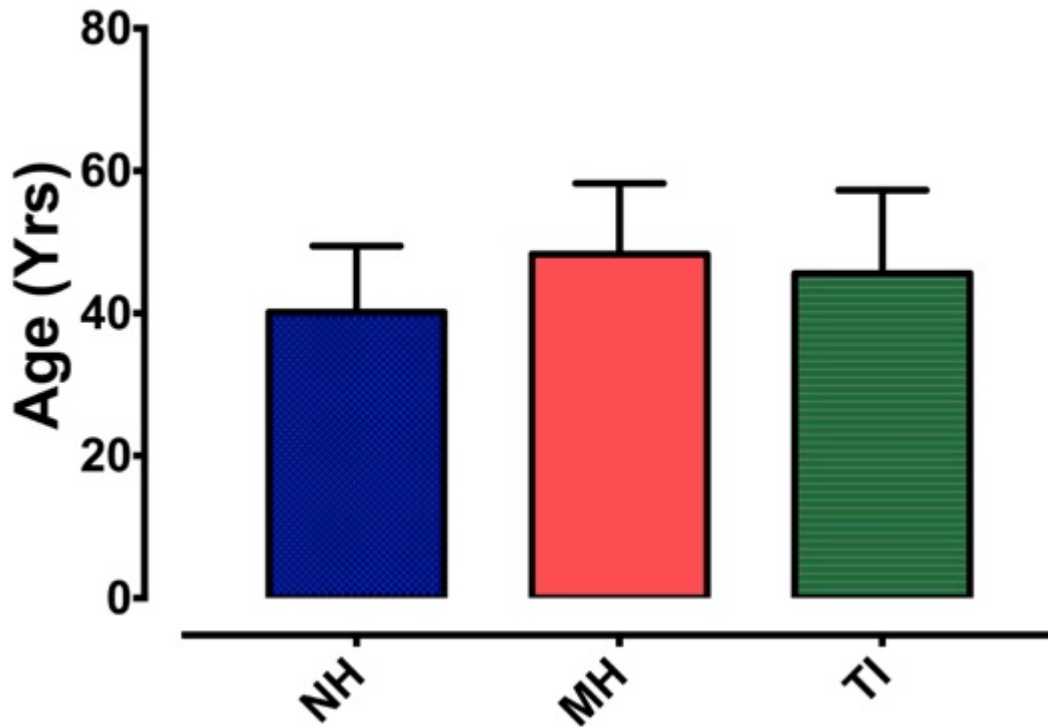
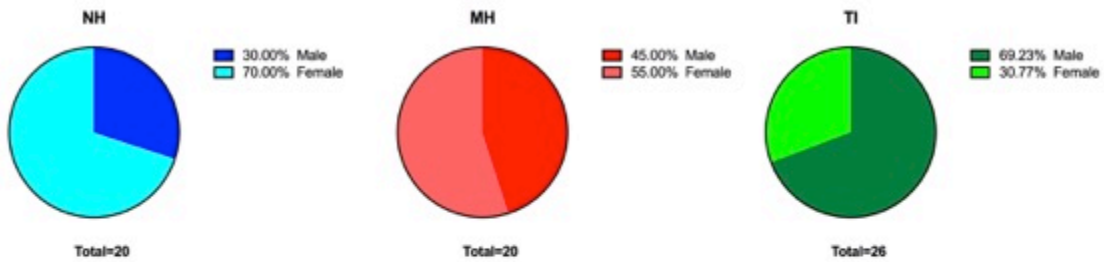


Figure 4.1: Age distribution of participants' groups who undertaken the experiments

Gender distribution



Handedness distribution

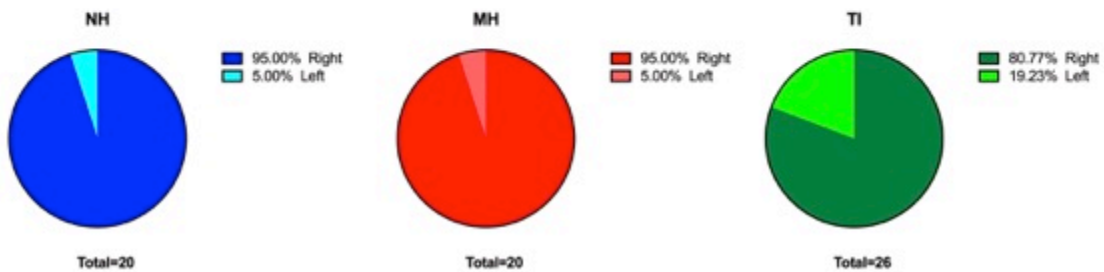


Figure 4.2: Gender and handedness distribution of participants' groups who undertaken the experiments

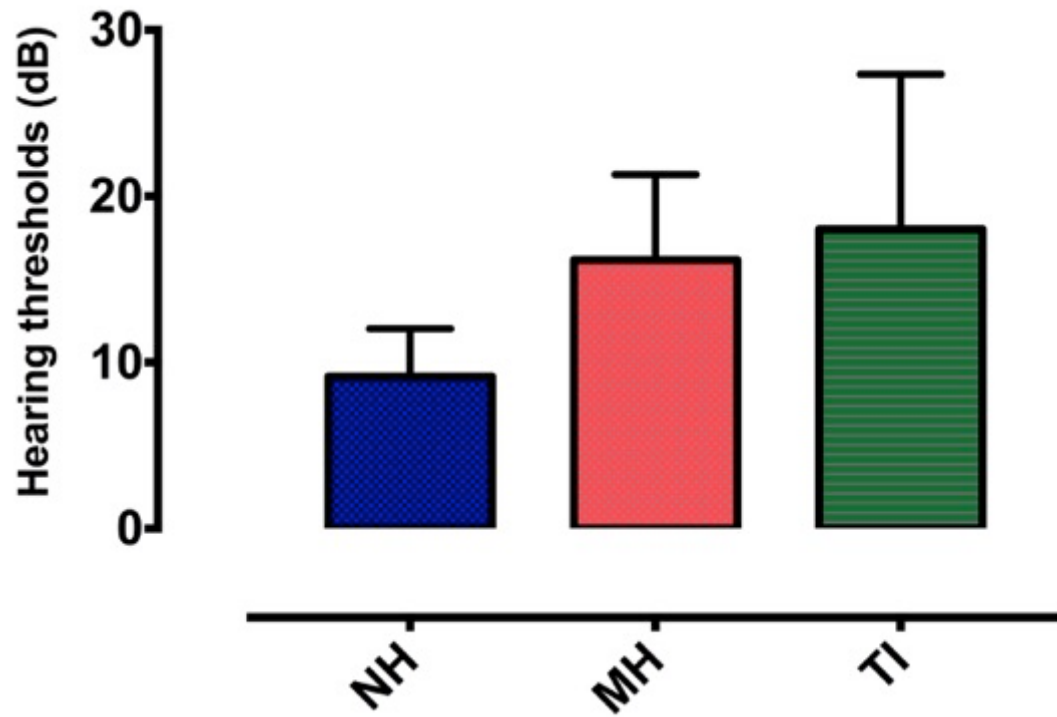


Figure 4.3: Hearing loss distribution of participants' groups who undertaken the experiment

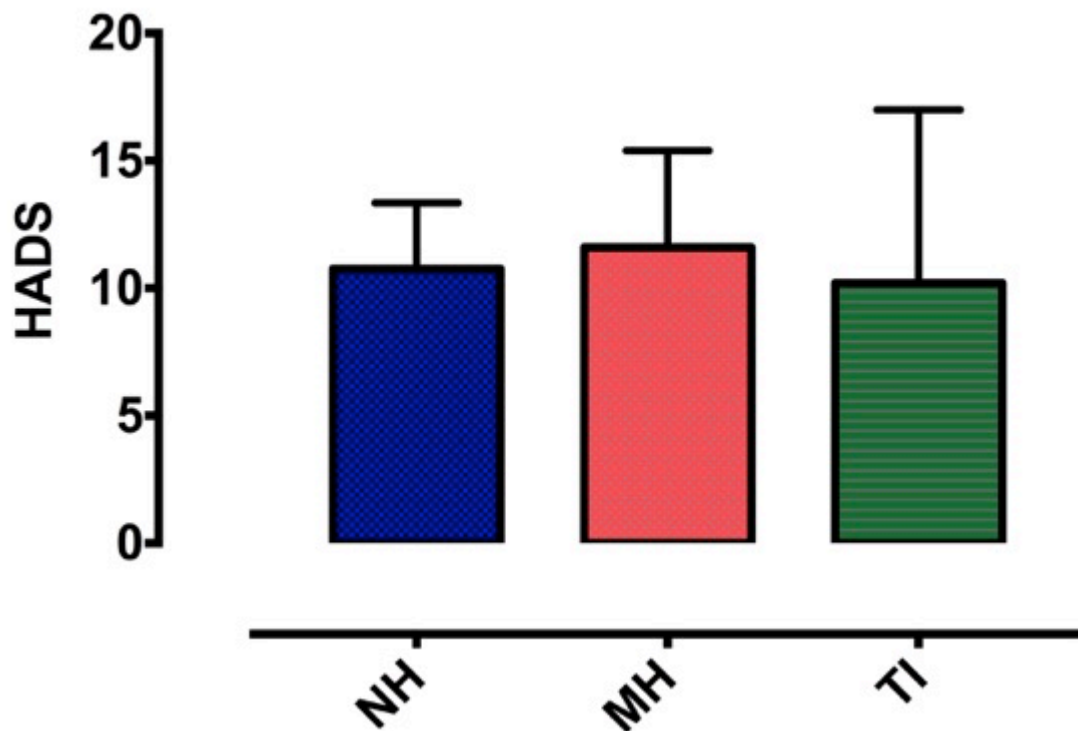


Figure 4.4: Anxiety and depression distribution of participants' groups who undertaken the experiment

4.7 Magnetic resonance imaging (MRI):

4.7.1 Nuclear Magnetic Resonance background

All molecules are made of atoms that have a standard structure, where nucleus and electrons are allocated at the central and peripheral levels respectively. Elementary particles (electrons, protons and neutrons) have unique properties. The basic theory of MRI is based upon the interaction between a static magnetic field and an atomic nucleus. The human body contains a very large amount of hydrogen in form of fat and water molecules, and each hydrogen atom behaves like a tiny bar magnet.

With no external magnetic field is applied, protons rotate about their axes in a random direction (figure 4.5 a). While, when an external magnetic field is applied, protons align their spin states either with or against the external field (figure 4.5 b). These orientations have different energies; parallel alignment is the low energy state, and anti-parallel alignment is a high-energy state .A radio frequency (RF) is applied to disturb the equilibrium status causing the tiny magnetic moments to fall out of alignment with the external magnetic field (figure 4.5 c). This RF must have the same frequency as the protons' precession frequency (resonance frequency). Energy exchange or transfer is easier when protons are moving at a specific frequency. In magnetic resonance, only protons with the same frequency as the RF pulse will respond. When protons pick up energy from an RF pulse of the same frequency, this is called resonance. After transmitting a RF pulse, nuclei become excited from lower to the higher energy because of the absorption of energy that leads to increase the number of spin down hydrogen nuclei. The effect of RF pulse is simply to cause a precession of the net magnetisation vector around the X-axis,

which is called forced precession. RF field is applied to create phase coherence between the M_x and M_y plane (figure 4.5 d).

The degree to which the net vector moves down is called the flip angle, which is a function of strength and duration of the RF pulse. The duration of pulse determines the flip angle degree e.g., 5 ms and 10 ms pulses give 45 and 90 degrees flip angle respectively. After excitation, the nuclei return to equilibrium as they emit energy in a form of electromagnetic waves (Weishaupt et al., 2006).

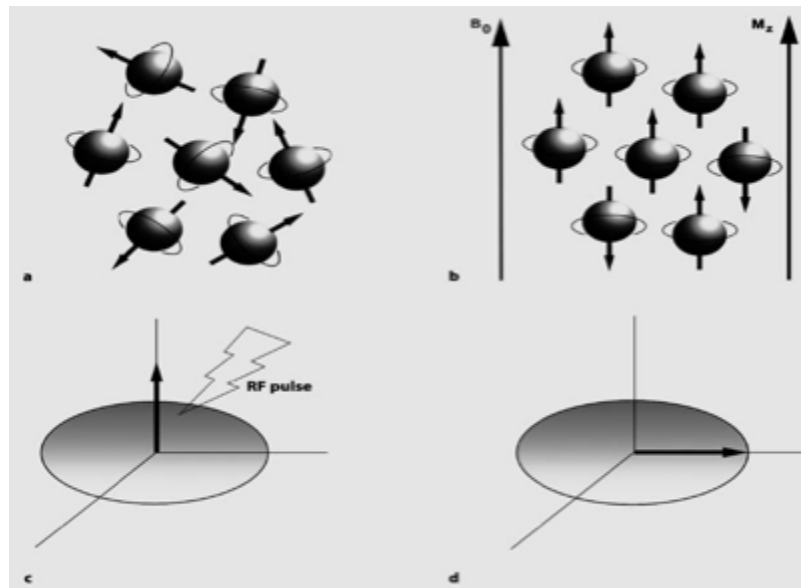


Figure 4.5: Protons with no external magnetic field present (a) and with external magnetic field (b), an RF pulse causing excitation (C) and net magnetization vector flips over and rotate into the transverse magnetization (d). Adapted from (Weishaupt et al., 2006)

4.7.2 Instrumentation and safety

Basically, the subject is placed in a very strong magnetic field that produces a highly uniform field B_0 across the subject. The magnetic field is the heart of MR system, and most magnets used in MRI machines are superconducting solenoids. Then, the radiofrequency (RF) is sent to the target by a transmitted coil that surrounds a part of the body that would be scanned. After that, the radio wave will be turned off, and

the subject emits signals that are received and used to construct the MR image (McRobbie, 2007).

The basic hardware components used in MRI examination are the magnet that has the gradient coils and the radiofrequency (RF) coils that send the pulse to the tissues and/or receive the signals from them. The gradient coil is built inside the bore magnet that generates clicking sound during scanning. The creation of MR image is based upon the signal emitted by the patient body tissue after turning on and then off of a radio wave (McRobbie, 2007).

Due to no ionising radiation is used in MRI, it is particularly safe for patients and people work in this environment. However, MRI uses electric and magnetic fields: static magnetic fields, magnetic field gradients and radio frequency wave, which means that there are some sources of hazard need to be considered carefully when using MRI technology. The static magnetic field is always on that could be dangerous for people entering the scanning room. Whereas the magnetic field gradient and radio frequency field are only on when the scanning is running. These are important for patients and people working with MRI scanner (McRobbie, 2007).

The static magnetic field can be considered not safe in some cases within the Five Gauss Line that specifies the perimeter around an MR scanner. The controlled area of MRI is defined as “an enclosed and of such a size to contain the 5 Gauss magnetic field contour” (Device_Bulletin, 2007). This is important in particular for patients with implanted pacemakers who should be kept outside the 5 Gauss.

MRI could cause serious damaging effects such as torque, heating and artefact in the human body that holds a metal. Implants can potentially be moved unpredictable

within the body. Type of metal used is one factor that determines the force exerted on them in the magnetic field. Non-ferromagnetic can cause heating due to their inability to dissipate the heat caused by RF absorption (McRobbie, 2007).

Acoustic noise should be limited within recommended safety guidelines because it can cause some reversible and irreversible effects (Price et al., 2001). All patients should be given hearing protection that can be seen in many forms. In many situations, earplugs are used, which are acceptable and inexpensive way to prevent these effects.

4.7.3 NMR signal and MRI contrast

After the RF pulses excite hydrogen nuclei, there are two relaxations processes occurred that are T_1 recovery and T_2 decay (figure 4.6). T_1 recovery restores the equilibrium population, which is termed spin-lattice relaxation due to the nuclei give up their energy to the neighbouring environment or lattice. When the RF pulse is switched off, the protons begin to become out of phase (dephase) due to protons do not all precess at exactly the same rate because of magnetic field inhomogeneities, which are influenced by small magnetic fields from surrounding nuclei. This is called T_2 relaxation that destroys the phase coherence created by the RF pulse, and as well is named spin-spin relaxation because nuclei exchange energy with surrounding environment (McRobbie, 2007).

An important advantage of MRI is the sensitivity of the MR signal to the number of different physical chemical properties of the sample. This is considered as the major feature of MRI compared with other image modalities (McRobbie, 2007). Changing the image contrast between tissues can be achieved by manipulating three main

parameters: the repetition time (TR), the echo time (TE), and flip angle. The TR and TE determine the magnitude of T1 and T2 relaxation respectively that have happened when the signal is read. When a tissue has a large transverse component of coherent magnetization at time TE, it has a high signal and vice versa. TR controls how far each vector recovers before the next RF pulse excites it. Whereas, TE controls the amount of T₂ decay which is allowed to happen before the signal is received (McRobbie, 2007).

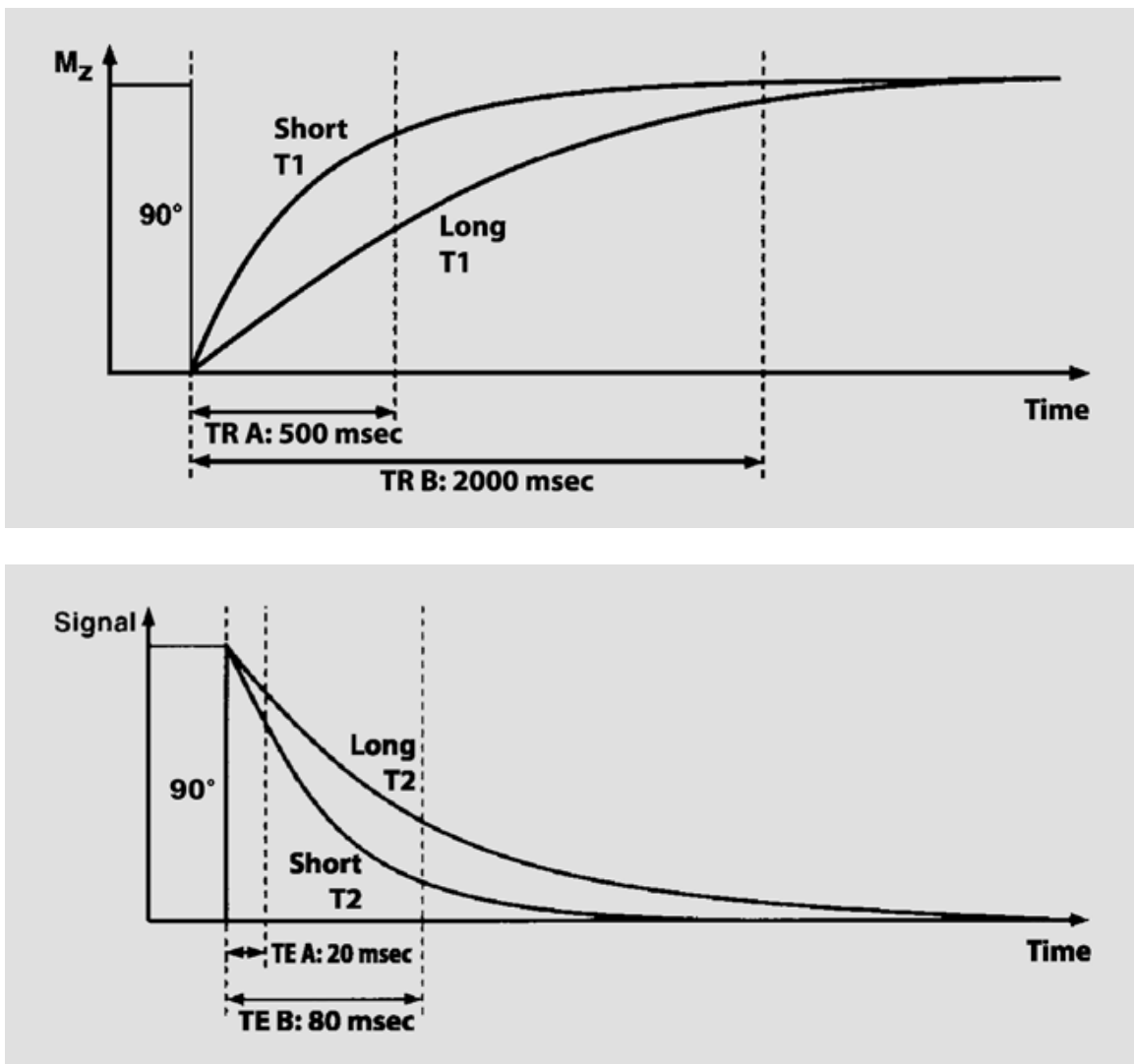


Figure 4.6: Diagrammatic representation of the relaxation depending of image contrast. (Adapted from (Weishaupt et al., 2006))

4.7.4 Sequences

The basic appearances of the body tissues in MRI are fluids, water and fat that have different signal intensities or brightness, which describes the contrast. There are three main types of contrast images: T₁ weighted (anatomical), T₂ weighted (pathological) and Proton Density (PD) weighted (anatomical) images (figure 4.7).

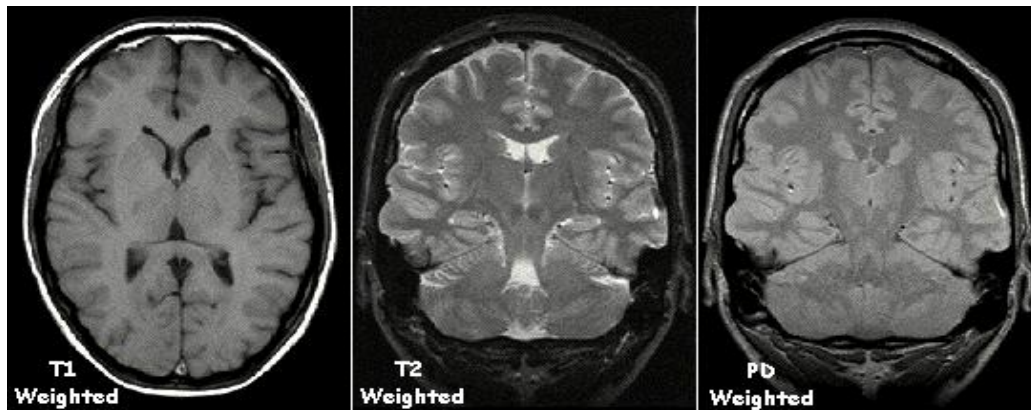


Figure 4.7: Examples of a T₁-weighted, T₂-weighted and Proton density. Adapted from <https://www.ole.bris.ac.uk>

Different contrasts can be achieved with the basic pulse sequences: spin sequences (SE) and gradient sequences (GE).

Spin echo (SE) consists of a 90° and a 180° pulse (Figure 4.8 a). The 90° pulse rotates the magnetization down onto the X'Y' plane. The transverse magnetization starts to dephase. At some point in time after the 90° pulse, the 180° pulse is applied that rotates the magnetization by 180° about the X' axis, and causes the magnetization to at least partially rephase and to produce a signal called an echo. The SE will be used for diffusion experiment.

Gradient echo (GRE) starts with a small RF pulse producing a flip angle (α) typically less than 90 (figure 4.8 b). GRE sequences can use shorter TR and TE than spin echo (SE). Thus, total time scan is much shorter than in SE experiments. However, they

are influenced by the quality of the main magnetic field (inhomogeneities) (Weishaupt et al., 2006). These effects appear on T_2 that becomes shorter. Practical spin and gradient echo sequences are shown in figure 4.4 with full explanation. GRE will be used for fMRI task and rest state based experiments.

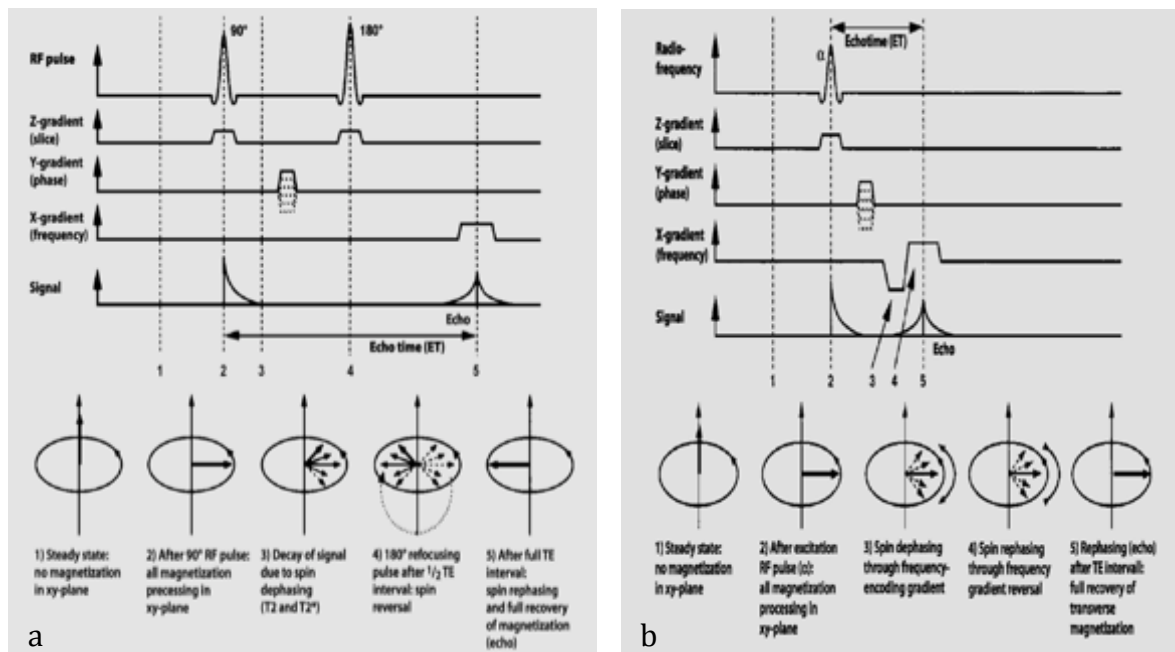


Figure 4.8: Practical spin (a) and gradient (b) echo pulse sequences. Adapted from (Weishaupt et al., 2006).

4.7.5 Functional magnetic resonance imaging (fMRI)

fMRI aims to obtain images of brain function using echo planer imaging (EPI) and mapping the hemodynamic related to the input and intracortical processing of a specific area rather than its spiking output (Logothetis et al., 2001). This technique uses the blood as an intrinsic contrast to pick up the changes of local blood flow during brain activity. These changes are relative amounts of oxyhaemoglobin (diamagnetic) and deoxyhaemoglobin (paramagnetic). The extra oxygen causes changes in the local T_2^* of the regions where there is extra blood flow. This leads to a change in signal. The effect is known as Blood Oxygenation Level Dependent (BOLD)

contrast. fMRI requires high field strengths as the changes being measured are very tiny. So it is important to maximize the signal at low noise level. Gradient echo (GE) is widely used, as BOLD sequence in fMRI due to it is sensitive to the magnetic field inhomogeneity.

4.7.6 MRI screening safety

Prior each MRI scan, all participants underwent safety screening that is performed by the research radiographer at the Magnetic Resonance and Imaging Analysis Research Centre (MARIARC) in the University of Liverpool. Safety screening aims to make sure participants are fitting with MRI environment criteria and do not have any contraindications of being exposed to high magnetic field. MRI contraindications are summarized as follows: metallic implants (e.g., Pacemaker and surgical clips), being on medications for specific diseases (e.g., renal failure and cardiovascular diseases), claustrophobia, tattoo or permanent makeup, body piercing jewelers and pregnant.

4.7.7 MRI quality assurance (QA) report

The MRI QA report of the period with respect to when the MRI data were acquired concludes that 1)- the SNR shows some variability, but is stable on the whole, 2)- the uniformity is within the suggested limits, 3)- the Geometric Distortion measures have shown some variability towards the end of last year, which may indicate that the system is ready for a full service, and 4)- the ghosting ratio and ghost-to-signal ratios are frequently above their limits (0.025 and 0.05 respectively), which is something to keep an eye on.

4.7.8 Image acquisitions

All image acquisitions were performed using a Siemens 3T Trio (Siemens, Erlangen, Germany) with a standard 8 channels head coil. In order to control head movement during the scan, we used foam padding and head resistance. As the main objective of this study was to investigate the structural and functional neural substrate of tinnitus, the study has been design to acquire structural, functional, diffusion images that summarized as the following: T1-weighted images (Structural MRI), T2-weighted images scan (clinical scan), functional images (resting-state and task-based fMRI), arterial spin labelling images (ASL) and diffusion tensor imaging (DTI). The image acquisition parameters were as follows:

- **Structural scan:** Anatomical T1-weighted MR images were acquired in the sagittal plane using 3D a modified driven equilibrium Fourier transform (MDEFT) sequence with the following parameters: TR/TE 7.92/2.48 ms, 176 volumes, slice thickness 1.00 mm, FOV 256*256 mm² and scan time 12 min 51 s.
- **Clinical scan:** T2-weighted MR images were acquired to identify whether there is any incidental findings, which are considered as exclusion criteria for this study. The image acquisition parameters of clinical scan were as follows: TR/TE 4000/102 ms, 24 volumes, slice thickness 5.00 mm, and scan time 01 min 51 second.
- **Resting state fMRI scan:** Resting state functional images were acquired using a T2-weighted gradient echo planer imaging (EPI) sequence with the following parameters: TR 2000 ms, TE 30 ms, slice thickness 3.5 mm, matrix size 64*64, interleaved slice order, slice gap 4mm and 180 volumes. One hundred and eighty

axial slices oriented parallel to AC-PC line were taken, covering the whole brain. Participants were instructed to close their eyes, try not to think about anything in particular and let their minds wonder. Scan time was 6 minutes.

- **Task-based fMRI scan:** Task-based functional images were acquired using a T2-weighted gradient echo EPI sequence with the following parameters: TR 3000 ms; TE 30 ms; a flip angle of 80°; slice thickness 2.5 mm; matrix size 128*128; imaging resolution 2*2*2 mm³; interleaved slice order; slice gap 3mm and 127 volumes. One hundred and twenty seven axial slices oriented parallel to AC-PC line were taken, covering the whole brain. Participants were listening to a pure tone during task-based fMRI session. Scan time was 6 minutes and 30 seconds.
- **Arterial spin labelling scan:** A pulsed ASL technique was used to measure the CBF with the following parameters: TR 2000 ms, TE 19 ms, 150 volumes, flip angle=90°, slice thickness= 5 mm, FOV= 256*256 mm, tag saturation time (TI₁)=0.7 s, tag saturation end-time (TI_{1stop})= 1.3 s, time between label and readout (TI₂)=1.4 s, tag-width=10 cm, a 10-mm tag-slice gap and crusher gradients with b=5 mms⁻¹. Scan time was 10 minutes.
- **Diffusion scan:** Diffusion-weighted images were acquired using a T2-weighted spin echo (EPI) sequence implemented with 60 isotropic gradients directions (TR=10100 ms, TE= 106 ms, slice thickness 2mm, field of view (FOV) 256*256 mm², voxel size= 2.1*2.1*2.1 mm, b-factor=1200s/mm³), and 5 volumes with no diffusion weighting (b=0s/mm³). Total acquisition time was 11 minutes.

Measurements obtained from these MRI modalities and statistical analyses performed on these measurements are summarized in table (4.6). Three neuroimaging sessions were undertaken in this PhD thesis: structural, functional and diffusion scans. Structural MRI session aims to study grey matter volume, cortical thickness and subcortical shape appearance differences using T1 weighted MR images. Functional MRI session aims to study auditory perception, functional connectivity and cerebral blood flow perfusion using echo planer images (EPI). Diffusion MRI session aims to study structural connectivity using EPI. Different statistical analysis methods were applied including t-test and correlation analysis using explanatory variables such as age, hearing loss (HL), THI, TFI and tinnitus duration.

Table 4.6: Measurements obtained from neuroimaging sessions including the output from preprocessing and outcome variable.

Neuroimaging sessions	Effects studies	Imaging techniques	ROI	Output from preprocessing	Statistical analysis	Explanatory variables	Outcome variables
Structural scan	VBM	T1w MRI	Whole brain	Segmented GM images	t-tests using GLM Correlation analysis	Age HL thresholds	Whole brain volume
	SBM	T1w MRI	ROI	Spherical maps			Cortical thickness
	Shape / appearance model	T1w MRI	ROI	Vertex displacement maps			Sub-cortical shape differences
Functional scan	Auditory Perception	EPI	Whole brain	GLM		THI scores	BOLD signals
	Functional connectivity	EPI	Whole brain	Group ICA		TFI scores	Functionally distinct networks
	Perfusion	EPI	Whole brain	GLM		Tinnitus duration	CBF difference
Diffusion scan	Structural connectivity	EPI	Whole brain	FA & MD maps		FA&MD difference	

VBM= Voxel Based Morphometry, SBM= Surface Based Morphometry, GM= Grey matter, T1w MRI= T1-weighted MR images, EPI= Echo Planer Images, ROI=Region of interest, GLM=General linear model, ICA=Independent Component Analysis, FA= Fractional Anisotropy, MD= Mean Diffusivity. BOLD= Blood Oxygen Level Dependent, CBF=Cerebral Blood Flow.

4.8 MR Imaging Analysis Techniques:

4.8.1 Structural MRI analysis techniques

4.8.1.1 *Voxel based morphometry (VBM)*

In the VBM analysis, the local grey matter volume is investigated using optimized VBM protocol (Good et al., 2001). This structural GM differences analysis is, which does not require a prior hypothesis of the locations of these possible grey matter volume differences (ROIs). In order to achieve this analysis, structural images are brain extracted and segmented in to GM, WM and CSF images (Douaud et al., 2007). Then, GM images need to be transformed into a standard space using non-linear registration. Next, aligned images needs to be smoothed with Gaussian kernel. Finally, voxel-wise GLM was carried out for comparison across subjects.

Extracting non-brain tissue is carried out using BET toolbox in FSL software (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET>) (Smith, 2002). This step is an automated step to extract non-brain tissues from the MR brain images. It does not require pre-registration or other pre-processing, which is considered as a very robust and accurate method to segment brain from non-brain tissue (Smith, 2002).

Segmentation step is carried out using FAST toolbox in FSL software (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST>). In this step, brain images are segmented into three classifications according to their intensities: grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). This process is fully automated, which is robust, reliable and sensitive to noise (Zhang et al 2001).

Affine (non-linear) registration is carried out using FNIRT toolbox part of FSL toolbox (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT>) (Good et al., 2001) to align all grey matter images into the same stereotactic space using affine registered. This helps to identify the localization of the GM differenced across subjects (Good et al., 2001).

All normalized GM images are smoothed using an isotropic Gaussian kernel with Full Width-Half Maximum (FWHM). This step aims to locally average the data of GM density with surrounding voxel intensity in order to render the data to be more normal distributed (Good et al., 2001).

The final step of VBM analysis is the voxel-wise statistical analysis, which aims to identify the differences of grey matter volume across subjects using permutation test (Winkler et al., 2014) (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise>). It employs the general linear model (GLM) that can be used to apply different tests (t-test, multiple regression). It provides corrected and non-corrected results for multiple comparisons. Locations of significant clusters are identified using FSLView 3.1.8 to localize their entire coordinates on template atlases.

4.8.1.2 Surface based morphometry (SBM)

Surface based analysis is carried out to investigate the influences of tinnitus perception in auditory and non-auditory brain structure. Structural MRI data were analyzed using FreeSurfer software package version 5.3 (<http://freesurfer.net>).

The surface based morphometry analysis consists of several stages. Firstly, the MRI volume data is affine registered to the standard space (MNI 305) in order to compute seed points in later stages (Collins et al 1994). The variation of white

matter intensity is measured by estimating the B1 bias field. The skull and non-brain tissue are extracted using a deformable template model. Voxels were then classified in to grey matter, white matter and CSF based on tissue intensity and neighbor constraints. The brain is separated into two hemispheres based on the location of corpus callosum and pons of the expected standard space (MNI305).

An initial surface is generated for each hemisphere, and then refined to follow the intensity gradients between the white and gray matter (white surface) and between the gray matter and CSF (pial surface). The white and pial surfaces are then overlaid on the original T1-weighted image. Cortical thickness is measured by estimating the distance between the white and the pial surfaces (Fischl and Dale, 2000).

4.8.1.3 Subcortical shape/appearance difference

The shape/appearance differences of brain sub-cortical structures is carried out using FIRST model tool as part of FSL software package (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST>) (Patenaude et al., 2011). It incorporates prior anatomical information via explicit shape model contains 15 different sub-cortical structures (left and right separately) (figure 4.9): accumbens nucleus, amygdala, brain stem, caudate, hippocampus, pallidum, putamen and thalamus.

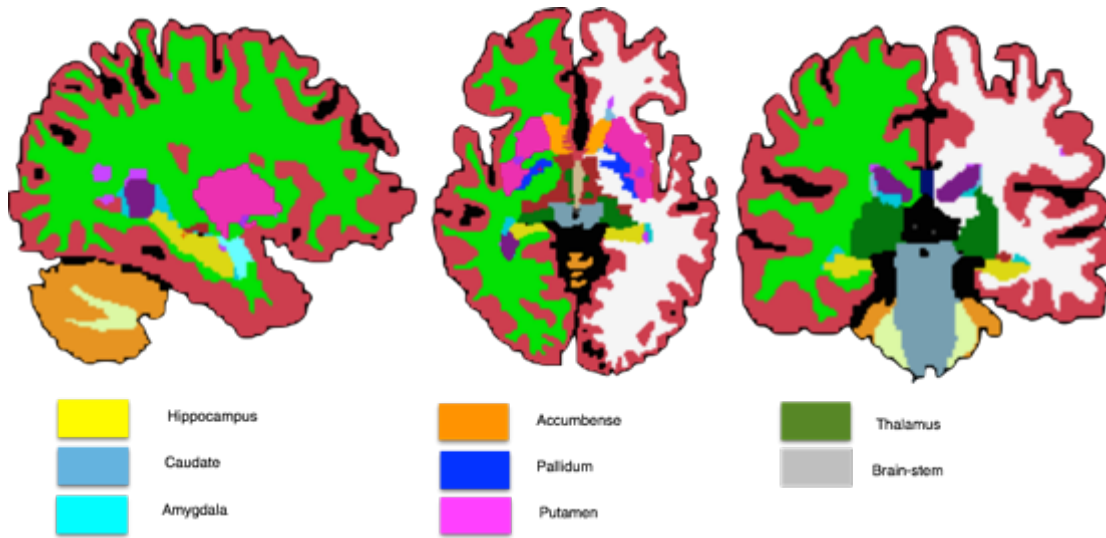


Figure 4.9: Subcortical structures segmentation. Orthographic view for illustration purposes using Freeview.

All structural images (T1 weighted) are affine-registered to a high-resolution 1 mm standard space (MNI 152) using FLIRT toolbox part of FSL software (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT>). This step involves 12 degree of freedom (DOF) affine registration with MNI-space sub-cortical mask.

The model is a 3D mesh that uses prior information of the sub-cortical structures anatomical shape and intensity. It is used to assess the iterative displacement of vertices across subjects. From vertex locations model, the average shape assessment of sub-cortical structures can be assessed using a Bayesian formulation (Patenaude et al., 2011). Sub-cortical intensities are re-scaled to a common range, and then the mode of the intensity in the structure is subtracted. In order to fit the model, the best shape model across the population is selected that can describe the ways in which the structure shape varies most typical over a population.

FIRST models all sub-cortical structures. Boundary correction aims to decide if boundary voxels should belong to the sub-cortical structures or not. This step uses

FAST classification method (segmentation) that ensures neighboring structures do not overlap in sub-cortical images. Vertex analysis measures difference in location (between means of two groups of subjects) using distance along surface normal. This analysis was performed in MNI space.

4.8.2 Functional MRI analysis techniques

Functional MRI (fMRI) can be used to explore a wide range of central nervous system (CNS) functions including information processing in auditory, motor and sensory systems (Cacace et al., 2000). fMRI aims to detect discrete areas of oxygen level change within the brain resulting from neuronal activity (Strainer et al., 1997). The preprocessing steps of fMRI data analysis include motion correction, slice-timing correction, spatial normalization and smoothing.

It is assumed that time course represents a value from single location. Therefore, motion correction is applied using rigid-body transformations in order to remove or eliminate the effect of head motion during fMRI scan (Ashby, 2011). Participants always move during the scan even with padding around head there is still some motion. It is essential to make sure that every voxel for every subject is correspond to a consistent anatomical point for each point in time. Any change of BOLD signals regarding to small motion near strong intensity boundaries can cause noise, which leads to make uncertainty of activated voxels (Ashby, 2011).

Due to volumes are not acquired all at the same time in fMRI scan, slice timing correction is applied using sinc interpolation in order to shift each voxel's time-series by approach fraction of a repetition time (TR), which is relative to the half of

the repetition time (TR) (Ashby, 2011). In this study, functional MR images were acquired in interleaved order (0, 2, 4.... 1, 3, 5...).

Each individual functional MRI scans was co-registered to a high-resolution T1 weighted images scans. More details can be seen in the high-resolution anatomical scan than the fMRI scan. Then, these high-resolution images are aligned to a standard image (MNI 152 average image). These two transformations then are combined, and align the low-resolution fMRI scans to the standard space. So, given voxels of fMRI scan represent and match the anatomical regions of participants' brain (Ashby, 2011).

Smoothness is parameterized as Full Width at Half Maximum (FWHM) of Gaussian kernel that is needed to smooth a white noise random field to roughness, which calculates minimum cluster sizes for significance. Spatial smoothing is carried out to replace voxel value with a weighted average of nearby voxels that is usually weighted with Gaussian kernel (Ashby, 2011). It is similar to interpolation, which aims to reduce noise without reduction valid activation, increase signal to noise ratio (SNR) by removing the high spatial frequencies and improve inter-subject registration. Default setting (5 mm) was used to smooth fMRI data.

For task based fMRI group analysis, inter-session and/or inter-subject random effect components were estimated for mixed-effects variance. Full model design was set up to specify the contrasts between the groups and/or variables regression within a group.

For resting state fMRI group analysis, multisession temporal concatenation was performed to run on the concatenated 2D data matrix. This approach is looking for

common spatial maps pattern of BOLD activation. Each subject has different time series that are extended to higher dimensions. Common activation patterns are estimated in the presence of subject-specific process. Independent component analysis (ICA)-based methodology for multi-subject analysis cannot be run on each subject separately because of correspondence problem (Resting state networks (RSNs) across subjects), and different splitting sometime caused by small change in the data. Group maps of ICA are related back to the individual subjects. Spatial IC maps were regressed into each subjects' maps to find subject-specific time courses in order to find subject-specific spatial ICs associated with groups ICs. Voxel-wise analysis is performed across subjects separately for each original group ICA map to define template maps, estimate subject-wise maps and test subjects differences.

4.8.3 Perfusion MRI analysis techniques

Perfusion scan is an MRI technique aims to assess the perfusion of tissue by blood. There are two MRI techniques to measure the absolute quantification of cerebral blood flow (CBF): arterial spin labeling (ASL) and dynamic contrast-enhanced (DCE). ASL is a non-invasive and non-ionizing MRI technique that aims to measure tissue perfusion using a freely diffusible intrinsic tracer (Petcharunpaisan et al., 2010). In comparison to other perfusion MRI techniques, DCE MRI relies on the signal decreases of T2 or T2* during the first passage of an exogenous endovascular susceptibility contrast agent via the cerebral vasculature (Rosen et al., 1990).

ASL Perfusion data set contains labels and controls images, which were added to produce a time-course of BOLD images, and then subtracted to produce a time-course of cerebral blood flow (CBF) images that were converted into quantitative

CBF maps (unit ml/min/100ml) using a single blood compartment model described by (Parkes and Tofts, 2002). BOLD images were normalized to the standard space, and then the CBF time courses data were written out to the normalized BOLD images. Next, normalized perfusion data were smoothed to 10 mm (FWHM) in order to increase the signal to noise ratio (SNR) in the exported time course. A group-wise analysis was then performed to identify the significant differences of the absolute global CBF.

4.8.4 Diffusion MRI analysis techniques

Diffusion tensor imaging (DTI) is an extension of diffusion weighting imaging (DWI), which measures the velocity and direction of diffusion. In grey matter, diffusion is isotropic (similar in all direction), while white matter diffusion is anisotropic (motion along with fibers). The amount of diffusion occurring in one pixel of MR images is called Apparent Diffusion Coefficient (ADC) or Mean Diffusivity (MD) (Pierpaoli et al., 1996).

In DTI, there are four measurements that are most common used: fractional anisotropy (FA) is quantifying the directional degree; mean diffusivity (MD) is measuring the overall level of diffusion; axial diffusivity (AD) is measuring the axial diffusivity on the primary eigenvector that is parallel to the axons and reflect organization, and radial diffusivity (RD) is measuring the diffusivity on the secondary and tertiary eigenvectors that flow perpendicular to axons and reflects myelination. These measures reflect the density of axons, degree of myelination, averaged fiber diameter and directional similarity of axons (Pierpaoli et al., 1996).

Tract Based Spatial Statistics (TBSS) (Smith et al., 2006) was applied to conduct voxel-wise analysis of whole brain white matter (WM) measurements. Brief summary of TBSS approach described as followings: non-brain tissues are extracted. All individuals FA and/or MD images are aligned into a common registration target using non-linear registration. Next, the mean FA and/or mean MD images are created and thinned to produce mean FA and/or mean MD skeleton that is the center of all tracts common to the group. This skeleton is aligned with each individuals FA and/or MD data, and the results data are fed into voxel-wise statistics. Randomization option (Monte Carlo permutation test) is performed to identify the differences of white matter integrity between two groups.

Eddy current correction is the first step in the DTI analysis. Due to eddy currents in the gradients coils induce stretches and shears in the diffusion weighted images (DWI), this preprocessing is applied to correct spatial distortions caused by the gradients for DTI data. Different directions have different distortions effects. During DTI scan, different strengths (b-values) and directions (b-vectors) are applied. Diffusion raw data contains b=0 images (from 1 to 5), which represent diffusivity status without direction effect. These B0 images have a little distortion effect which looks like T2 weighted images. Eddy current correction is using affine registration to align all directions volumes to the b0 (reference) image.

A tensor is composed of three vectors. The first vector is considered as the longest vector, which points along the principle axis. The second and third vectors are orthogonal to the first. Tensor (DTI) has trouble distinguishing voxels with crossing fibers from isotropic. DTIFIT toolbox aims to estimate a diffusion tensor at each

voxel of the brain. The output is a set of images including fractional anisotropy (FA), mode (MO), mean diffusivity (MD), tensor eigenvalues and vectors.

The aim of non-linear alignment is to achieve initial alignment. Each multiple FA images are aligned into each. This will help to make no change of the fundamental nature of the images during this co-registration and project the FA data into a tract skeleton using intermediate degree of freedom (DoF).

It is very important to identify the real FA images as the target to register every subject to every other subject. This can be achieved by wrap the field by its mean displacement, and choose the target subject with minimum mean distance compared to other subjects.

All subjects' FA images are aligned into the most typical subject that was chosen as the target for alignment. Then, this aligned data is affine transformed into 1*1*1 mm³ standard space (MNI 152). This gives the advantages of convenience for interpretation and display, and no significant interpolation blurring (Smith et al., 2006). Averaging the transformed FA images creates the mean FA image that is locally relatively smoothed and fed into the tract skeleton generation. This aims to represent all tracts to all subjects.

Each subject's aligned FA images are projected onto the mean FA skeleton, which aims to assess residual misalignment between subjects after the initial nonlinear registration. Small misalignment in the direction can have great impact on the final FA statistics (Smith et al., 2006). Voxel-wise statistics analysis is carried out for each point on the common skeleton. GLM is set up across subject to identify the significant local FA differences between study groups.

4.9 Conclusion

In this chapter, participants' details, materials and methods were reviewed in details. Three groups of participants (NH, MH and TIN) underwent the procedures. Different behavior assessments were applied to investigate the influence of hearing loss, handedness, anxiety and depression on tinnitus perception. Also, tinnitus severity was assessed using THI and TFI questionnaires. Different MRI techniques were utilized to identify brain structural and functional alterations in tinnitus patients.

In the next six chapters, I will go through the technical parts of this thesis to investigate the influence of tinnitus perception on (1) the quality of life, (2) brain morphology, (3) brain auditory perception, (4) brain perfusion, (5) brain functional connectivity and (6) brain white matter microstructure integrity.

Chapter 5: An Investigation the Impact of Tinnitus Perception on the Quality of Life

5.1 Introduction

Tinnitus is the sensation of sound in the absence of any external source. Millions of people around the world are affected by tinnitus and it's origin not yet fully understood. The prevalence of tinnitus has been estimated to be between 10-15% of the adult population (Baguley et al., 2013). The majority of tinnitus sufferers have subjective tinnitus that cannot be heard by examiner, but only described by the tinnitus subjects (Baguley et al., 2013). The condition can sometimes have serious psychological impacts on the individual such as dealing with problem, depression and anxiety, low concentration and loss of control (Holmes and Padgham, 2009, Pinto et al., 2010).

One of the tinnitus challenges is that most individuals who experience tinnitus cope well whilst some do not (Eggermont and Roberts, 2004). Various tinnitus questionnaires such as Tinnitus Handicap Inventory (THI) (Newman et al., 1996) and the Tinnitus Functional Index (TFI) (Meikle et al., 2012) have been published that aim to investigate the influence of tinnitus on different impacts of tinnitus sufferer's life such as emotional, functional, claustrophobic, hearing, anxiety and depression. Due to there be no objective tool to diagnose most tinnitus cases, clinical practitioners have been using these questioners widely in order to assess the impact and severity of tinnitus.

There are some psychoacoustic characteristics used to evaluate tinnitus such as tinnitus intensity, frequencies and suppression levels. No association was found between the tinnitus severity and psychoacoustic characteristics (Jastreboff and Hazell., 2004). On the other hand, other studies found the correlation between tinnitus intensity and severity (Holgers et al., 2005, Newman et al., 1994).

The influence of hearing loss on tinnitus severity has been investigated with wide divergent results. It has been demonstrated that the correlation between hearing loss and tinnitus severity is uncertain (Baskill JB, 1992). On the other hand, tinnitus annoyance was linked with hearing loss at low and high frequencies (Weisz et al., 2004, Searchfield et al., 2007). The prevalence of tinnitus is increased with age (Coles, 1984). Tinnitus severity was found not correlated to age in one study (Meric et al., 1998), whereas another study found older male showed higher severity of tinnitus symptoms (Hiller and Goebel, 2006).

As individuals react differently to different symptoms and disorders, this study aims to assess the impact of tinnitus perception on the quality of life of tinnitus sufferer's. This will be justified by taking into account the influence of some behavior variables: age, gender, handedness, anxiety and depression status, hearing loss and tinnitus characteristics such as onset, laterality, loudness and severity.

5.2 Material and methods

5.2.1 Subjects

The ethical application of this study was approved by the National Research committee in North West (Liverpool, UK). Certain inclusion and exclusion criteria were set in this study. Inclusion criteria were as following: age between 30-65 years olds, subjective tinnitus for at least 6 months, and no conductive hearing loss.

34-tinnitus sufferers were recruited in this study with a wide range of hearing loss thresholds and tinnitus severity status. Participants were recruited from announcements in the University of Liverpool's website (20 participants) and referral via Aintree University Hospital NHS Foundation Trust (14 participants).

A post hoc power analysis was conducted using the software package, Gpower (Erdfelder et al., 1996). The sample size of 34 was used for the statistical power analyses and a 9-predictor variable equation (age, gender, handedness, hearing loss, hospital anxiety and depression scale (HADS) scores, tinnitus handicap inventory (THI), tinnitus functional index (TFI), tinnitus duration, tinnitus loudness) was used as a baseline. The recommendation effect sizes used for this assessment were as follows: small ($f_2=0.02$), medium ($f_2=0.15$) and large ($f_2=0.35$) (Cohen, 1977). The alpha level used for this analysis was $P < 0.05$. The post hoc analyses revealed that the statistical power for this study was 0.06 for detecting a small effect, 0.23 for detecting a medium effect, and 0.70 for detecting a large effect. Thus, the sample size was underpowered for detecting small and medium effects, but very close for detecting a large effect (power=0.70).

5.2.2 Audiological examination

Pure tone air conduction audiometry was performed to assess the hearing level of the participants in this study. Audiograms were measured with a calibrated diagnostic audiometer (Amplivox 2160, with Audiocups to reduce noise and permit accurate pure tone audiometry). The pure tones were presented at seven different octave frequencies (0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 kHz), and at different sound intensities that ranged from -10 to 120 dBHL. A consultant audiological physician performed the hearing assessment at the University of Liverpool site in a quiet office with ambient noise levels less than 35dB. Hearing function was defined as the mean hearing loss thresholds, averaged over the seven frequencies tested for both ears.

5.2.3 Behaviour assessments

Handedness was assessed in this study by using Edinburgh Handedness Inventory (EHI) (Oldfield, 1971). In addition, anxiety and depression was screened for all participants by completing Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983).

In order to assess the effect of tinnitus on participants' lifestyle, the tinnitus group completed two tinnitus questionnaires that called THI and TFI. The THI has been used in this study as it has been used widely in clinics, to assess the impact of tinnitus at three sub-scales: emotional, functional and catastrophic subscales. On the other hand, the TFI is a relatively new index, which is currently under validation and examines the impact of tinnitus at eight tinnitus dominants: intrusive, sense of control, cognitive, sleep, auditory, relaxation, quality of life and emotion.

5.3 Results

5.3.1 Demographics findings

The characteristics of the tinnitus participants were listed in table 5.1. This study included 34 tinnitus patients: 20 male (59%), and 14 women (41%). The age range of tinnitus participants was from 30 to 65 years olds. The mean and standard deviation age of tinnitus patients was 48 ± 11 years. The hospital anxiety and depression scale (HADS) showed that 15 tinnitus subjects (44%) have high level of anxiety and depression ($HADS > 10$), while 19 subjects (56%) have low level of anxiety and depression ($HADS \leq 10$). The handedness of tinnitus participants was 28 rights handed (82%) and 6 left handed (18%).

23 tinnitus subjects (68%) are suffering from tinnitus symptoms in both ears (bilateral), while eleven tinnitus subjects (32%) are suffering from tinnitus symptoms in one ear either right or left (unilateral). Furthermore, hearing loss occurred in 23 tinnitus patients (68%), and 11 tinnitus subjects (32%) had normal hearing as measured with pure tone audiometry.

Tinnitus subjects were asked whether they could cope with their tinnitus or not. Half of the tinnitus participants showed that they could cope with their tinnitus. The onset of tinnitus has been categorized into two groups: 5 years or less and more than 5 years. Thirteen tinnitus (38%) subjects have been suffering from tinnitus for five years or less, while twenty-one (62%) have been suffering from tinnitus for more than five years. Tinnitus participants described tinnitus sounds as whistling (35.1%), hissing (27.7%), ringing (12.9%), roaring (9.2%), pulsating (5.6%), high pitch noises (5.6%), clanging (1.85%) and chirping (1.85%).

Table 5.1: The demographics and characteristics of tinnitus participants

Variables	Tinnitus population
Sample size	34
Gender	
Male	20 (59%)
Female	14 (41%)
Handedness	
Right handed	28 (82%)
Left handed	6 (18%)
Anxiety and depression	
Mean \pm SD	10.2 \pm 6.7
Yes	15 (44%)
No	19 (56%)
Hearing level	
Mean \pm SD	24.8 \pm 16.7
Normal hearing	11 (32%)
Hearing loss	23 (68%)
Severity	
Coping	17 (50%)
Suffering	17 (50%)
Laterality	
Unilateral	11 (32%)
Side	7 (Left) & 4 (Right)
Bilateral	23 (68%)
Duration	
Mean \pm SD	14.2 \pm 15.6
\leq 5 years	13 (38%)
$>$ 5 years	21 (62%)
Tinnitus descriptions	
Whistling	35.1%
Hissing	27.8%
Ringing	12.96%
Roaring	9.26%
Pulsating	5.6%
High pitch noises	5.6%
Clanging	1.85%
Chirping	1.85%

5.3.2 Audiological findings

Clinical pure tone audiometry was performed over seven frequencies from 0.5 to 8.0 kHz, and the mean and standard deviation for each ear is shown in figure 5.1. Normal hearing was defined as pure tone hearing thresholds of 20 dB or better at these frequencies, and hearing loss was defined as pure tones hearing loss thresholds of 25 dB or worse at any frequency. Normality checks were carried out on the residuals, which were approximately normal distributed. A repeated measure ANOVA with a Greenhouse-Geisser correction showed that mean hearing loss differed significantly between frequencies [$F(3,31)=19.32$, $P < 0.001$] and ears tested [$F(1,33)=5.50$, $P=0.025$].

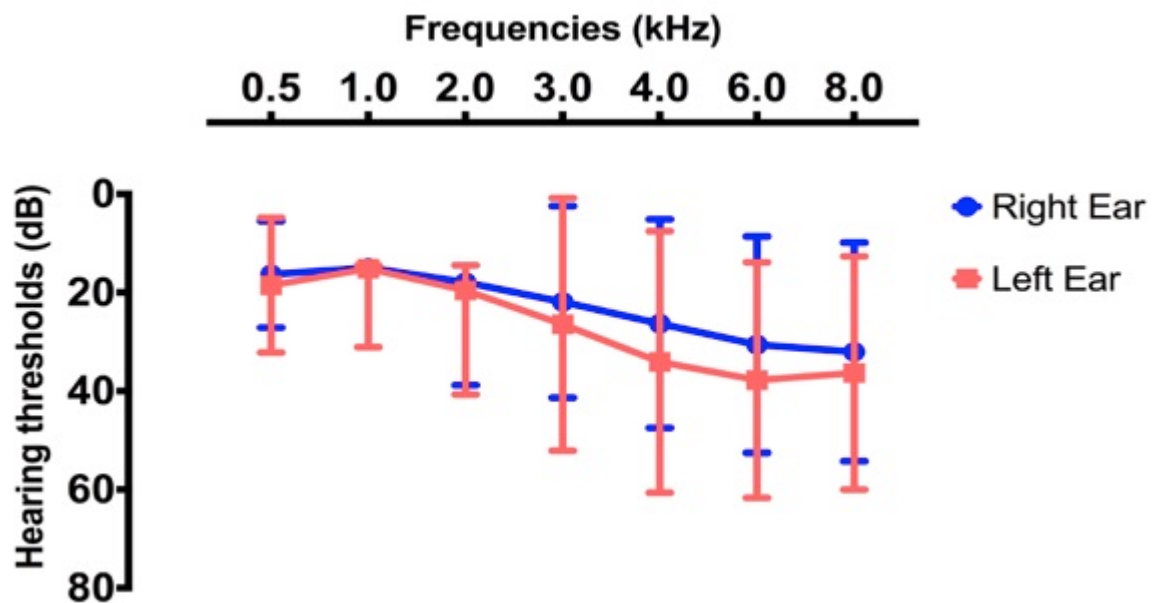


Figure 5.1: The mean and standard deviation of right and left ear audiograms. Y-axis is the hearing thresholds (dB) and x-axis is the tested frequencies (kHz).

The left ear audiograms showed higher hearing loss thresholds than the right ear at 3, 4, 6 and 8 kHz, however, it did not reach significant level ($P \geq 0.05$) (table 5.2). Furthermore, no significant different was found of the hearing loss level between gender (male and female), handedness (right and left handed), anxiety and

depression subgroups (yes and no), tinnitus-severity groups (coping and suffering), tinnitus laterality (bilateral and unilateral) and tinnitus onset groups (5 years or less and more than 5 years) (table5.1). The hearing loss threshold was found a borderline significant level ($P=0.06$) between left handed (67 ± 26) and right handed (44 ± 23) tinnitus participants (table 5.2). Tinnitus subjects with hearing loss group (50 ± 11 years old) were significantly older ($P=0.02$) than tinnitus subjects with normal hearing group (41 ± 9 years old) (table 5.2).

Table 5.2: T-test results of the effect of different variables (gender, handedness, hearing loss, tinnitus bothering, tinnitus laterality and tinnitus duration on hearing acuity.

Variables	Right ear	Left ear	Average	HLT
Gender				
Male (n=20)	27±18	29±18	28±18	51±23
Female (n=14)	17±13	23±19	20±15	43±27
<i>P</i> -value	0.063	0.36	0.16	0.32
Handedness				
Right (n=28)	22±14	25±17	23±14	44±23
Left (n=6)	27±25	37±24	32±24	67±26
<i>P</i> -value	0.64	0.27	0.42	0.06
Anxiety and depression				
Yes (n=15)	18±11	23±13	20±11	45±21
No (n=19)	26±18	29±19	28±20	51±28
<i>P</i> -value	0.12	0.3	0.19	0.47
Hearing loss				
Yes (n=23)	29±16	34±18	31±16	61±20
No (n=11)	10±5	11±6	11±5	22±5
<i>P</i> -value	0.001	0.0003	0.0003	0.0000005
Severity				
Coping (n=17)	25±19	26±22	25±20	47±28
Suffer (n=17)	21±13	28±15	24±13	49±22
<i>P</i> -value	0.5	0.8	0.86	0.87
Laterality				
Unilateral (n=11)	24±18	25±21	25±2	45±3
Bilateral (n=23)	20±10	30±11	25±9	54±2
<i>P</i> -value	0.45	0.44	0.97	0.29
Onset				
≤ 5 years (n=13)	18±12	22±13	20 ± 12	40±17
> 5 years (n=21)	26±18	30±21	28±19	53±28
<i>P</i> -value	0.2	0.2	0.15	0.12

5.3.3 Tinnitus severity findings

The impact of tinnitus perception on the quality of life was assessed using THI and TFI. The overall results of THI and TFI are summarized in table 5.3 and figure 5.2. In THI questionnaire, the percentages of tinnitus subjects according to the impact of tinnitus in their life are 41.2% (slight impact), 32.4% (mild impact), 17.6% (moderate impact) and 8.8% (catastrophic impact). In TFI inventory, 44.2% showed a mild impact, 23.5% of tinnitus subjects showed a slight influence and 32.4% showed a sever impact.

Table 5.3: The impact of tinnitus perception results using THI and TFI.

Tinnitus inventories	Mean scores levels	Prevalence Percentage
THI	Slight	41.2%
	Mild	32.4%
	Moderate	17.6%
	Catastrophic	8.8%
TFI	Mild	44.2%
	Moderate	23.5%
	Sever	32.4%

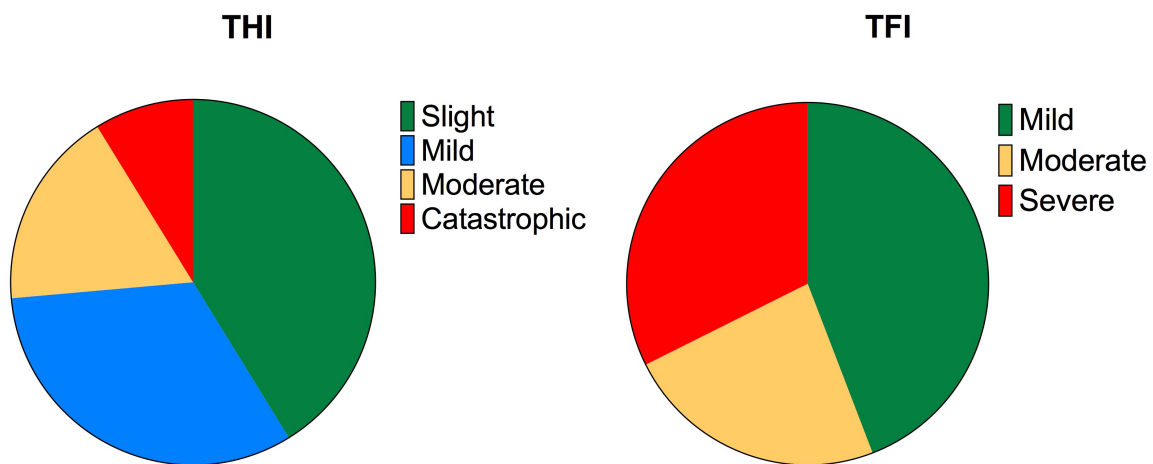


Figure 5.2: The impact of tinnitus perception results using THI and TFI.

The influence of tinnitus perception on the eight TFI dominates assessed in this study (table 5.4). The intrusive subscale was found the highest (58%) distributed TFI dominate, while the quality of life subscale (20%) was the lowest distributed TFI dominate. Half of tinnitus participants showed the reduced sense of control. Also, sleep disturbance was assessed in tinnitus participants using sleeping subscale dominant in TFI inventory, which was found that 41% (15 out of 34) of tinnitus subjects have sleep distributed. Relaxation and enjoyment was found interfering in 28% of tinnitus participants. The auditory difficulty attributed to tinnitus was found in 35% of tinnitus subjects. Attention and focusing ability was found influenced in 26% of tinnitus subject. 23% of tinnitus participants thought that tinnitus could increase their anxiety and depression levels.

Table 5.4: The mean (M), standard deviation (SD) and the distributed percentage for each TFI dominant. Distributed percentage was determined as the overall score of each dominant exceed the scale.

TFI dominates	Conceptual content	TFI score (M±SD)	Distributed percentage (%)
Intrusive	Unpleasantness, intrusiveness, persistence	15.7±6.8	58%
Sense of control	Reduced sense of control	14.5±7.1	50%
Sleep	Sleep disturbance	10.8±9.6	41%
Relaxation	Interference with relaxation	14.1±8.1	38%
Auditory	Auditory difficulties attributed to tinnitus	11.7±8.5	35%
Cognitive	Cognitive interference	10±8.4	26%
Emotional	Emotional distress	7.76±8.5	23%
Quality of life	Quality of life reduced	10.1±0.7	20%

No significant difference of the impact of tinnitus perception on the quality of life was found between gender (male and female), handedness (right and left handed), hearing loss levels (normal hearing and hearing loss) and the duration of tinnitus (5 years or less and more than 5 years). Tinnitus participants with hearing loss (50 ± 11 years) were found significantly older ($P=0.02$) than tinnitus participants with normal hearing (41 ± 10 years). The TFI score was found significantly higher ($t=2.55$, $P=0.01$) in the unilateral tinnitus group (55 ± 23) compared to the bilateral tinnitus group (30 ± 18) (figure 5.3). Suffering group showed higher impact of tinnitus perception than coping group in both inventories THI and TFI (table 5.5).

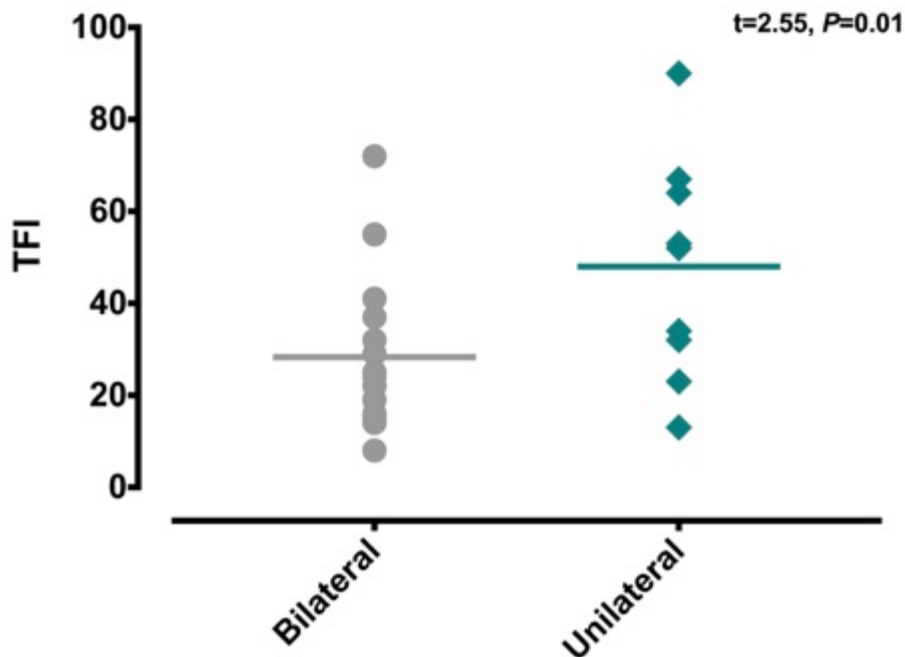


Figure 5.3: Boxplot charts showed the effect of tinnitus laterality on tinnitus severity.

Table 5.5: T-test results of the effect of different variables (gender, handedness, hearing loss, tinnitus bothering, tinnitus laterality and tinnitus duration on tinnitus severity).

Variables	Age (Years)	HADS (Scores)	THI (Scores)	TFI (Scores)
Gender				
Male (n=20)	48±13	11±7	29±26	38±25
Female (n=14)	46±9	9±6	31±22	38±21
<i>P</i> -value	0.6	0.5	0.88	0.96
Handedness				
Right (n=28)	46±11	10±7	29±23	37±23
Left (n=6)	51±12	11±3	34±31	44±24
<i>P</i> -value	0.37	0.5	0.71	0.5
Hearing loss				
Yes (n=23)	50±11	9±5	27±21	38±22
No (n=11)	41±10	12±9	35±29	38.3±26
<i>P</i> -value	0.02	0.2	0.43	0.97
Bothering				
Coping (n=17)	43±8	9±6	15±7	21±9
Suffer (n=17)	50±13	12±7	45±26	55±21
<i>P</i> -value	0.06	0.22	0.00009	0.000002
Laterality				
Unilateral (n=11)	51±10	11±5	41±25	55±23
Bilateral (n=23)	45±11	10±7	25±22	30±18
<i>P</i> -value	0.14	0.7	0.09	0.007
Duration				
≤ 5 years (n=13)	44±12	12±4	36 ± 32	42±30
> 5 years (n=21)	48±11	9±7	26±17	35±18
<i>P</i> -value	0.35	0.2	0.23	0.4

Multivariate regression analysis entering tinnitus participants' age, HADS scores, hearing loss average, hearing loss thresholds and tinnitus duration variables confirmed a significant influence of the quality of life measured by THI and TFI (table 5.6). The correlation analysis between variables (age, HADS, hearing loss, tinnitus onset, THI and TFI) was assessed (table 5.7), and found the followings: a significant positive correlation between subjects' ages and hearing loss ($r=0.48$, $P<0.0001$) (figure 5.4), no significant correlation between hearing loss and tinnitus duration ($r=0.16$, $P=0.40$) (figure 5.4) and a significant positive correlation between HADS (anxiety and depression) and tinnitus severity: THI ($r=0.57$, $P=0.0003$) and TFI ($r=0.48$, $P= 0.003$) (figure 5.5).

Table 5.6: Summary of multivariate regression analysis for variables predictions among THI and TFI (N=34). Asterisks indicate a significant effect of variables on the constants (THI and TFI).

Constant	Variables	β	R^2	ΔR^2	F	P
THI	Age	0.41	0.37	0.27	3.97	0.012*
	HADS	1.92				
	HL	-0.46				
	Onset	-0.20				
TFI	Age	0.51	0.29	0.19	2.86	0.043*
	HADS	1.63				
	HL	-0.24				
	Onset	-0.24				

Table 5.7: Summary of multivariate regression analysis for variables predictions among THI and TFI (N=34). Asterisks indicate a significant effect of variables on the constants (THI and TFI).

	THI	TFI	Age	HADS	HL	Duration
THI						
TFI	.907*					
Age	.133	.229				
HADS	.574*	.488*	.000			
HL	-.013	-.135	.687**	-.069		
Duration	-.168	-.135	.141	-.045	.160	

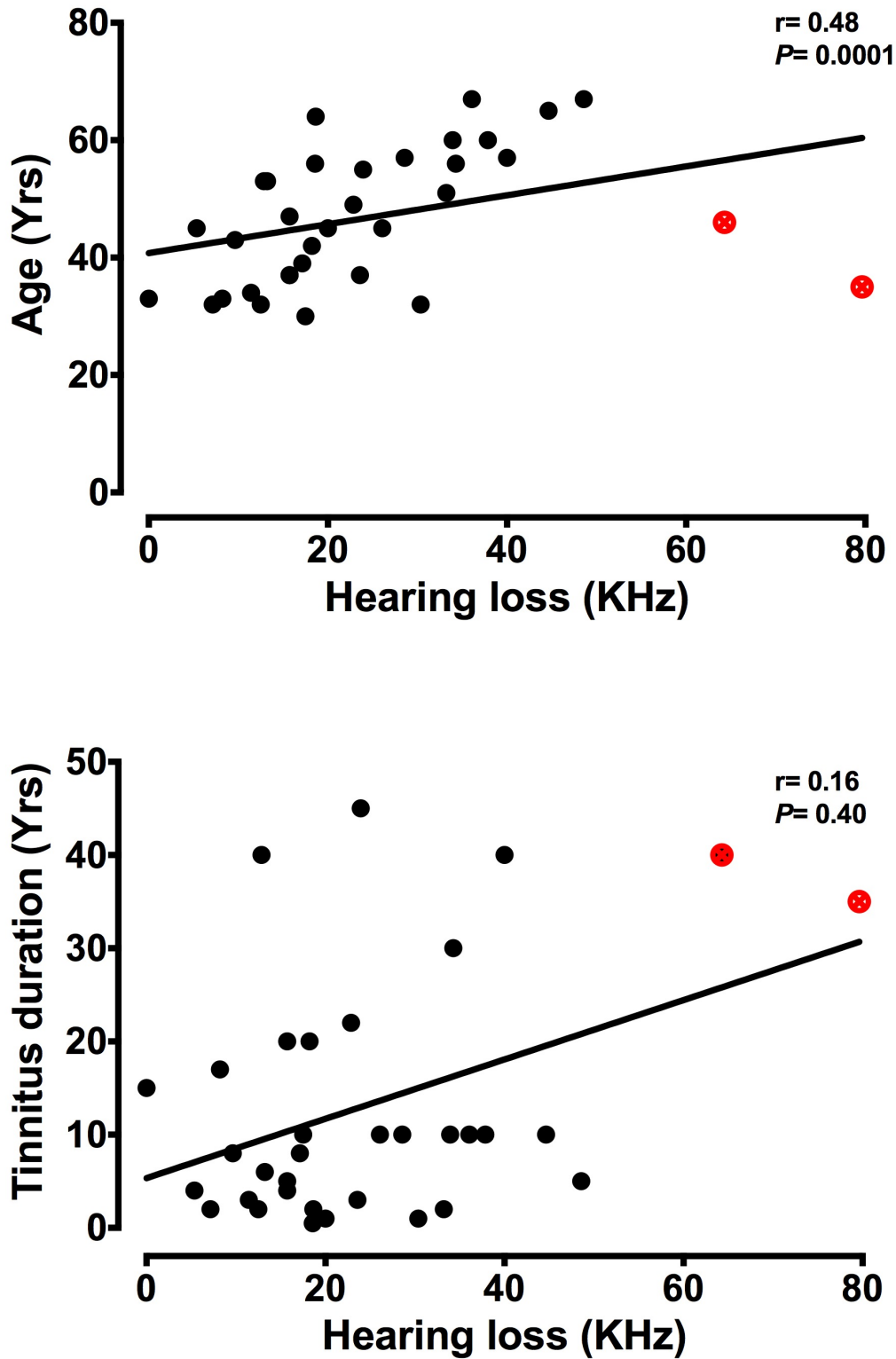


Figure 5.4: The relationship between hearing loss average and age (*Top graph*), hearing loss and tinnitus duration (*Bottom graph*). The hearing loss of two tinnitus subject (P14 and W01) were excluded (highlighted in red) due to their hearing loss were outliers ($HL > \text{mean} \pm 3*SD$) (highlighted in red).

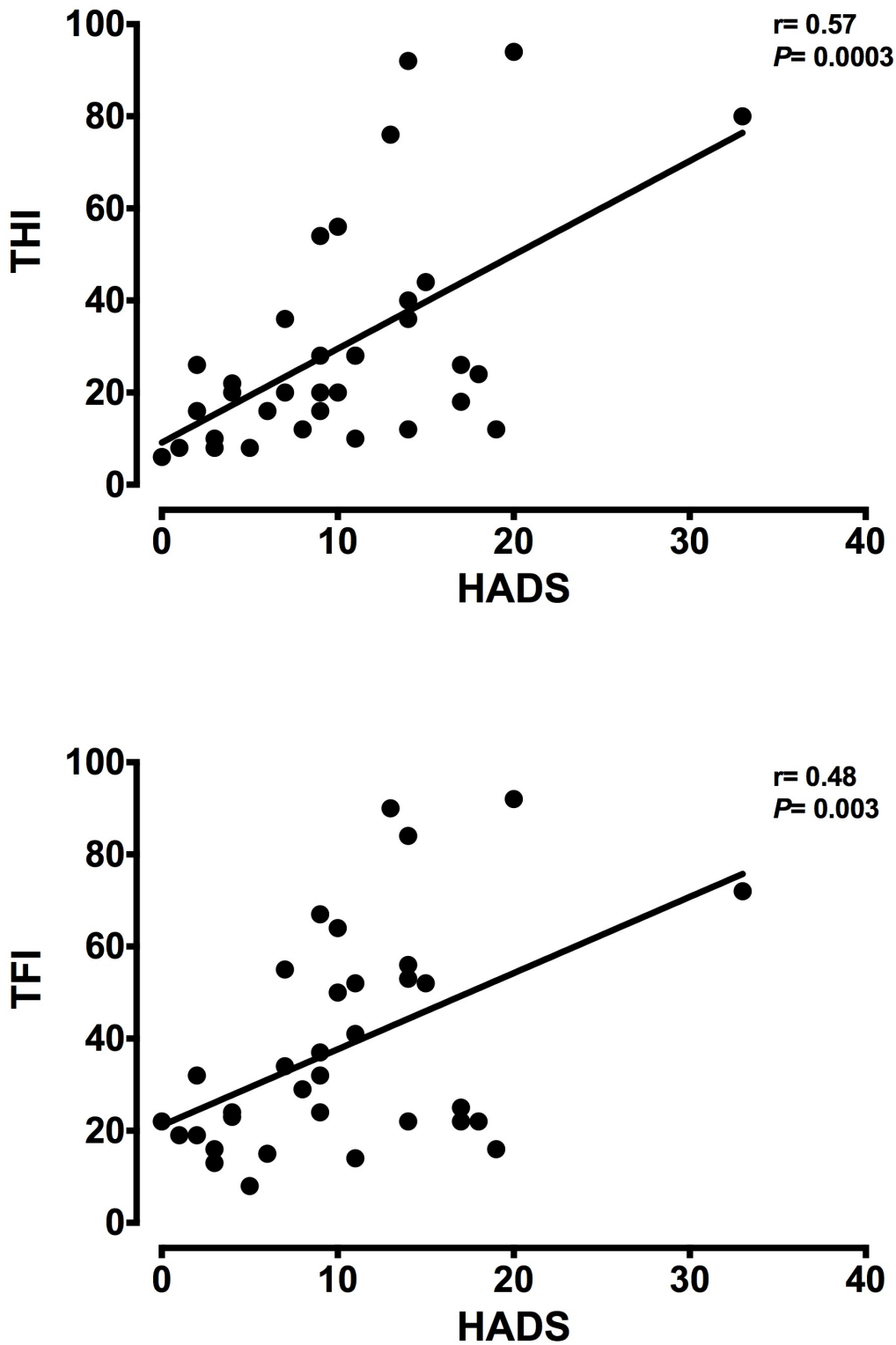


Figure 5.5: Positive significant correlations were identified between Hospital Anxiety and Depression Scale (HADS) and Tinnitus Handicap Inventory (THI) scores, and Tinnitus Function Index (TFI).

5.4 Discussion

This study aims to investigate the effect of tinnitus perception on the quality of life using pure tone audiometry (PTA), hospital anxiety and depression scale (HADS), tinnitus handicap inventory (THI) and functional index (TFI).

5.4.1 Results summary

Results of the present study revealed as the followings: 1)- tinnitus subjects with hearing loss were significantly older than tinnitus subjects with normal hearing, 2)- unilateral tinnitus patients showed a significant higher impact measured by TFI compared to the bilateral tinnitus patients, 3)- a significant positive correlation between subjects' ages and hearing loss, 4)- a significant positive correlation between hearing loss and tinnitus duration, 5)- a significant positive correlation between HADS score (anxiety and depression) and tinnitus severity measured by THI and TFI.

5.4.2 Tinnitus prevalence and gender

In this study, it was found that the prevalence of tinnitus in male (n=20) is more than in female (n=14) that is consistence with other studies (Alsanosi, 2011). On the other hand, another study of tinnitus prevalence was found lower in females compare to males under 75 years olds (Møller, 2011a). The link between gender and tinnitus severity was seen variables as some studies found women showed higher annoyance scores compared to male (Davis, 1983), while others have shown the opposite (Holgers et al., 2005) (Lockwood et al., 2002) or no correlation (Pinto et al., 2010, Meric et al., 1998). In our study, no significant difference between males and females in terms of hearing loss and tinnitus severity was identified.

5.4.3 Tinnitus prevalence and age

The prevalence of hearing loss and tinnitus are associated with age. However, it is unknown whether the age factor could play an important role in tinnitus severity. Some studies have suggested that there is no correlation between tinnitus severity and age (Pinto et al., 2010, Meric et al., 1998); however, Hiller and Goebel found a positive correlation between tinnitus severity and age (Hiller and Goebel, 2006). In our study, we found that suffering tinnitus group is older than coping tinnitus group but this different did not reach significant different ($P=0.06$). In correlation analysis, no significant correlation was found between participants' ages and the scores of THI or TFI.

5.4.4 Tinnitus perception and hearing loss

Hearing loss is considered as one of the major risk factor of tinnitus perception. (Baguley et al., 2013). The link between tinnitus perception and hearing loss is variable (Alsanosi, 2011, Savastano, 2008). In our study, we found nearly two-third of the tinnitus participants had some degree of hearing loss, while one-third had normal hearing that is nearly consistent with a large epidemiology study (Nondahl et al., 2002). A remarked effect of hidden hearing loss was found on the auditory brain that might be sufficient to cause tinnitus (Schaette and McAlpine, 2011).

5.4.5 Tinnitus perception and the quality of life

The influence of tinnitus perception on the quality of life was assessed using two questionnaires: (THI) and (TFI). There are no significant differences determined in the THI and TFI scores between tinnitus participants with normal hearing and tinnitus participants with hearing loss, which has been found as well in these

previous studies (Lim et al., 2010, Pinto et al., 2010, Savastano, 2008, Hallberg and Erlandsson, 1993). This may suggest that there is no association between hearing loss and tinnitus severity.

5.4.6 Tinnitus perception and duration

We detected that recent onset tinnitus can have a stronger negative impact of tinnitus on the quality of life (at least initially), but the effect does not reach the significant level ($P \geq 0.05$). Participants with tinnitus duration for less than 5 years showed a higher impact of tinnitus in their life than participants with tinnitus duration for more than 5 years. It seems that tinnitus participants need more time to learn how to cope with tinnitus symptoms. Again, the affect did not reach a significant level, which may require a larger sample size that has been reported in this study (Alsanosi, 2011).

5.4.7 Tinnitus perception and laterality

Tinnitus laterality was not found to play a significant role on tinnitus severity (overall THI score) (Alsanosi, 2011). This study only used THI to identify the impact of tinnitus on the quality of life. In our study, we also did not identify a significant difference of THI score between unilateral and bilateral tinnitus subjects. However, unilateral tinnitus participants showed a significant higher negative impact of tinnitus (Overall TFI score) than bilateral tinnitus subjects. TFI enclose more dominants subscales than THI, which may identify more clearly and deeply the influence of tinnitus on the quality of life.

5.4.8 Study limitations

The main limitation of this study was a small sample size and we need a larger population including younger and older ages as well as tinnitus subjects with wide range of hearing loss.

Hearing loss was examined up to 8 kHz that is used in clinics. However, hidden hearing loss could be identified at higher frequencies such as 12 or 16 kHz (Schaette and McAlpine, 2011). Because of the subjectivity of tinnitus, questionnaire inventory is the only way to estimate the influence of tinnitus on the quality of life. In addition, different variables should be taken into account such as medical history, medications in use, socio-demographic, occupation and education levels.

5.5 Conclusion

This research study showed the impacts of tinnitus perception on the quality of life. The ageing factor was found to be associated to hearing acuity in tinnitus population. Also, tinnitus laterality seems playing an important role on tinnitus severity. No association was found between hearing loss and tinnitus severity. Audiometry, interview and self-assessment questionnaires are considered the only assessment methods to assess the influence of tinnitus on a participants' life. Therefore, it is essential to develop an inventory that can be used to track the progress of tinnitus treatment in the future.

Chapter 6: Morphological Alterations of Cortical and Subcortical Structures in Tinnitus

6.1 Introduction

Magnetic resonance-based morphometry is a window into the structural plasticity of the brain (May and Gaser, 2006). In the past, brain structure morphology was based on examining post-mortem cases, which requires a skilled radiologist to assess brain structures morphology manually that is time consuming. Recently, brain structure investigation in-vivo has been possible invasively by magnetic resonance imaging (MRI) technique that has the ability to distinguish brain tissue types and can provide quantitative information to assess brain morphology changes using high-resolution T1-weighted images.

There are three morphometric techniques, namely, voxel-based morphometry (VBM), surface-based morphometry (SBM) and vertex based morphometry, which have been used widely to investigate the influence of psychological and neurological disorders on brain structures by making comparisons between normal health and patients groups.

VBM is one of the most common applied techniques, which is based on the segmentation of MR images into the main brain tissue types: grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). VBM assessed the grey matter distribution for each individual brain at the level of each voxel. SBM has several advantages over using volumetric analysis as it has been shown that the brain surface meshes increase the accuracy of brain registration (Desai et al., 2005). SBM

analyses cortical surface by computing and calculate surface parameters such cortical thickness, surface area and gyrification indexes. Vertex-based morphometry is using a univariate test at each vertex to measure the differences in locations (between means of two groups of subjects) using distance along surface normal. These morphometric techniques mentioned above have great interests in neuroscience, neurology and psychology communities to understand normal brain development; such as handedness (Herve et al., 2006), intelligence (Haier et al., 2004), age (Good et al., 2001), and gender (Davatzikos and Resnick, 1998) and the pathophysiology mechanisms of neurological and psychological disorders such as autism (Libero et al., 2014), schizophrenia (Douaud et al., 2007) and semantic dementia (Mahoney et al., 2011).

To the best of our knowledge, no neuroimaging study have been combined these three morphometric techniques (VBM, SBM and vertex-based morphometry) to investigate brain structural morphometry in the tinnitus population with hearing loss. However, few studies have used VBM technique to investigate the alteration of grey matter volume in tinnitus patients, which reported GM changes in prefrontal cortex (PFC) (Melcher et al., 2013), auditory cortex (AC), thalamus (Schneider et al., 2009), and insula (Schecklmann et al., 2013). Cortical thickness analysis was performed by (Aldhafeeri et al., 2012a, Leaver et al., 2012), and found that tinnitus sufferers have thinner cortices at frontal and auditory brain regions. To the best of our knowledge, no previous studies have examined the effect of tinnitus perception on subcortical shape appearance differences.

The aim of this study is to identify the neuroanatomical correlate of tinnitus using magnetic resonance imaging. The correlation between some psychoacoustics and tinnitus characteristics and brain structural changes were examined. Different morphometric techniques were applied: VBM, SBM and vertex based morphometry in order to estimate grey matter volume, cortical thickness, and subcortical shape analysis respectively.

6.2 Materials and Methods

6.2.1 Subjects

This study has been approved from the National Research Ethics Services (NRES) Committee North West-Liverpool Central. Data was collected from three well-matched groups: 20 subjects in the normal hearing (NH) group, 20 subjects the mild to moderate hearing loss (MH) group and 26 subjects in the tinnitus with hearing loss (TI) group. Participants' characteristics were listed in table 6.1. Certain inclusion and exclusion criteria have been set for this study to recruit homogeneous groups of individuals who are in healthy conditions, have similar etiologies and matching in terms of age and genders.. All participants signed a consent form in the first visit prior audiometry and MRI scan as they are agreed to take part in this study.

6.2.2 Audiometry examination

Clinical pure tone audiometry was performed for each subject to check hearing loss thresholds at seven different frequencies: 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 kHz. According to participants' hearing test results, Three adults groups have been

recruited in this study: 20 subjects with normal hearing (NH), 20 subjects with mild to moderate hearing loss without tinnitus (MH) and 26 tinnitus patients with mild to moderate hearing loss (TI) groups. These three groups are matched in terms of age, gender, handedness and anxiety and depression scores.

6.2.3 Behaviour assessments

Handedness was assessed in this study using Edinburgh Handedness Inventory (EHI). In addition, anxiety and depression was measured for all participants using Hospital Anxiety and Depression Scale (HADS). In order to assess the effect of tinnitus on participants' lifestyle, tinnitus participants were asked to completed two tinnitus questionnaires: Tinnitus Handicap Inventory (THI) and Tinnitus Functional Index (TFI). In addition, tinnitus participants were asked about tinnitus duration, laterality and sounds descriptions

6.2.4 Anatomical imaging session

Using a Siemens 3T Trio (Siemens, Erlangen, Germany) with a standard 8 channels head coil performed in this study. In order to control head movement during the scan, we used foam padding and head resistance. Structural images were acquired to perform spatial normalization and localization using 3D a modified driven equilibrium Fourier transform (MDEFT) sequence. The acquisition parameters were as follows: TR/TE 7.92/2.48 ms, 176 volumes, slice thickness 1.00 mm, FOV 256*256 mm² and scan time 12 min 51 s.

6.3 Anatomical investigation methods

6.3.1 Quantitative measurement of Brain size

A quantitative measurement of brain size changes is an important clinical imaging tool to identify brain atrophy state and rate. In the current study, brain size was estimated using SIENAX toolbox (part of FSL software) (Smith et al., 2002) by identifying GM, WM and intracranial volumes (ICV). Non-brain tissues were removed using brain extraction (BET) FSL's toolbox. Then, brain tissue images were registered to the standard space (152 MNI). The standard brain image mask is transformed into the original image space and applied to the brain image in order to ensure that no artifact such as eyeballs is involved from the original brain extraction. The masked brains were segmented into GM, WM and CSF tissues using FAST toolbox. Finally, the volume of GM and ICV were estimated separately in cm³ that were imported to SPSS software (Version 22) for further analysis.

6.3.2 Voxel-based morphometry

Voxel-based morphometry is an automated morphometrical methods that aims to identify the structural brain differences based on voxel-wise comparisons of local volume and concentration of grey mater (GM) and white matter (WM) between different groups. It can be applied as whole brain and a region of interest (ROI) analyses: the former does not require a prior hypothesis, while the latter examined specific brain regions. It provides a comprehensive assessment of the anatomical distribution of specific brain tissue.

In the current study, structural raw data was analyzed with FSL-VBM version 5.0 ([http:// www.fmrib.ox.ac.uk/fsl/fslvbm/index.html](http://www.fmrib.ox.ac.uk/fsl/fslvbm/index.html); Oxford University, Oxford, UK),

a voxel-based morphometry analysis tool (Douaud et al., 2007) that is using an optimized VBM protocol (Good et al., 2001). Brain extraction is the first step to remove non-brain tissue from structural images (Smith, 2002). Then, brain images were segmented into three main brain tissues: GM, WM and CSF. GM images using FMRIB's automated segmentation tool. Next, the segmented GM images were co-registered to a standard space (152 MNI) using a 12-parameter affine transformation accomplished with FMRIB's linear image registration tool (FNIRT) (Andersson et al., 2007). In order to create the study specific GM template, the co-registered images were averaging and flipped along the x-axis using FMRIB's non-linear image registration tool (FNIRT). All native GM images were registered (non-linear) to the study specific GM template, and modulated in order to correct the local expansion and/or contraction caused by non-linear component of the spatial transformer. Next, the modulated GM images were smoothed with an isotropic Gaussian kernel with a sigma of 3mm (full width at half-maximum = 7 mm).

Group comparisons and regression analyses were performed to examine the differences of GM volume between groups, and relationships between GM volume changes across the whole brain and some tinnitus and acoustic characteristics such as tinnitus severity and hearing loss thresholds respectively by applying permutation-based non-parametric testing (5,000 permutations) that is corrected for multiple comparisons. Significant clusters were defined by using threshold-free cluster enhancement (TFCE).

6.3.3 Surface-based morphometry

Surface-based morphometry (SBM) was analyzed using Freesurfer software version 5.3. It is an automated procedure derives morphometric measures from geometric models of the cortical surface.

High-resolution structural MR images were corrected for intensity bias. Grey and white matter surfaces were reconstructed by segmenting corrected T1-weighted images into GM and WM. The values of pial surface area, thickness, gyrification indexes were calculated based on the vertex of reconstructed surfaces. These data were then smoothed using 10 mm FWHM Gaussian kernel in surface-space.

Whole brain analyses were performed included two-tailed t-test, ANOVA analyses and correlation analyses. Two-tailed t-test (Student's t) was applied to identify group differences of morphometric measurements between controls and tinnitus patients. ANCOVA analyses were performed to measure differences between groups that are controlled by specific covariates: participants' ages, gender, mean hearing loss (PTA) and the combined anxiety and depression scores (HADS). Correlation analyses (Pearson's r) aim to identify the association between tinnitus characteristics (tinnitus duration and severity) and cortical morphological changes.

6.3.4 Subcortical shape/appearance difference

The shape/appearance differences of brain sub-cortical structures were carried out using FIRST model tool as part of FSL software package (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST>) (Patenaude et al., 2011). It incorporates prior anatomical information via explicit shape model, which contains

15 different sub-cortical structures (left and right separately): accumbens nucleus, amygdala, brain stem, caudate, hippocampus, pallidum, putamen and thalamus).

All structural images (T1 weighted) were affine-registered to a high-resolution 1 mm standard space (MNI 152) using FLIRT toolbox part of FSL software (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT>). This step involves 12 degree of freedom (DOF) affine registration with MNI-space sub-cortical mask.

The model is a 3D mesh that uses prior information of the sub-cortical structures anatomical shape and intensity. It is used to assess the iterative displacement of vertices across subjects. From vertex locations model, the average shape assessment of sub-cortical structures can be assessed using a Bayesian formulation (Patenaude et al., 2011). Intensity is then sampled along the surface normal and stored. Sub-cortical intensities were re-scaled to a common range, and then the mode of the intensity in the structure is subtracted. In order to fit the model, the best shape model across the population was selected that can describe the ways in which the structure shape varies most typical over a population.

FIRST models all sub-cortical structures. Boundary correction aims to decide if boundary voxels should belong to the sub-cortical structures or not. This step uses FAST classification method (segmentation) that ensures neighboring structures do not overlap in sub-cortical images. Vertex analysis is using a university test at each vertex to measure difference in location (between means of two groups of subjects) using distance along surface normal. This analysis was preformed in MNI space. Fractal dimensions (FD) were estimated for each subject in each subcortical structure in order to investigate the vertex displacement of subcortical structures.

Positive values of FD indicate the outward vertex displacement (expansion), whereas negative values indicate the inward vertex displacement (contraction).

6.4 Results

6.4.1 Demographics

Three participants groups were included in this study (table 6.1). One-way ANOVA was applied to identify the group differences. No significant difference was found between three groups in their ages ($F=0.46$, $P=0.63$), handedness ($F=0.6$, $P=0.56$), and anxiety and depression scores ($F=0.56$, $P=0.57$). Gender distribution was found not significant between the MH and TI groups ($P=0.12$), and between the NH and MH groups ($P=0.45$), while a significant difference of gender distribution was identified between the NH and TI groups ($p=0.01$). Therefore, I controlled the effect of gender in all the comparison between the NH and TI groups.

In the tinnitus group, the number of women subjects is smaller than men subjects that may reflect the prevalence of tinnitus in the general population. Out of 26 tinnitus subjects, half of participants (13 subjects) fell into the 'coping' group, half into 'suffering' group. No significant difference between tinnitus coping and suffering subgroups in terms of age ($t=-1.2$, $P=0.23$), gender ($t=-1.08$, $P=0.29$), hearing loss ($t=-0.27$, $P=0.79$) and tinnitus duration ($t=-1.71$, $P=0.10$). Tinnitus laterality was assessed, and found that 16 subjects have bilateral tinnitus and 10 subjects have unilateral tinnitus. No significant difference between two groups in terms of age ($t=-1.6$, $P=0.12$), gender ($t=-0.33$, $P=0.74$), hearing loss ($t=-1.08$, $P=0.28$), anxiety and depression ($t=-0.39$, $P=0.70$), and tinnitus duration ($t=0.34$,

P=0.73). The duration of tinnitus was found between 6 months and 30 years (9 ± 8 ; mean \pm SD). The impact of tinnitus on quality of life was examined using THI and TFI, which was range between slight to claustrophobic with the mean of the THI 26 ± 19 (mean \pm SD) and the TFI 36 ± 21 (mean \pm SD).

Table 6.1: Participants' characteristics for each group

Characteristics	NH	MH	TI
N	20	20	26
Age (yrs)			
Range	(30-63)	(30-65)	(30-65)
M \pm SD	43 ± 9	47 ± 10	45 ± 12
Gender	6 /14	9/11	18/8
M/F			
Handedness			
R/L	19/1	19/1	21/5
H.A.D.S			
Range	(7-18)	(6-16)	(0-32)
M \pm SD	10.9 ± 2.7	11.6 ± 3.8	10.2 ± 6.7
Hearing loss			
Range	(5-15)	(10-30)	(0-60)
M \pm SD	9.5 ± 3.14	16 ± 5	18.3 ± 6.1
THI			
Range	N/A	N/A	(6-80)
M \pm SD			26 ± 19
TFI			
Range	N/A	N/A	(8-90)
M \pm SD			36 ± 21
Coping			
Coping/non-coping	N/A	N/A	13/13
Tinnitus location			
Unilateral/ Bilateral	N/A	N/A	10/16
Tinnitus duration			
Range	N/A	N/A	(1-30 yrs)
M \pm SD			9 ± 8

6.4.2 Audiometry findings:

The mean and standard deviation by subject groups is shown in figure 6.1. No significant differences was found of the averaged of hearing loss thresholds between three groups at low frequencies tested (0.5-2.0 kHz). NH group showed normal hearing thresholds (<20 dB) at all frequencies tested. The averaged pure tone thresholds ranged between normal to mild to moderate hearing loss for MH and TI groups. No significant difference of hearing loss thresholds was found between MH and TI groups. Hearing loss was found more pronounced in MH than TI groups at high frequencies gradually from 4 to 8 kHz.

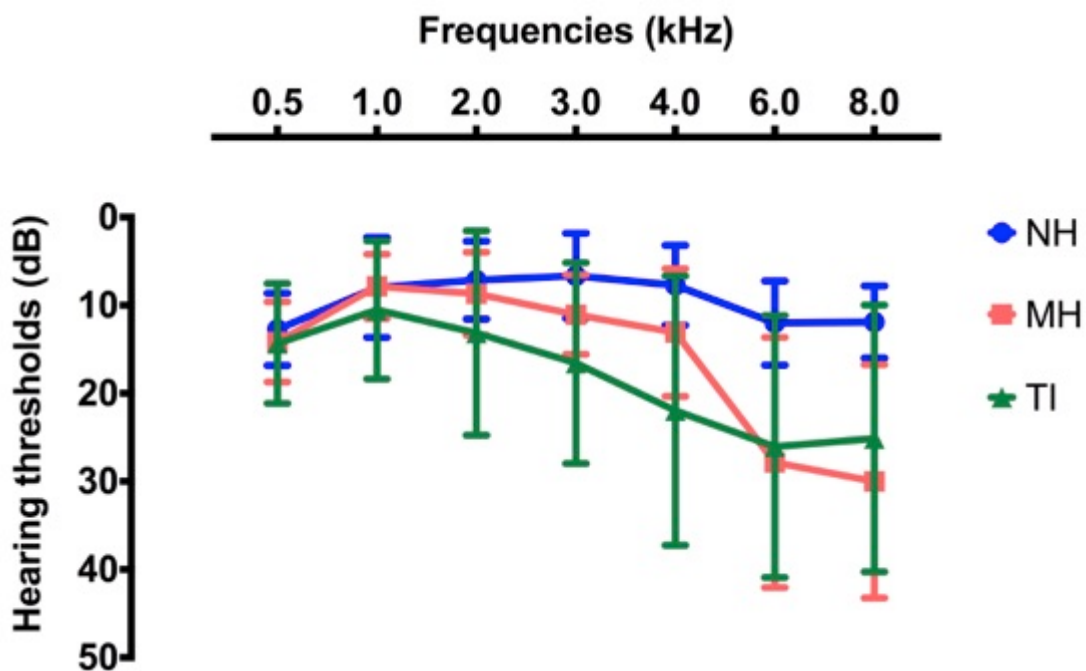


Figure 6.1: Audiogram for each participant's groups: normal hearers (NH blue), mild to moderate hearing loss (MH red) and tinnitus patients (TI green).

6.4.3 Quantification of total intracranial volume and grey matter volume.

The total intracranial volume (ICV) was found a statistically significant difference between three groups (NH, MH and TI) as determined by one-way ANOVA ($F(2,63)=3.89, P=0.026$) (Figure 6.2). A Tukey post-hoc test revealed that the ICV

was significantly lower ($t=-2.56$, $P=0.038$) in subjects with tinnitus group (1368.6 ± 45.7 cm³) compared to NH (1405.5 ± 60.9 cm³). No significant difference of ICV ($t=-2.10$, $P=0.117$) was found between TI (1368.6 ± 45.7 cm³) and MH (1399.0 ± 36.5 cm³). Gender factor was controlled for these results.

The global grey matter (GM) volume was found a statistically significant difference between three groups (NH, MH and TI) as determined by one-way ANOVA ($F(2,63)=7.75$, $P=0.001$) (Figure 6.3). A Tukey post-hoc test revealed that the GM was significantly lower ($t=-3.90$, $P=0.001$) in subjects with tinnitus group (731.42 ± 35.1 cm³) compared to NH group (772.23 ± 39.8 cm³). No significant difference of GM ($t=-2.15$, $P=0.106$) was found between TI (731.42 ± 35.1 cm³) and MH (753.92 ± 29.7 cm³). Gender factor was controlled for these results.

Pearson's correlation analysis showed a significant correlation of hearing loss with global grey matter volume ($R^2=0.16$, $P=0.03$) (figure 6.4) and a borderline significant correlation with total intracranial volume ($R^2=0.12$, $P=0.07$) (figure 6.5) in tinnitus patient group. However, this significant correlation did not survive ($P>0.05$) after controlling for the age factor. No significant correlation was found between hearing loss and global grey matter and total intracranial volume in normal hearing and mild to moderate hearing loss groups. Also, no significant correlation was found between total GM or ICV and tinnitus duration and severity measured by THI and TFI.

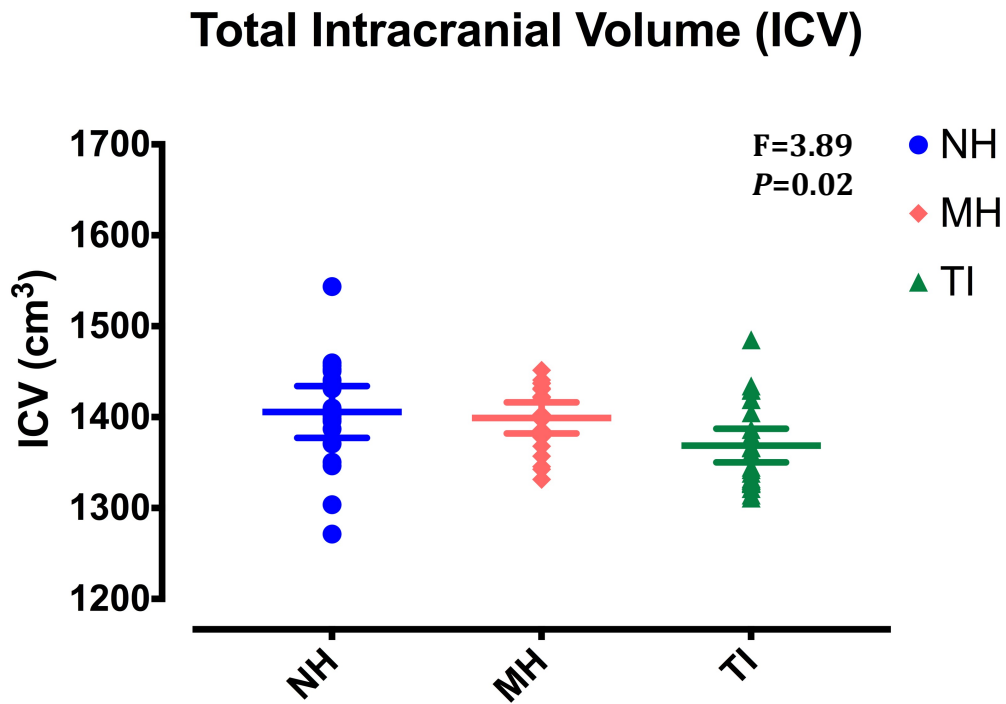


Figure 6.2: Plots with the mean and 95% confidence interval for total intracranial volume (TIV) in normal hearing (NH), mild to moderate hearing loss (MH) and tinnitus (TI) groups.

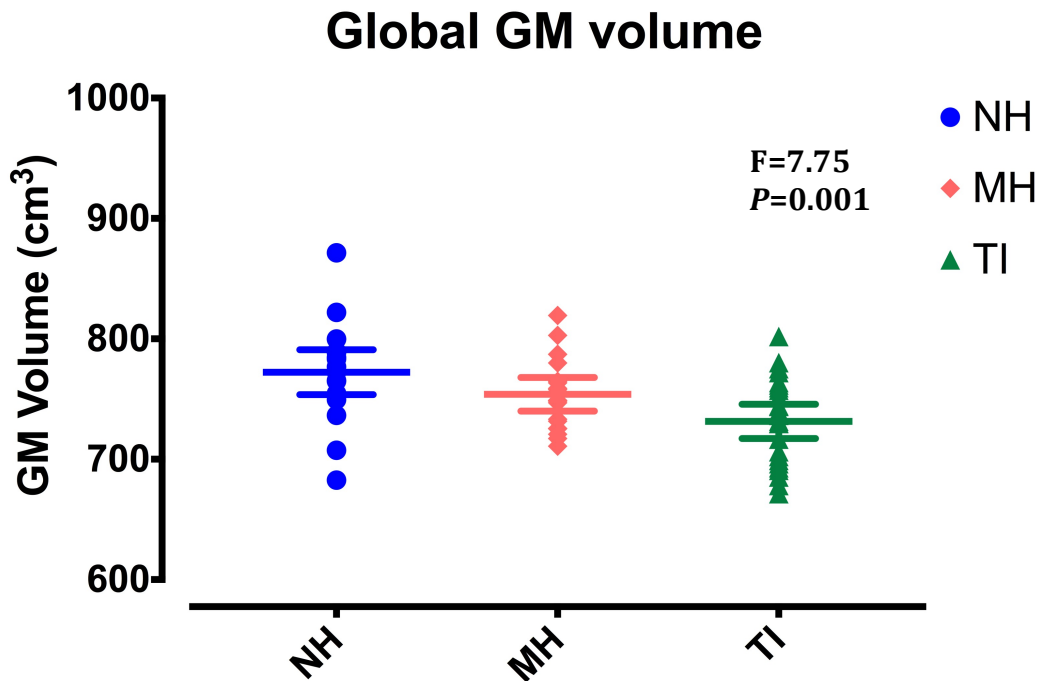


Figure 6.3: Plots with the mean and 95% confidence interval for grey matter (GM) in normal hearing (NH), mild to moderate hearing loss (MH) and tinnitus (TI) groups.

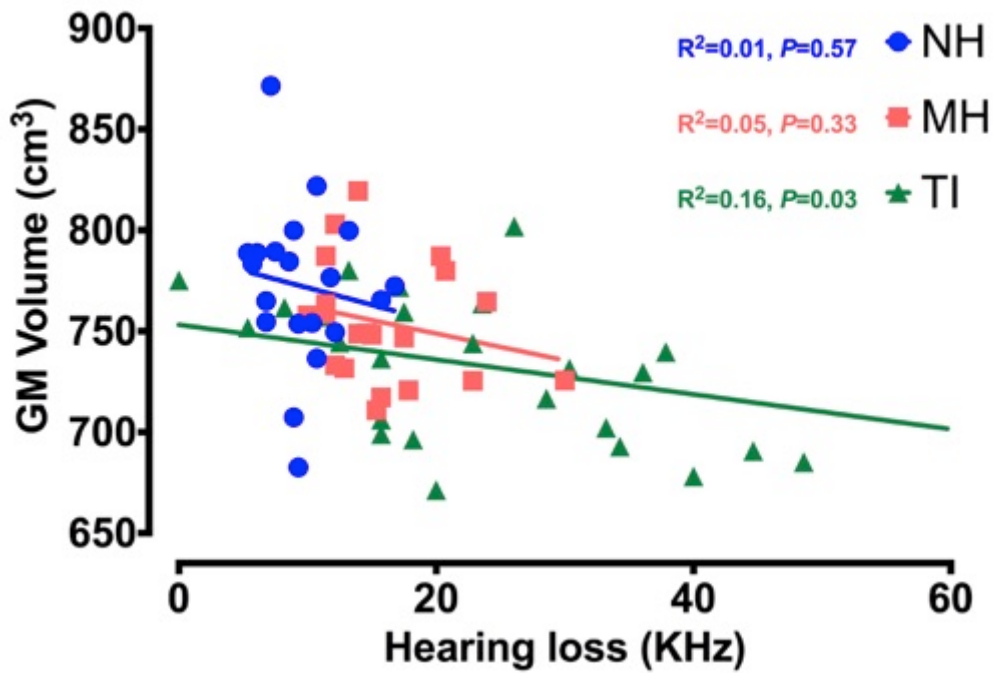


Figure 6.4: Correlations between total GM and hearing loss average. Scatter plot of hearing loss average (KHz) and total grey matter volume (voxels, cm³).

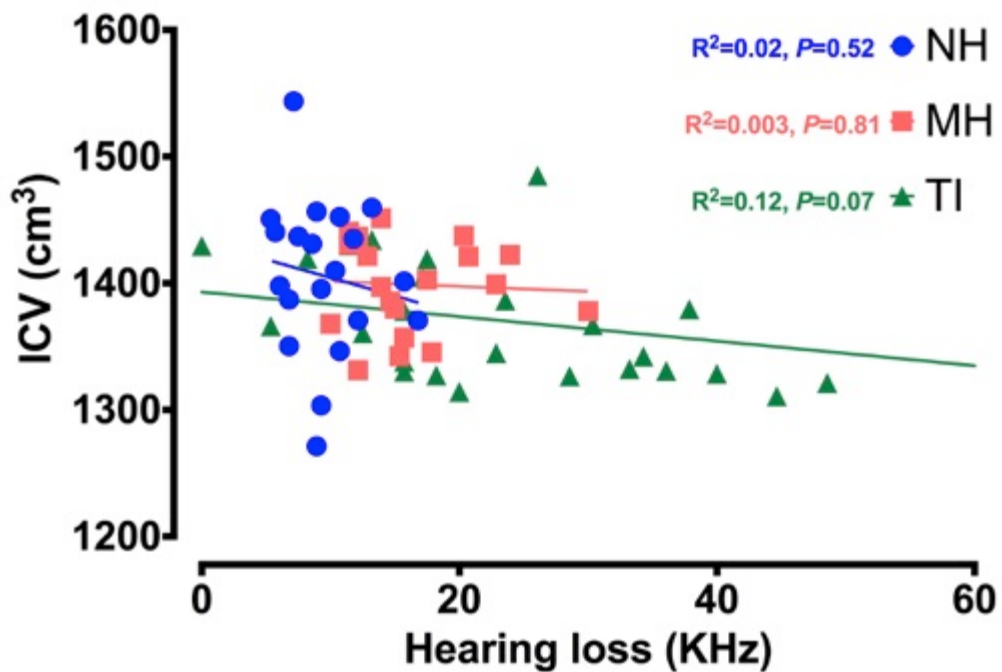


Figure 6.5: Correlations between ICV and hearing loss average. Scatter plot of hearing loss average (KHz) and total intracranial volume (voxels, cm³).

6.4.4 Voxel-based morphometry

- Cortical and subcortical grey matter reduction

Whole brain analysis of GM volume between NH and TI groups revealed that tinnitus subjects showed a significant reduction of grey matter volume (Figure 6.6, table 6.2) in left inferior temporal (788 voxels, $t=6.52$, $P=0.01$, coordinates -46 -4 -34), bilateral orbital frontal (OFC) (right: 210 voxels, $t=5.32$, $P=0.01$, coordinates 32 40 -12, left: 205 voxels, $t=4.90$, $P=0.01$, coordinates -22 32 -14), right insula (606 voxels, $t=4.86$, $P=0.01$, coordinates 34 18 -8), supramarginal (240 voxels, $t=5.46$, $P=0.01$, coordinates 60 -40 28) and occipital fusiform gyri (OFG) (138 voxels, $t=6.03$, $P=0.01$, coordinates -32 -64 -10) compared with NH. No significant difference of GM volume between the MH and TI groups, between tinnitus coping and suffering subgroups, and between tinnitus unilateral and bilateral subgroups. Also, no significant correlation was found between GM volume and tinnitus severity. Gender factor was controlled for these results.

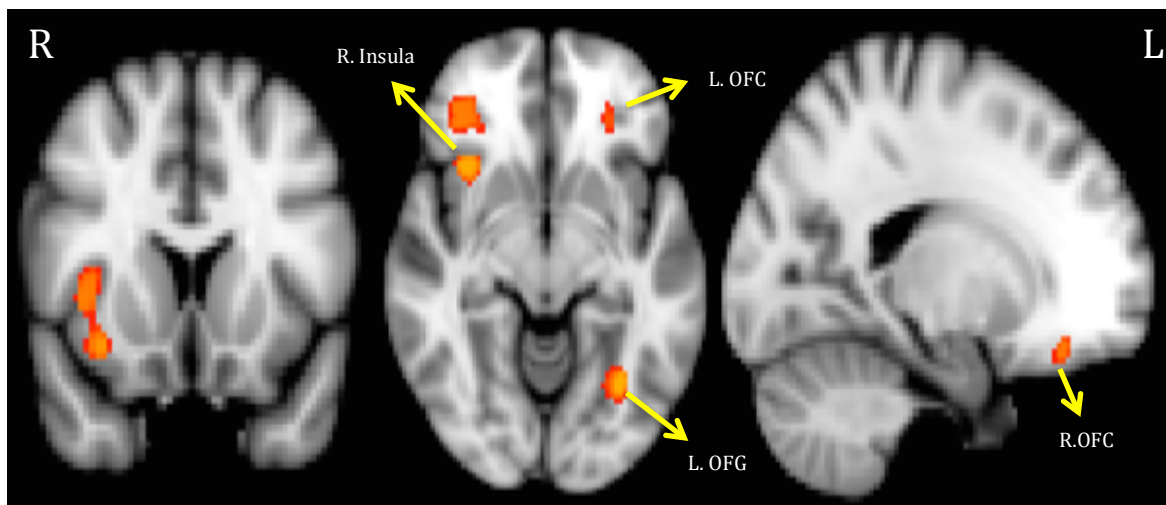


Figure 6.6: Statistical parameter map (extent threshold $P_{\text{corr}}=0.01$) showing clusters with statistically significant of decreased GM volume in tinnitus patients **compared** to normal hearing group.

Table 6.2: *P* values and MNI coordinates of cluster peak regions of VBM analysis.

Cluster peak region	Voxels	Zmax	<i>P</i>_{corr}	x	y	z
Left inferior temporal	788	6.52	0.01	-46	-4	-34
Right orbital frontal	210	5.32	0.01	32	40	-12
Left orbital frontal	205	4.90	0.01	-22	32	-14
Right insula	606	4.86	0.01	34	18	-8
Left occipital fusiform gyri	138	6.03	0.01	-32	-64	-10

6.4.5 Surface-based morphometry

- Cortical thickness analysis

The mean cortical thickness was found a statistically significant difference between three groups (NH, MH and TI) as determined by one-way ANOVA ($F(2,63)=4.12$, $P=0.021$) (Figure 6.7). A Tukey post-hoc test revealed that the mean cortical thickness was significantly thinner in subjects with tinnitus group (2.42 ± 0.10 mm, $P=0.023$) compared to NH (2.51 ± 0.11 mm) and MH (2.49 ± 0.11 mm). Gender factor was controlled for these results.

Pearson's correlation analysis showed a significant negative correlation between mean cortical thickness and hearing loss average in tinnitus patient group ($R^2=0.19$, $P=0.02$), whereas, healthy control with normal hearing did not show a significant correlation between hearing thresholds and the mean cortical thickness ($R^2=0.002$, $P=0.84$) and mild to moderate hearing loss group ($R^2=0.14$, $P=0.10$) (figure 6.8). However, this significant correlation was not survived ($P>0.05$) after controlling for the age factor. No significant correlation was found between the mean cortical thickness and tinnitus duration and severity measured by THI and TFI.

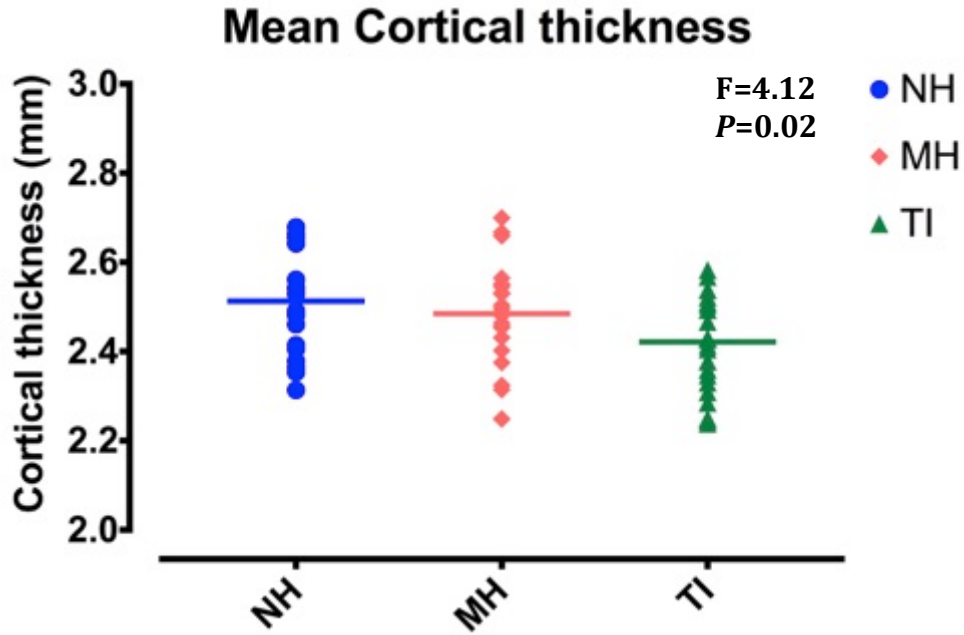


Figure 6.7: 95% confidence interval for mean thickness in normal hearing (NH), mild to moderate hearing loss (MH) and tinnitus (TI) groups.

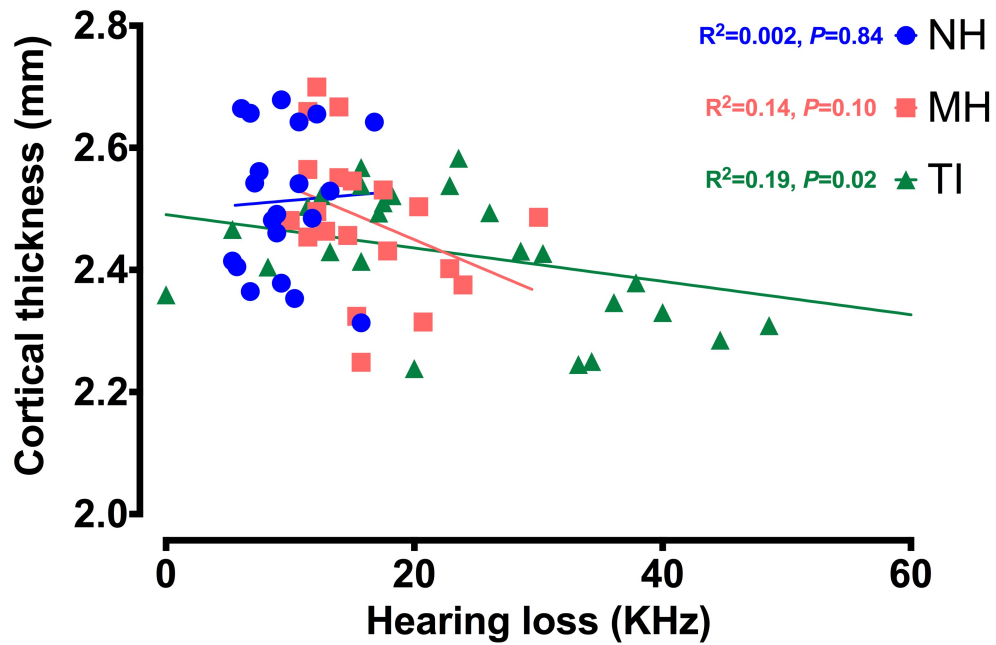


Figure 6.8: Correlations between mean cortical thickness and hearing loss average. Scatter plot of hearing loss average (KHz) and mean cortical thickness (mm).

Cortical thickness analysis (CTA) across the whole brain regions revealed that cortical thickness was statistically significant difference between three groups. Tinnitus patients showed a thinner cortex at left primary auditory cortex, superior frontal gyrus (SFG) and Parstriangularis, and thicker cortex at bilateral inferior parietal gyri (IPG), medial orbital frontal (mOFC) cortex, left inferior (ITG) and superior temporal gyri (STG) and precuneus compared to healthy controls with normal hearing (NH) (figure 6.9, table 6.3). In comparison with MH group, tinnitus subjects showed thinner cortices at right middle (MTG) and superior temporal (STG) and superior frontal gyri (SFG), bankssts and left medial orbital frontal cortex (mOFC), and thicker cortex was found at bilateral postcentral gyri, right inferior parietal gyrus (IPG) and left superior parietal gyrus (SPG) and precuneus (figure 6.10, table 6.3). Consistent findings were found in three brain regions that showed significant differences of cortical thickness: right inferior parietal gyrus (IPG), left precuneus and postcentral gyri.

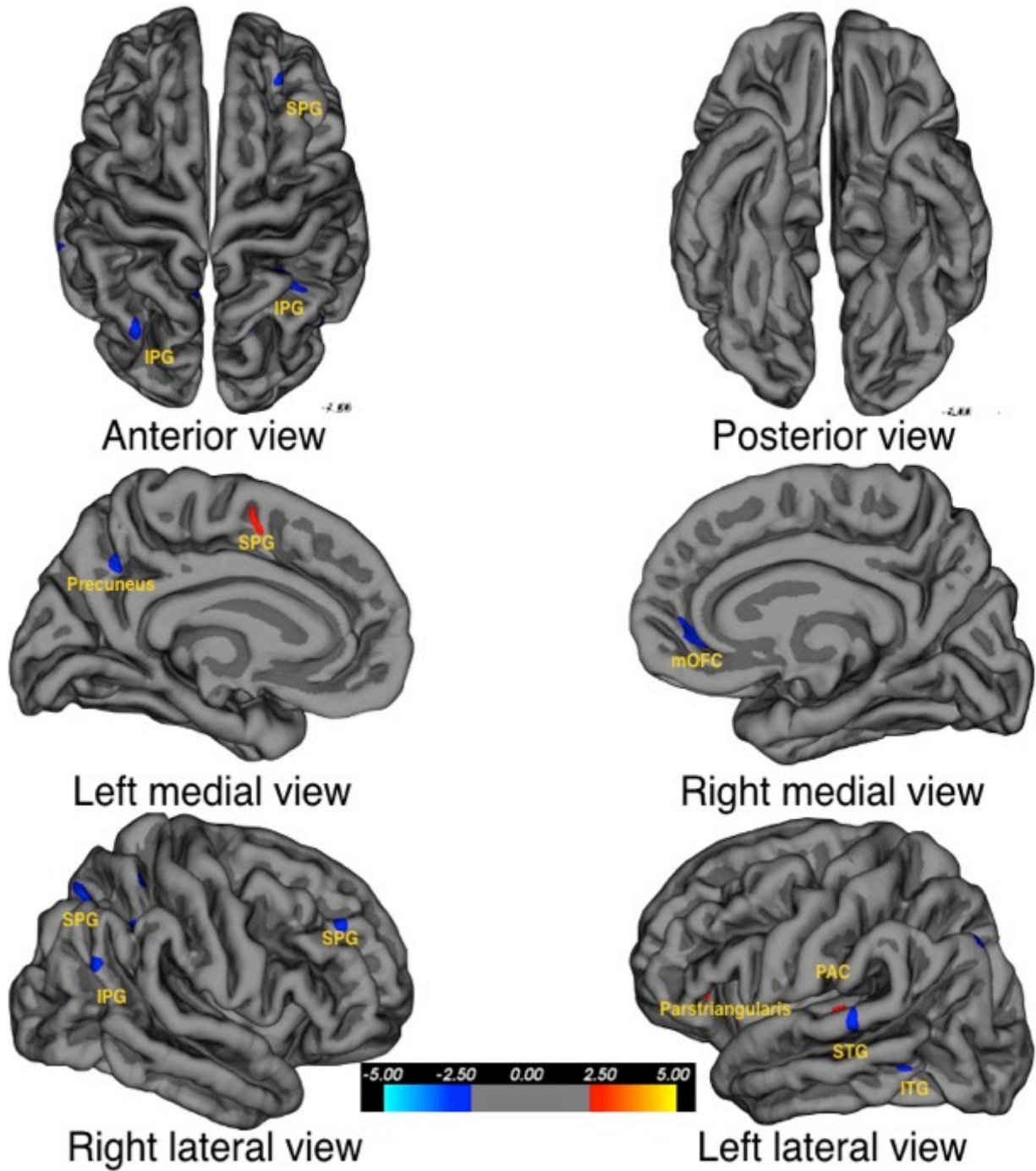


Figure 6.9: T-statistics maps of group difference in cortical thickness between NH and TI groups. Red blobs show the increase of cortical thickness, while the blue blobs show the decrease of cortical thickness in the tinnitus group comparing to the normal hearing group.

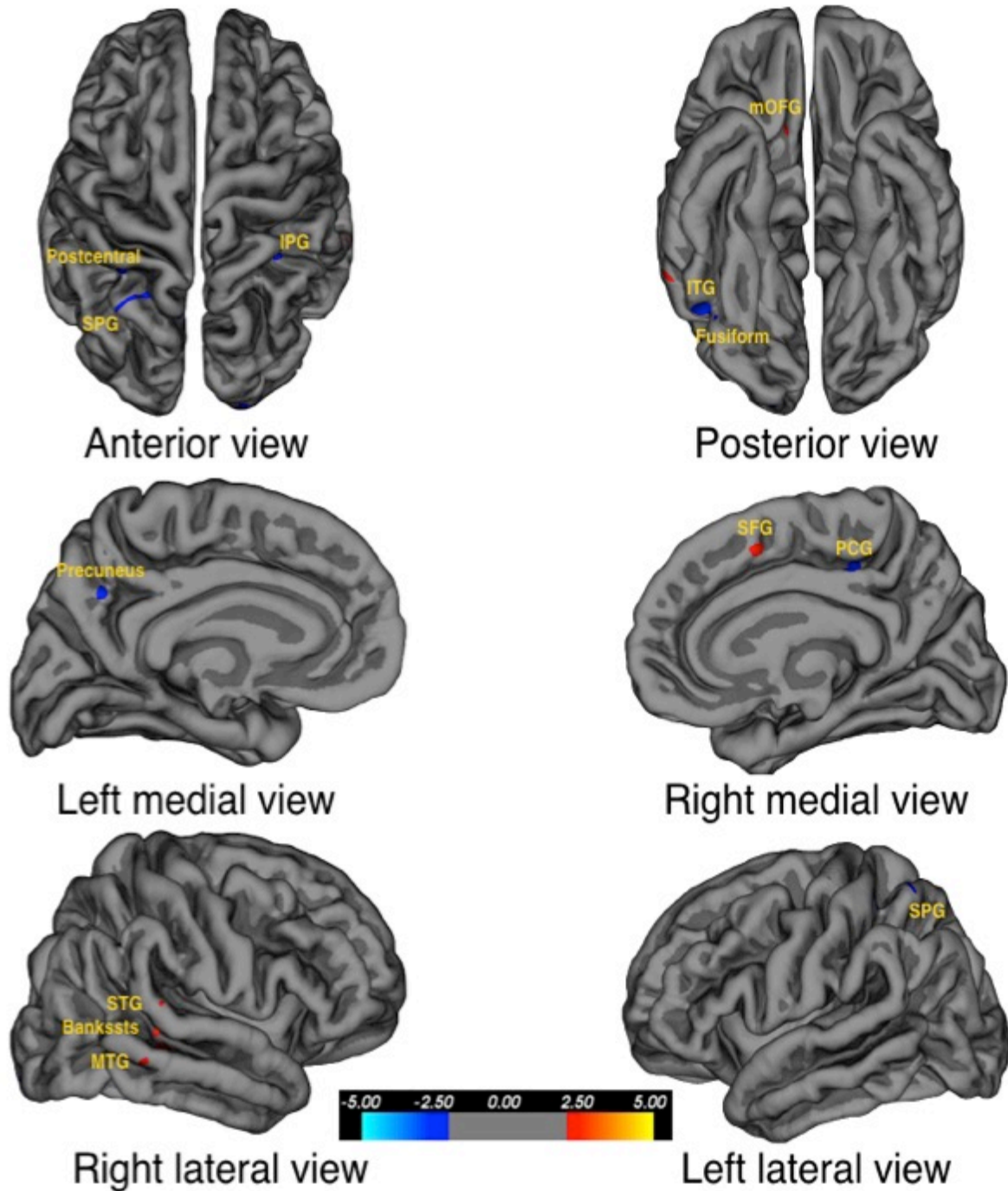


Figure 6.10: T-statistics maps of group difference in cortical thickness between MH and TI groups. Red blobs show the increase of cortical thickness, while the blue blobs show the decrease of cortical thickness in the tinnitus group comparing to the mild to moderate hearing loss group.

Table 6.3: T-statistics results of cortical thickness measurements in normal hearing, mild to moderate hearing loss, and tinnitus groups.

Contrast	Hemisphere	Cortical structures	T value	DoF	Talairach coordinate
NH>TI	Right	postcentral	2.30	42	23, 7, -5
	Left	PAC	2.52	42	-47, 18, -25
		SFG	2.30	42	30, 34, 48
		Parstriangularis	2.22	42	-12, 65, -30
		Pericalcarine	2.19	42	25, -79, -8
NH<TI	Right	SPG	-2.99	42	-15, -73, 42
		IPG	-2.96	42	14, -44, 38
		SFG	-2.59	42	-8, 72, 36
		mOFC	-2.57	42	-33, 79, -33
	Left	precuneus	-2.59	42	29, 54, 37
		STG	-2.26	42	-39, -26, -14
		IPG	-2.02	42	-6, -87, 10
		Postcentral	-2.38	42	-22, -4, 45
		ITG	-2.34	42	-33, -56, -32
MH>TI	Right	Bankssts	3.10	42	37, -29, 19
		MTG	2.64	42	39, -26, -31
		SFG	2.52	42	-31, 54, 40
		mOFC	2.11	42	-22, 45, -51
		STG	2.10	42	38, -22, -4
MH<TI	Right	Fusiform	-2.06	42	19, -68, -43
		ITG	-2.85	42	29, -57, -41
		PCG	-2.79	42	-31, -8, 32
		Paracentral	-2.77	42	-30, -12, 48
		IPG	-2.47	42	14, -42, 38
		LOG	-2.17	42	-10, -106, -25
	Left	Postcentral	-2.77	42	-8, -28, 55
		Precuneus	-2.45	42	29, -58, 36
		SPG	-2.42	42	10, -42, 67

- *Association between cortical thickness and tinnitus severity and laterality.*

In comparison between the tinnitus coping and suffering subgroups, cortical thickness analysis (CTA) revealed that the tinnitus suffering subgroup showed thicker cortex at left anterior cingulate cortex (ACC) ($t=-2.44$, $P=0.023$) (figure 6.11) and right posterior cingulate cortex (PCC) ($t=-2.22$, $P=0.038$) (figure 6.12) compared to the coping tinnitus subgroup. In comparison between the tinnitus bilateral and unilateral subgroups, CTA revealed that the bilateral tinnitus subgroup showed a significant cortical thinner in left Cuneus ($t=2.26$, $P=0.0036$) (figure 6.13), left ACC ($t=-2.28$, $P=0.03$) (figure 6.14), and left temporal pole (TP) ($t=-2.36$, $P=0.027$) (figure 6.15) compared to the unilateral tinnitus subgroup.

The correlation test between cortical thickness measurements and tinnitus severity revealed that three brain regions are associated with tinnitus handicap inventory (THI). Tinnitus severity was found significantly positive correlated to cortical thickness measurements at the left caudal anterior cingulate gyri (cACG) ($r=0.45$, $P=0.023$) (Figure 6.16) and the right transverse temporal cortex ($r=0.45$, $P=0.02$) (figure 6.17). Also, Significant negative correlation was identified between THI scores and cortical thickness measurements at the right temporal pole (TP) ($r=-0.39$, $P=0.047$) (Figure 6.18).

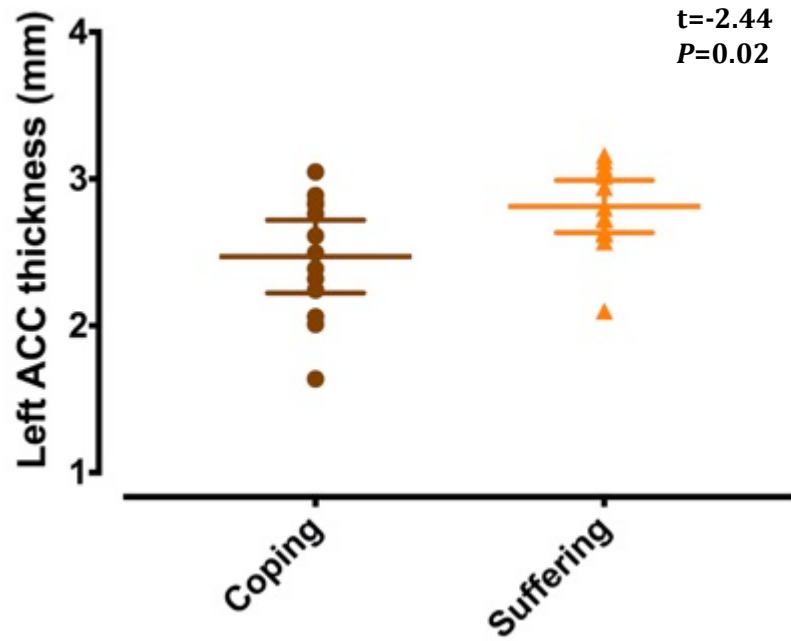


Figure 6.11: : Plots with the mean and 95% confidence interval for cortical thickness of the left anterior cingulate cortex (ACC) in the tinnitus coping and suffering subgroups.

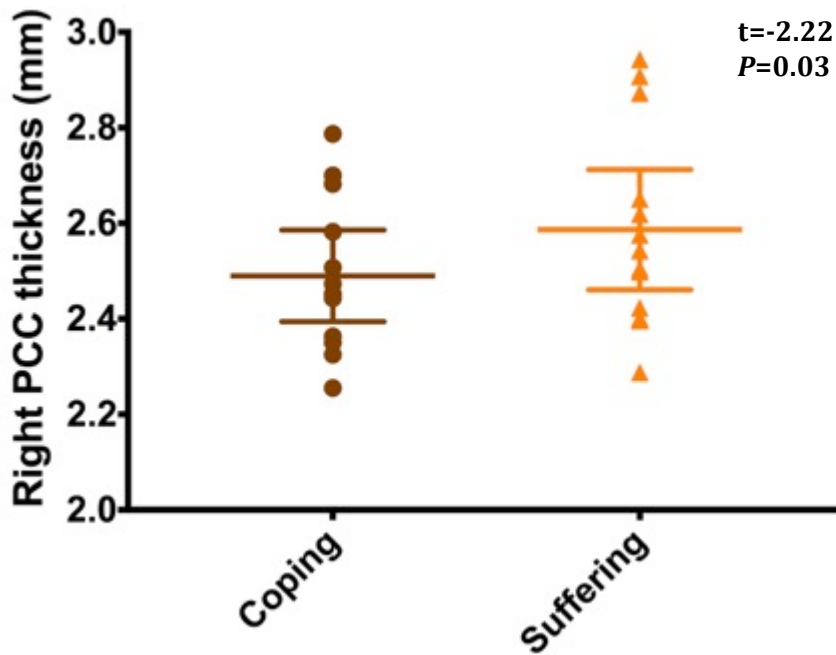


Figure 6.12: : Plots with the mean and 95% confidence interval for cortical thickness of the right precentral cortex (PCC) in the tinnitus coping and suffering subgroups.

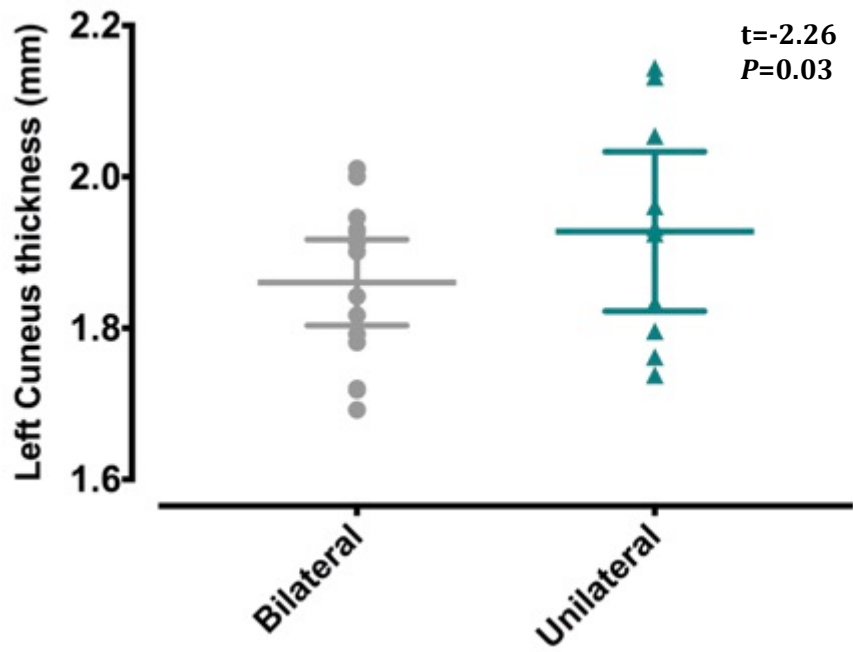


Figure 6.13: Plots with the mean and 95% confidence interval for cortical thickness of the left Cuneus in the tinnitus bilateral and unilateral subgroups.

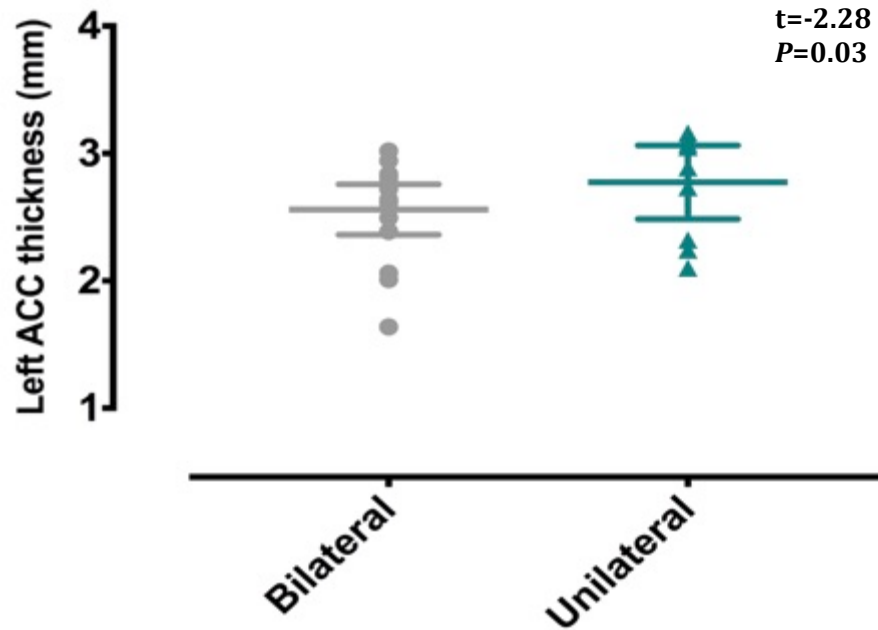


Figure 6.14: : Plots with the mean and 95% confidence interval for cortical thickness of the right anterior cingulate cortex (ACC) in the tinnitus bilateral and unilateral subgroups.

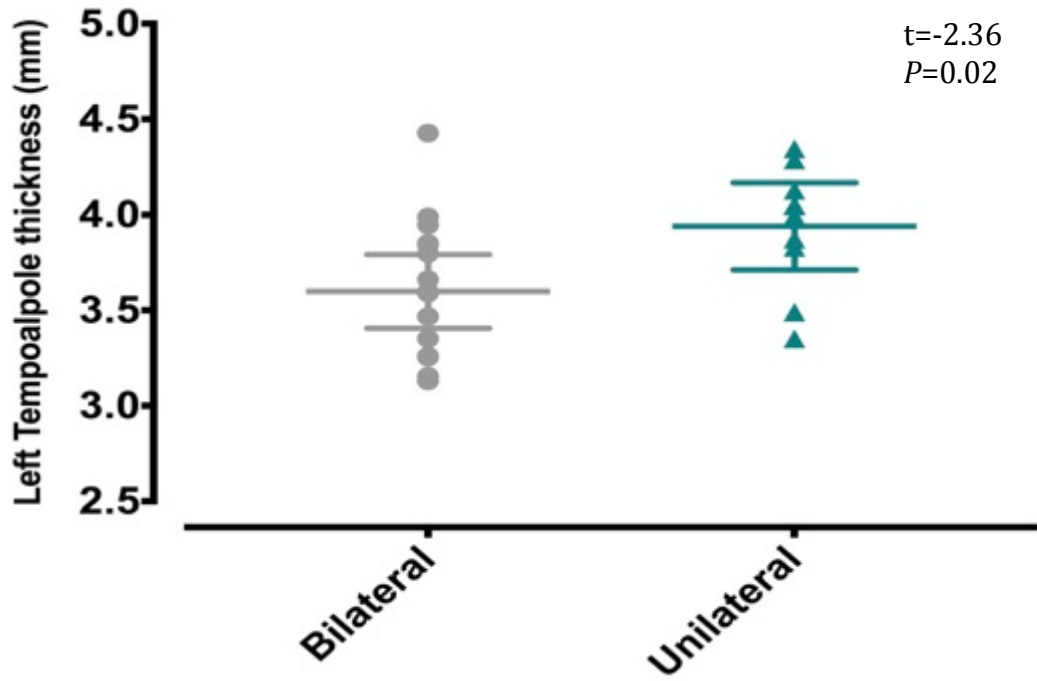


Figure 6.15: : Plots with the mean and 95% confidence interval for cortical thickness of the left temporalpole in the tinnitus bilateral and unilateral subgroups.

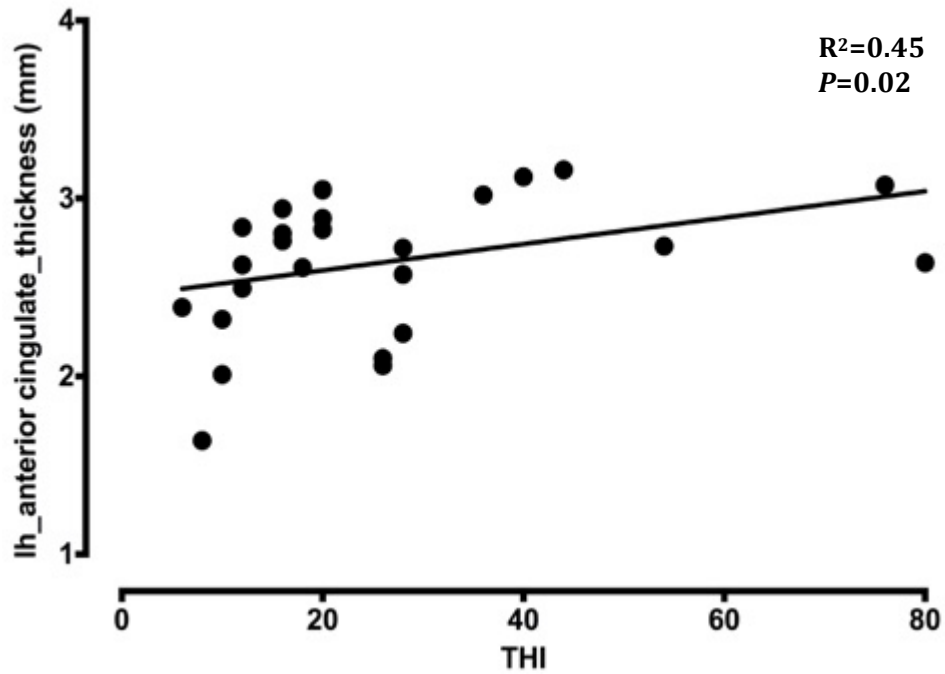


Figure 6.16: Scatter plot of the correlation between cortical thickness in the left anterior cingulate cortex and THI scores.

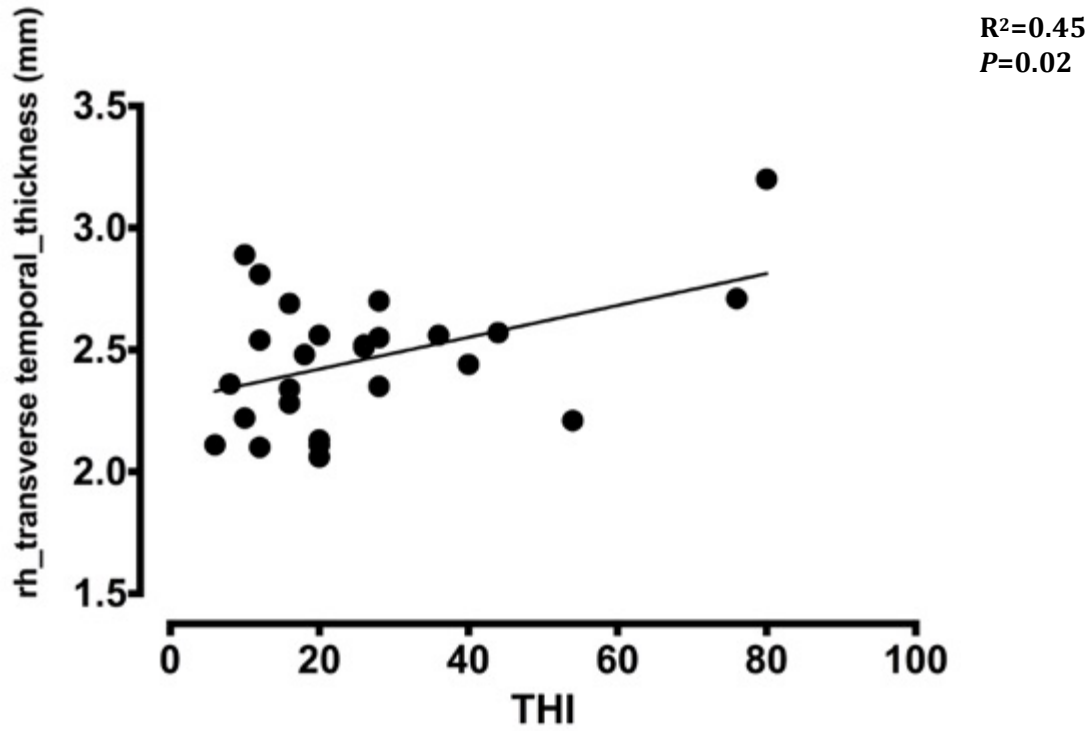


Figure 6.17: Scatter plot of the correlation between cortical thickness in the right transversetemporal cortex and THI scores.

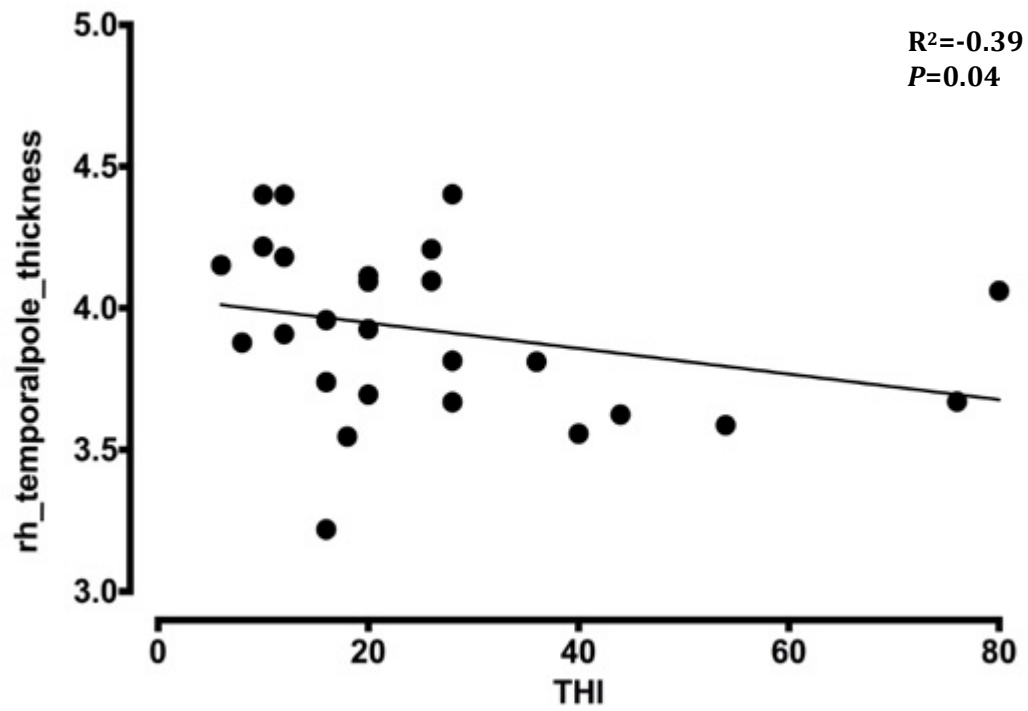


Figure 6.18: Scatter plot of the correlation between cortical thickness in the right temporalpole and THI scores.

6.4.6 Tinnitus-Related Differences in Subcortical Structures volume

Total subcortical grey volume was estimated for each participant in each group. One-way ANOVA statistics test revealed that no significant difference of total subcortical grey volume was found between the three groups ($F(2,64) = 0.19$ ($P=0.82$)) (figure 6.19). Gender factor was controlled for this analysis.

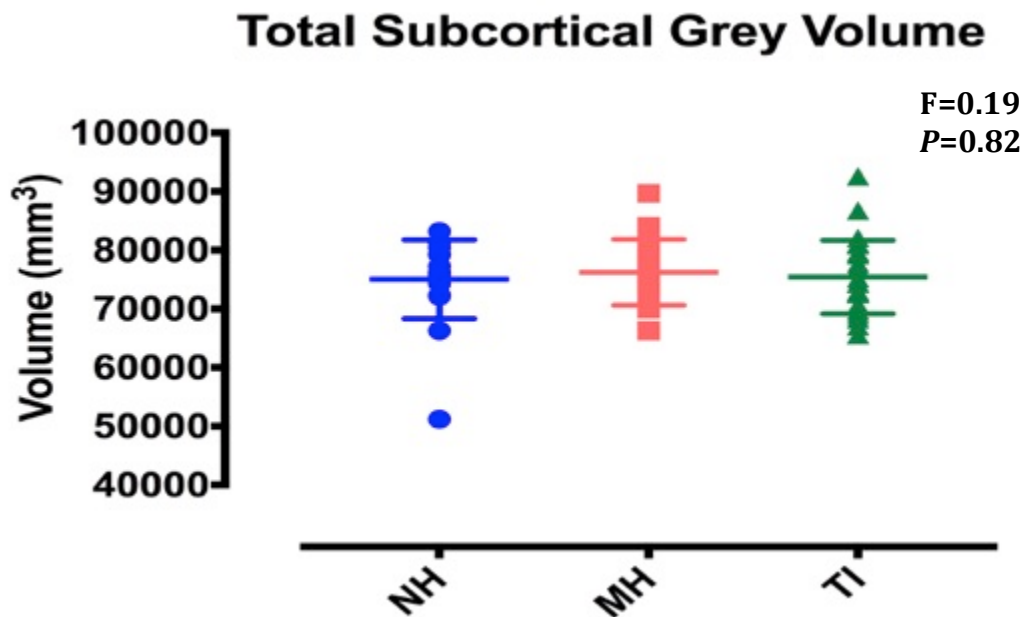


Figure 6.19 Plots with the mean and 95% confidence interval for total subcortical grey volume in the normal hearing (NH), mild to moderate hearing loss (MH) and tinnitus (TI) groups.

The volumes of each subcortical structure were extracted for each group including the brainstem and bilateral nucleus accumbens, thalamus, caudate, putamen, pallidum, hippocampus, amygdala and (table 6.4). ANOVA for repeated measurements showed that there were no group-hemisphere interaction effect ($F(2,64) = 0.24$ ($P=0.78$)) and no group=hemisphere-subcortical volume interaction effect ($F(2,64) = 1.63$ ($P=0.09$)). A single one-way ANOVA and Post hoc tests were

conducted between the NH, MH and TI groups for each subcortical structure separately. Gender factor was controlled for this analysis.

ANOVA test revealed that the left pallidum is the only subcortical structure significantly differs between groups ($F(2,63)=4.183$, $P=0.02$) (figure 6.20). A Tukey post-hoc test shows a significant reduction of the left pallidum volume ($t=2.9$, $P=0.03$) in the tinnitus patients group compared to the MH.

Table 6.4: The volume of subcortical structures in the normal hearers, mild to moderate hearing loss and tinnitus groups.

Sub-cortical structures	R/L	Subcortical volume (mm ³)		
		NH	MH	TI
Thalamus	R	8659.5±828.1	8922.7±730.4	8979.5±1024.8
	L	8488.5±860.5	8627.9±600.9	8784.1±1074.1
Caudate	R	4574.3±378.9	4571.6±434.2	4653.8±629.2
	L	4374.7±404.1	4502.7±372.6	4615.3±598.8
Putamen	R	8474.1±1082.2	8781.1±650.7	8615.1±1033.7
	L	8725.7±1283.3	9088.1±502.3	8735.7±1122.2
Pallidum	R	2127.9±316.9	2244.9±215.2	2136.7±314.8
	L	2126.8±347.8	2207.7±258.8	1944.6±334.8
Hippocampus	R	5236.6±658.4	5247.9±294.2	5366.8±649.1
	L	5288.8±683.5	5223.1±446.1	5355.9±624.9
Amygdala	R	2104.6±277.1	2017.1±224.7	2084.1±244.1
	L	1809.5±275.1	1839.1±251.5	1905.1±293.6
Nucleus	R	979.7±110.9	951.5±146.5	975.1±108.9
Accumbens	L	942.4±172.9	945.7±129.3	1000.9±134.9
Brainstem	N/A	27587.9±1818.6	28402.4±2317.9	28016.7±2915.7

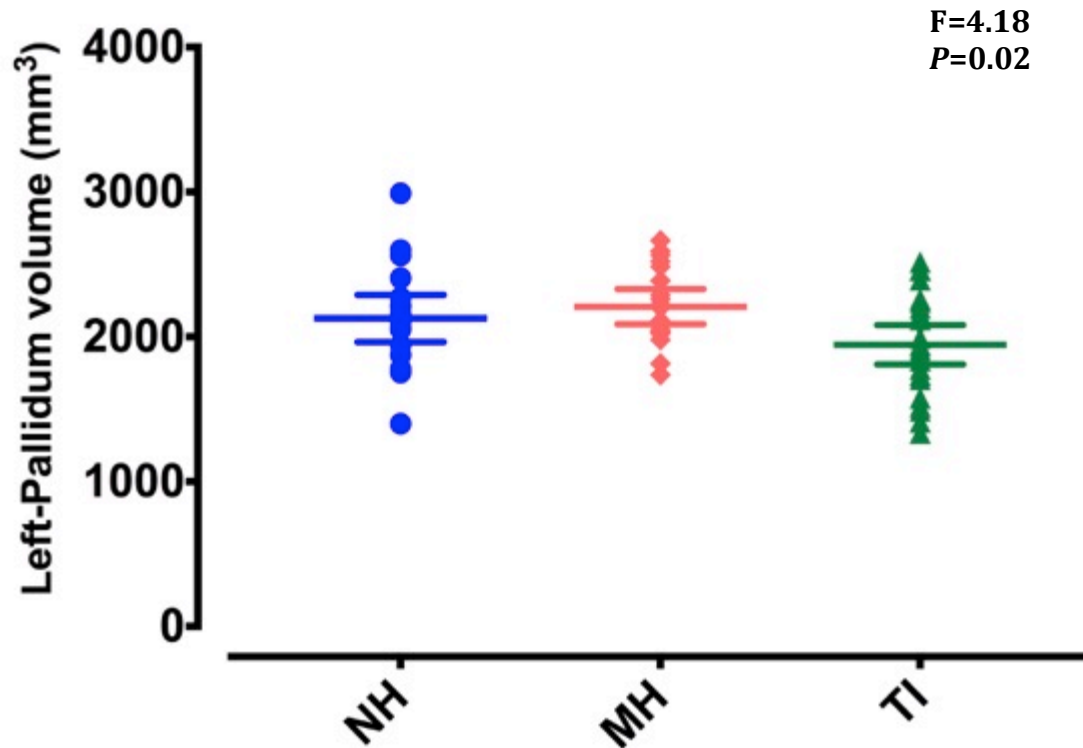


Figure 6.20: Plots with the mean and 95% confidence interval for the volume of the left Pallidum in the normal hearing (NH), mild to moderate hearing loss (MH) and tinnitus (TI) groups.

- *Association between Subcortical Structures and tinnitus severity.*

The correlation test between subcortical volumes and tinnitus severity revealed that the volume of the left hippocampus is significantly negative correlated to the total score of TFI ($r=-0.39$, $P=0.044$) (figure 6.21). This correlation did not survive correction for multiple comparisons. No significant correlation was identified between other subcortical volumes and tinnitus severity.

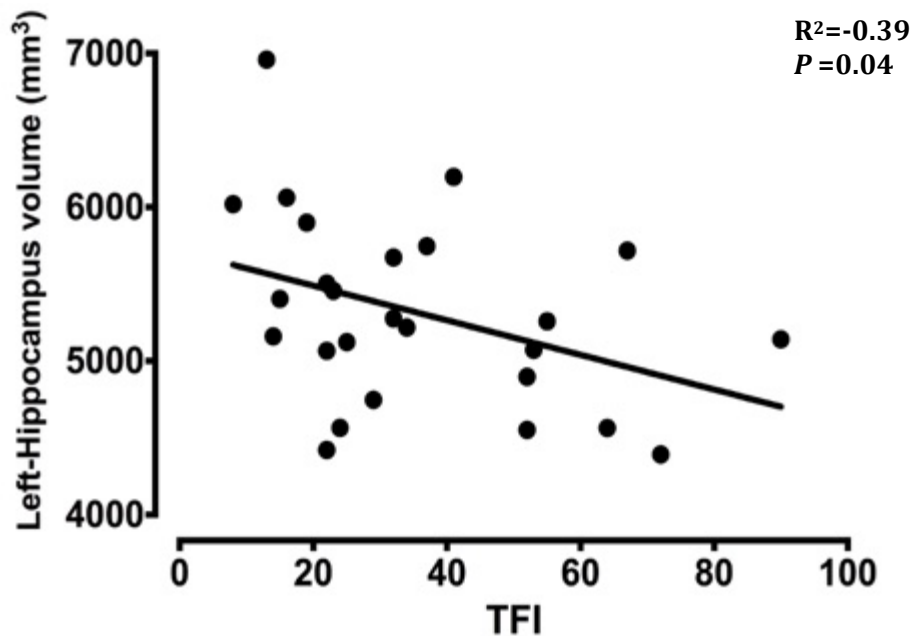


Figure 6.21: Scatter plot showing the association between the left hippocampus volume and tinnitus severity (TFI).

- *Association between Subcortical Structures and tinnitus laterality.*

The association between subcortical volumes and tinnitus laterality was assessed, and revealed that the unilateral tinnitus subgroup ($886.7 \pm 74 \text{ mm}^3$) showed a significant reduction ($t=2.8, P=0.01$) of the subcortical volume in the right nucleus accumbens (NAc) compared to the bilateral tinnitus subgroup ($1008.6 \pm 112 \text{ mm}^3$) (figure 6.22).

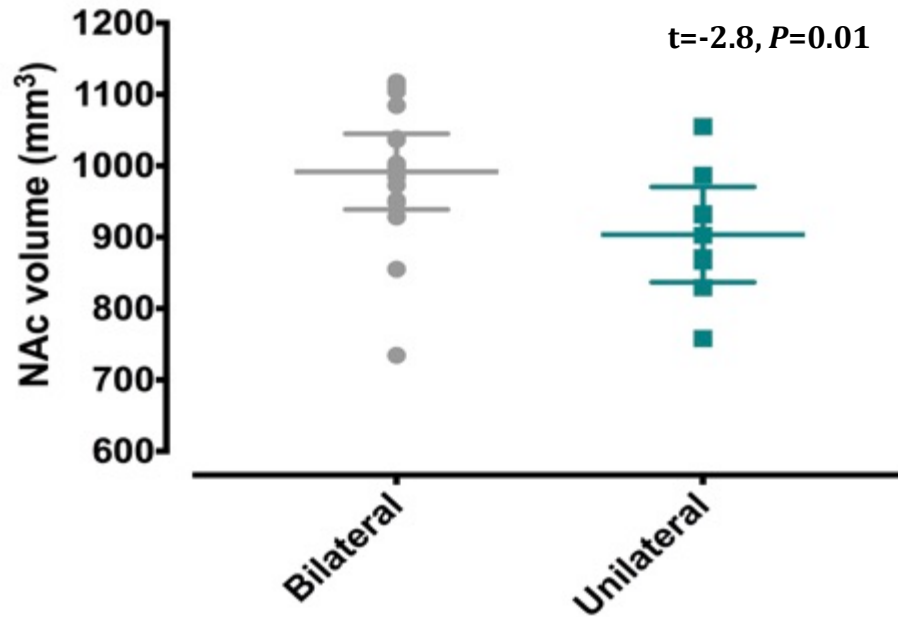


Figure 6.22: : Plots with the mean and 95% confidence interval for the right nucleus accumbens (NAc) in the tinnitus bilateral and unilateral subgroups.

6.4.7 Subcortical shape difference analysis

- Influence of tinnitus perception on subcortical structures surface shape

The group differences of subcortical structure differences were significant between tinnitus patients and normal hearers in the bilateral pallidum (figures 6.23 and 6.24), and the brainstem (figure 6.25), and between tinnitus patients and the mild to moderate hearing loss group was found in the right hippocampus (figure 6.26) and the left pallidum (figure 6.27). Within the tinnitus group, significant differences of subcortical surface shape were found between the tinnitus coping and suffering groups in right putamen (figure 6.28) and right amygdala (figure 6.29). No significant difference of subcortical structures surface shape was found between the tinnitus bilateral and unilateral subgroups, and between tinnitus patients with high level of anxiety and depression and tinnitus patients with low level of anxiety and depression. Gender factor was controlled for this analysis.

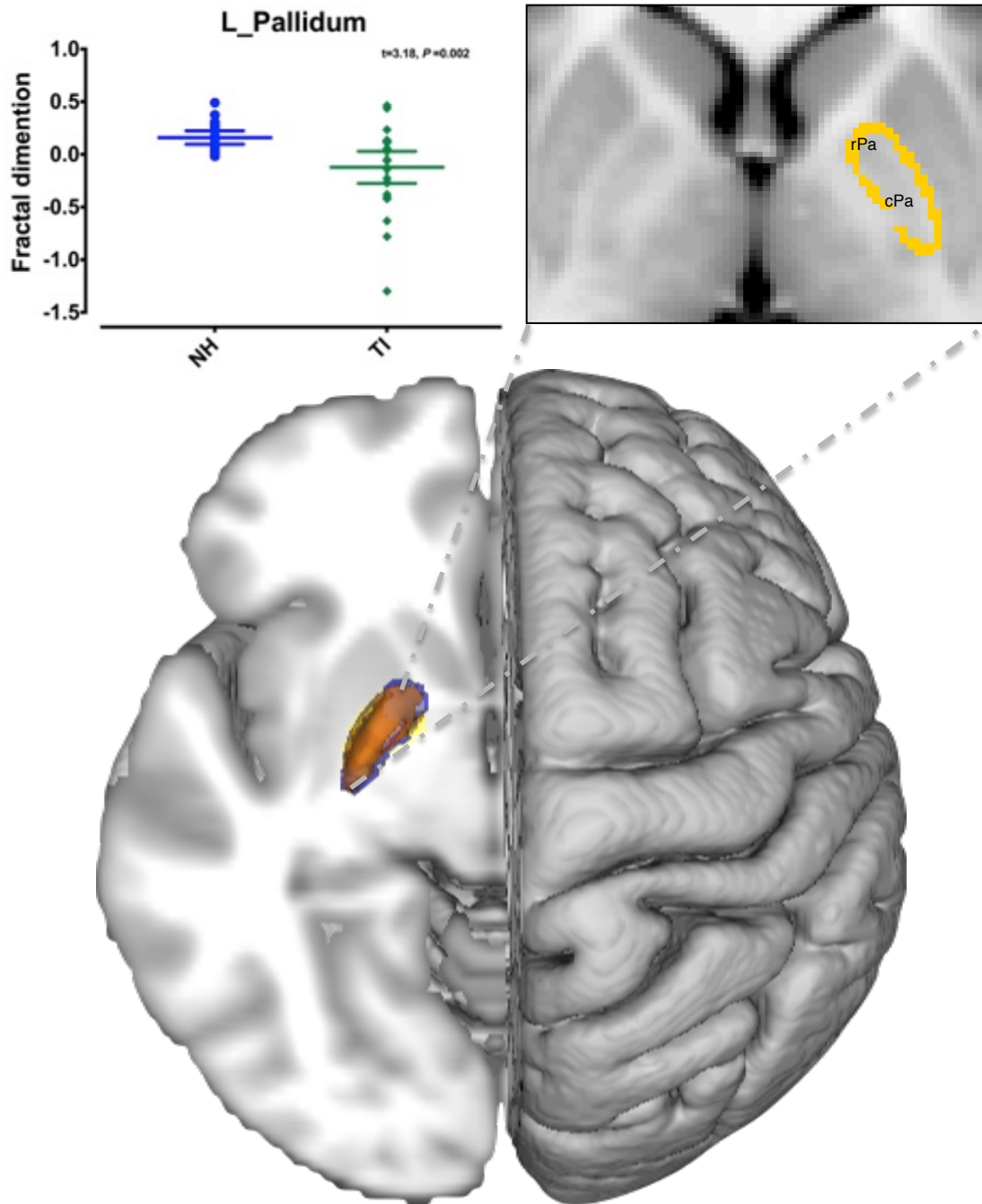


Figure 6.23: Statistic map and fractal dimension of shape difference in the rostral (rPa) and caudal (cPa) parts of pallidum between the tinnitus and NH groups. Subcortical masks and affected regions are coloured in blue and orange respectively.

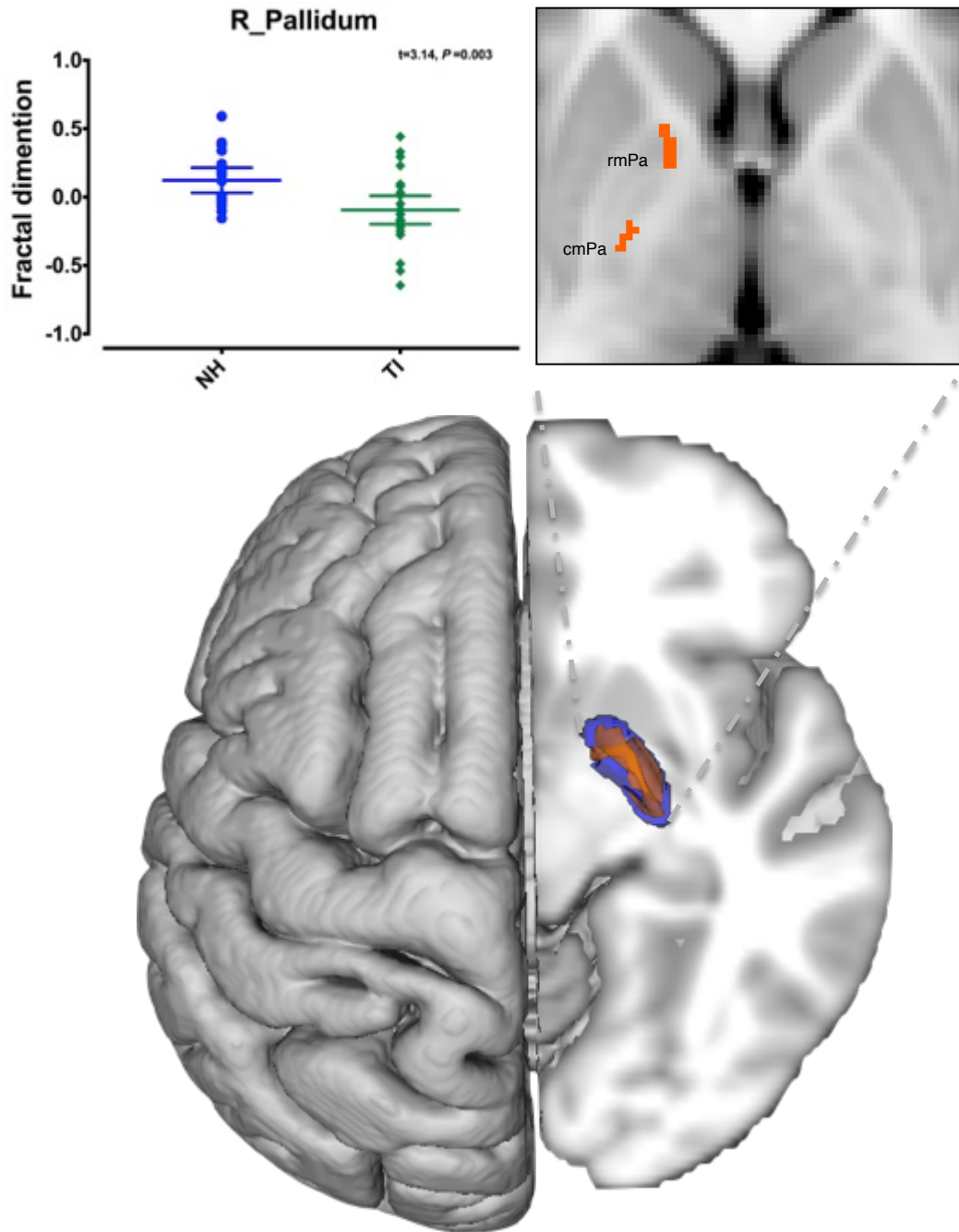


Figure 6.24: Statistic map and fractal dimension of shape difference in the rostral medial (rmPa) and caudal medial (cmPa) parts of right pallidum between the tinnitus group and NH groups. Subcortical masks and affected regions are coloured in blue and orange respectively.

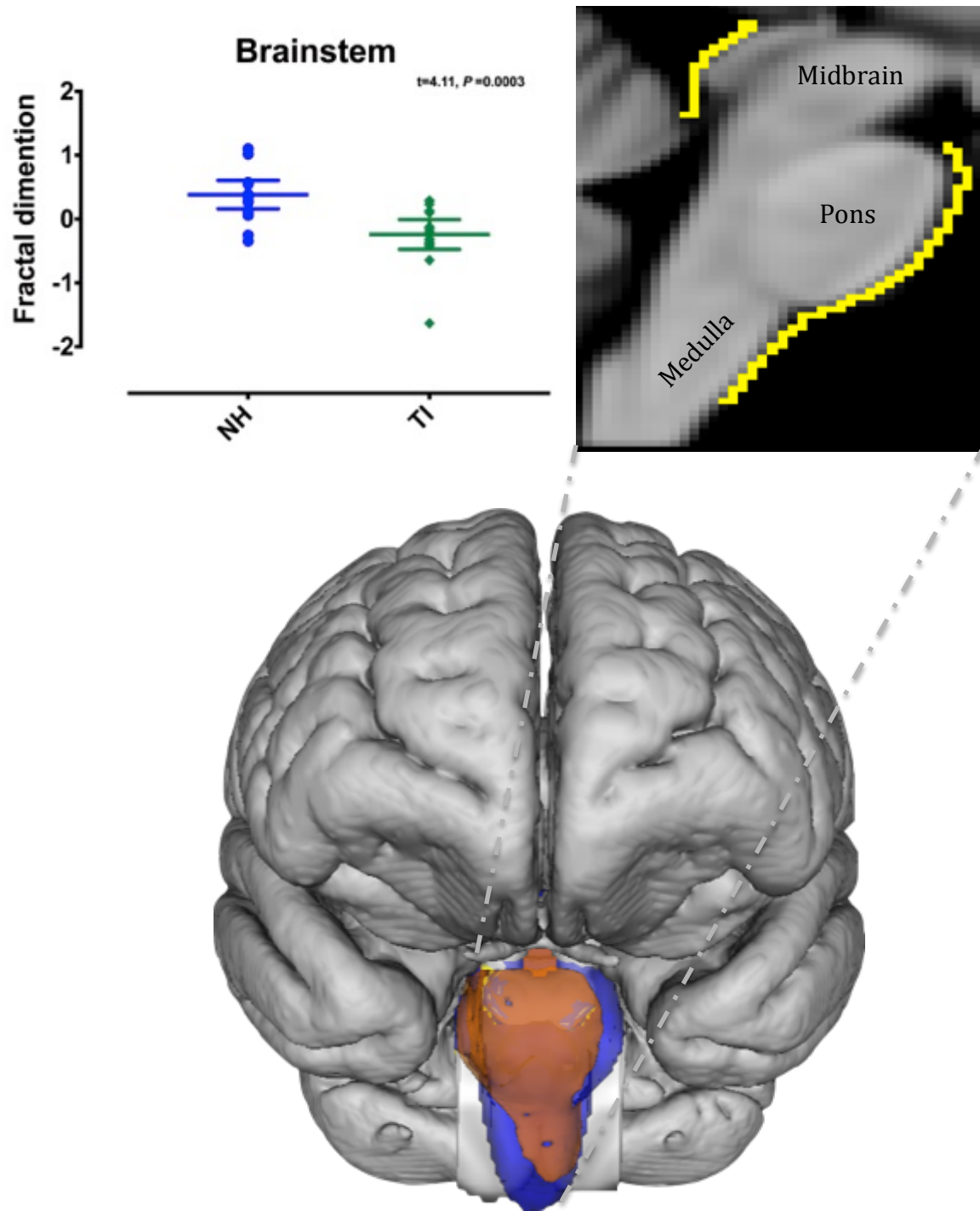


Figure 6.25: Statistic map and fractal dimension of shape difference in in the brainstem between the tinnitus and NH groups. Subcortical masks and affected regions are coloured in blue and orange respectively.

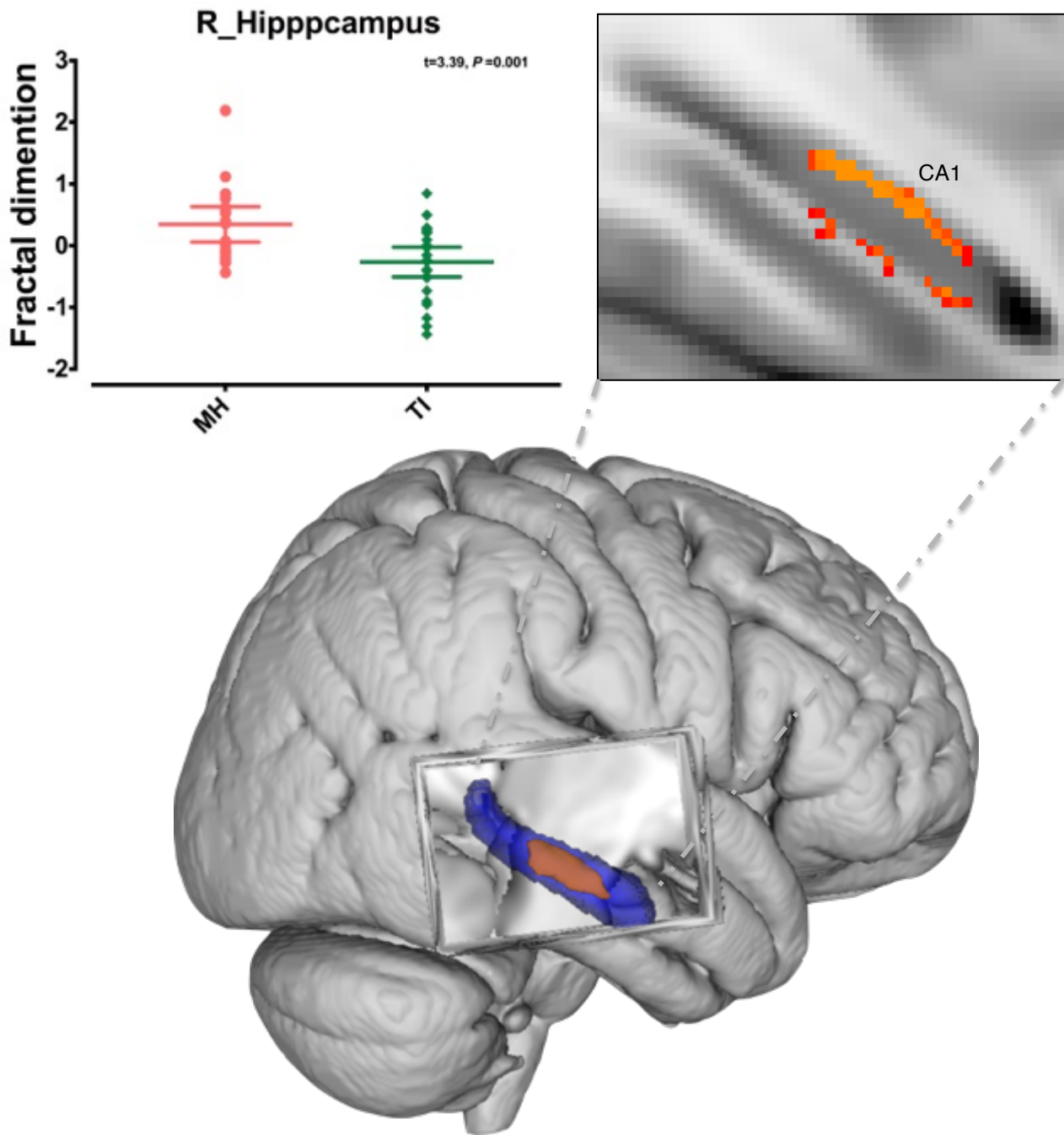


Figure 6.26: Statistic map and fractal dimension of shape difference in in the right hippocampus (Cornu Amonis (CA1)) between the tinnitus and MH groups.. Subcortical masks and affected regions are coloured in blue and orange respectively.

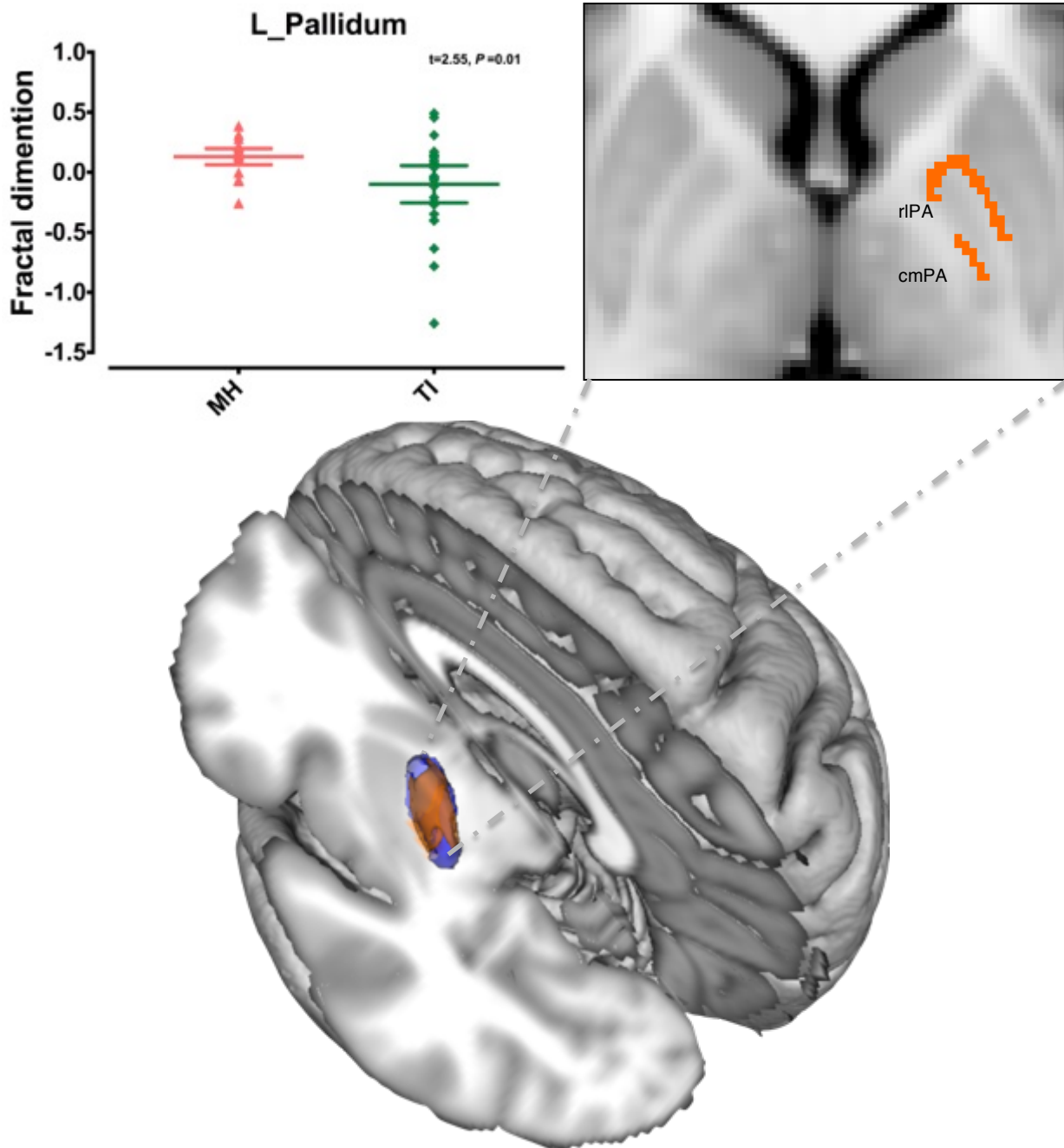


Figure 6.27: Statistic map and fractal dimension of shape difference in in the rostral lateral and caudal medial of left pallidum between the tinnitus and MH groups.. Subcortical masks and affected regions are coloured in blue and orange respectively.

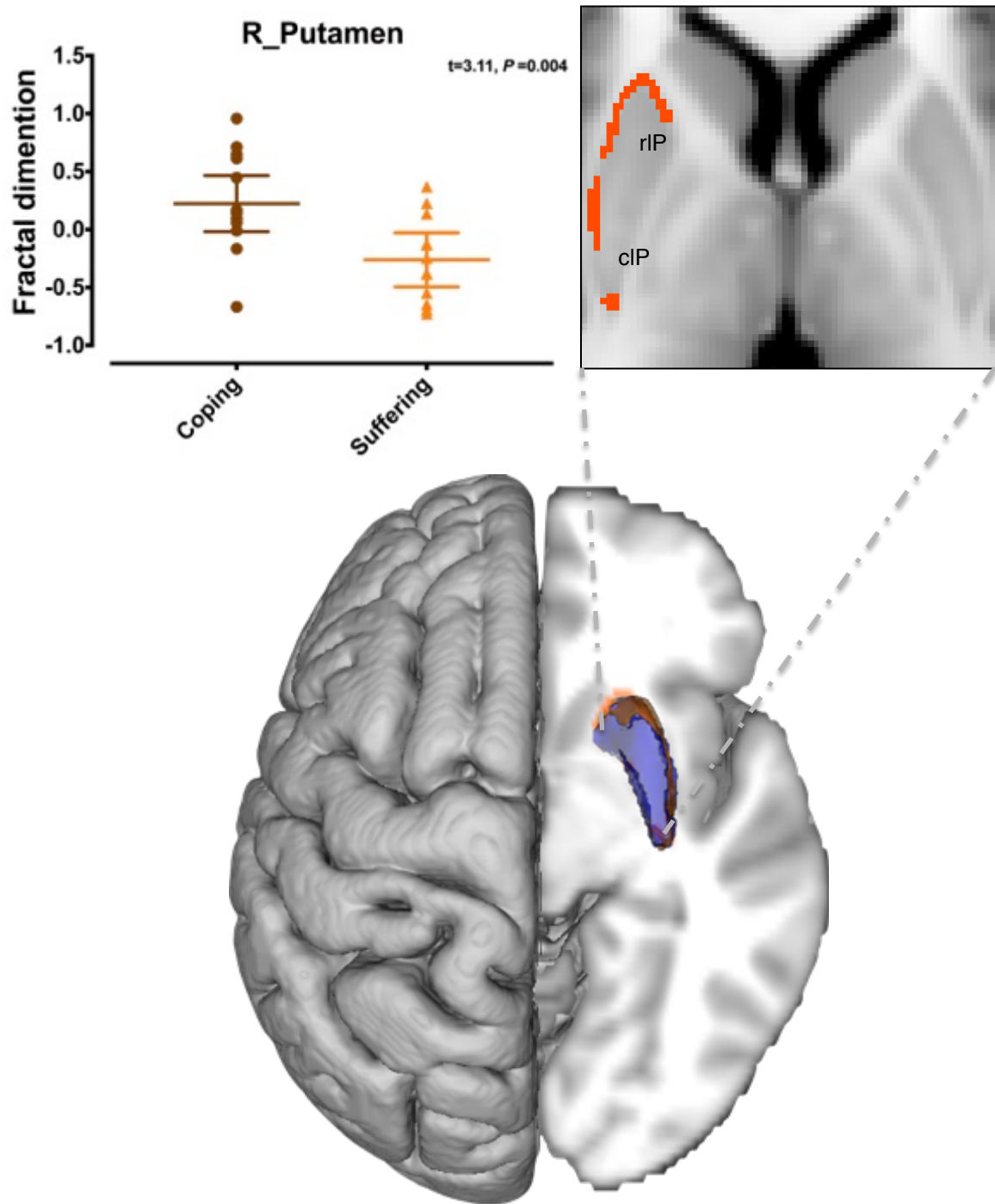


Figure 6.28: Statistic map and fractal dimension of shape difference in the rostral lateral (rIPu) and caudal lateral (cIPu) parts of right putamen between the coping and suffering tinnitus subgroups. Subcortical masks and affected regions are coloured in blue and orange respectively.

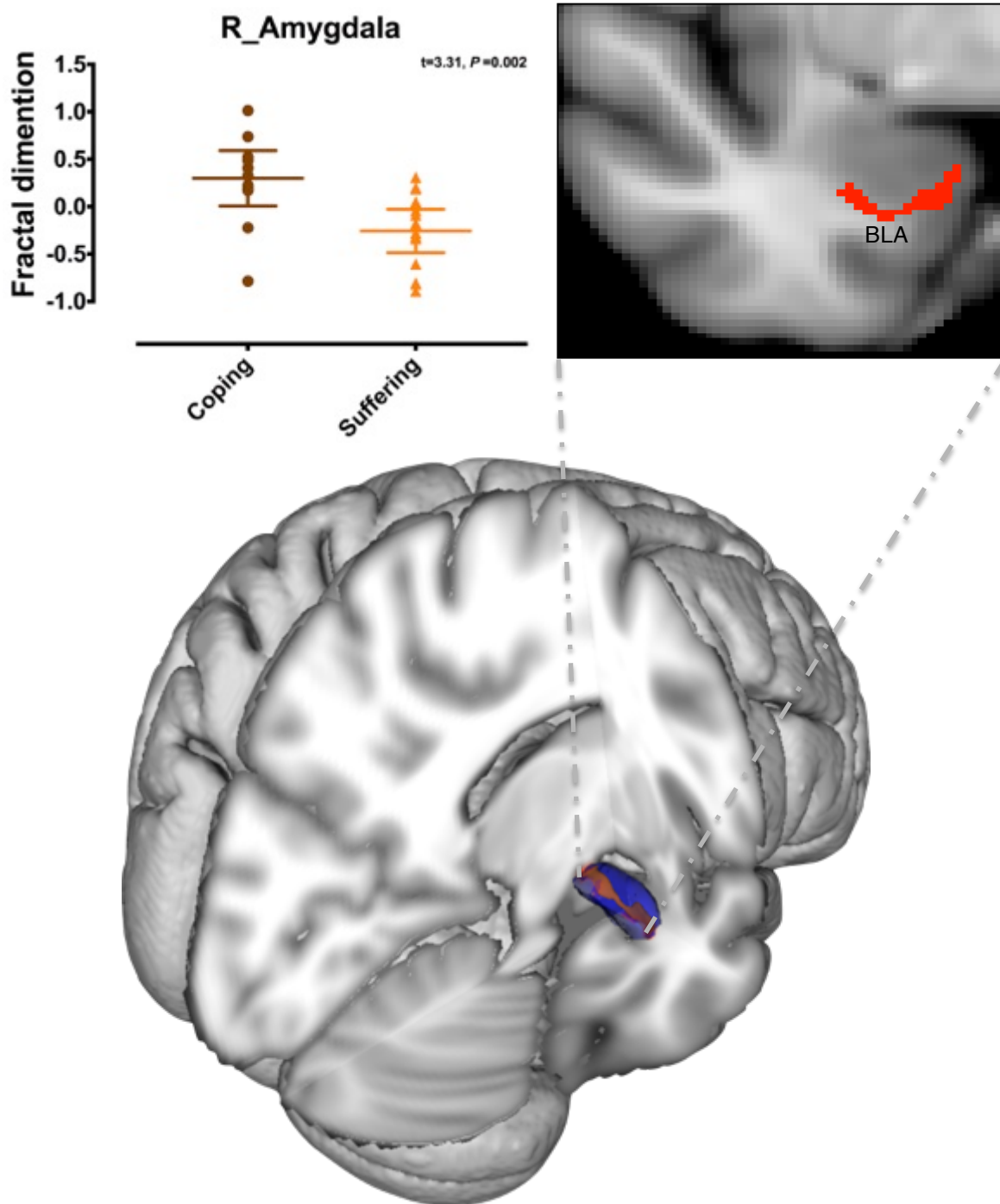


Figure 6.29: Statistic map and fractal dimension of shape difference in in the right basolateral amygdala (BLA) between the coping and suffering tinnitus subgroups. Subcortical masks and affected regions are coloured in blue and orange respectively.

6.5 Discussion

The aim of the present study was to investigate brain structural differences in three participants' groups: normal hearers, mild to moderate hearing loss and tinnitus patients with hearing loss. Also, the influence of tinnitus severity, duration, laterality, and anxiety and depression were assessed. Three neuroimaging structural techniques were performed: voxel based morphometry, cortical thickness analysis and subcortical structures shape differences.

6.5.1 Results summary

Results of the present study revealed as the followings: 1)- the means of intracranial brain volume and grey matter volumes were found to be significantly lower in tinnitus patients compared to normal hearers, 2)- GM reduction was found at orbital frontal, insula and visual cortices in tinnitus patients compared to normal hearers, 3)- the mean of cortical thickness was found significantly lower in tinnitus patients compared to the two control groups, 4)- cortical thickness reduction was found at inferior frontal, insula, auditory, para-hippocampus and visual cortices in tinnitus patients compared to control groups, 5)- the volume of left pallidum subcortical structure was found significant lower in tinnitus patients compared to MH group, 6)- a significant negative correlation was found between the volume of left hippocampus and tinnitus severity (measured by TFI) (this correlation was not survived after correction for multiple comparisons), 7)- bilateral tinnitus subgroup showed a significant cortical thinner in left ACC, Cuneus and temporal pole compared to unilateral tinnitus subgroup, 8)- Tinnitus suffering subgroup showed thicker cortex at left anterior cingulate cortex (ACC) and right posterior cingulate

cortex (PCC) compared to coping tinnitus subgroup, 9)- unilateral tinnitus subgroup showed a significant reduction of the subcortical volume in right nucleus accumbens (NAc) compared to bilateral tinnitus subgroup, 10)- tinnitus related to subcortical structure surface shape differences were found in bilateral pallidum, brainstem, right putamen, hippocampus and amygdala.

6.5.2 Tinnitus perception and auditory pathway

The involvement of auditory pathway in tinnitus perception has been demonstrated previously in many studies using wide range of image acquisition, contrast designs and imaging processing software (Aldhafeeri et al., 2012a, Landgrebe et al., 2009, Schneider et al., 2009, Arnold et al., 1996, Schecklmann et al., 2013). Tinnitus patients showed a reduction of cortical thickness in primary auditory cortex compared to controls (Aldhafeeri et al., 2012a). This result is consistent with our finding as tinnitus patients showed a thinner primary auditory cortex compared to normal hearers. However, mild to moderate hearing loss group did not show a significant difference compared to tinnitus patients of cortical thickness at primary auditory cortex. This may indicate the influence of hearing loss beside tinnitus perception to cause brain atrophy at the center of auditory system.

6.5.3 Tinnitus severity and brain morphology

Recently, the association between GM volume changes and tinnitus severity was determined as a negative correlation in temporal lobe (Boyen et al., 2013). Also, tinnitus severity was found to be positively correlated with cortical thickness in anterior insula (Leaver et al., 2012). In contrast to our study, tinnitus severity was found associated with GM volume changes in left hippocampus (this correlation did

not survive correction for multiple comparisons), and cortical thickness measurements in left anterior cingulate cortex and right temporal pole. Hippocampus is primarily associated with emotion, memory and autonomic nervous system processing (Duvernoy et al., 2013). Several studies have implicated hippocampus in chronic depression (Sapolsky, 2001, Campbell and MacQueen, 2004), persistent pain (Mutso et al., 2012) and chronic stress (Conrad, 2008). Anterior cingulate cortex (ACC) has a unique role in cognition and emotion that connects limbic system and prefrontal cortex (Stevens et al., 2011). The activity of the ACC was found significantly lower in posttraumatic stress disorder (PTSD) during fear condition (Etkin and Wager, 2007). The association between tinnitus and PTSD was determined (Fagelson, 2007). Temporal pole (TP) is considered as part of the limbic lobe, which connects posteriorly the orbital frontal cortex and laterally amygdala based on its anatomical location in the brain (Olson et al., 2007). TP atrophy was found in the frontal temporal dementia (FTD), which makes changes in FTD patients' personality and social appropriate behavior (Thompson et al., 2003).

6.5.4 Tinnitus perception and insula

Insula cortex is a multimodal brain region that plays important roles in the processing of emotional (Kurth et al., 2010), pain (Nagai et al., 2007) and auditory (Bamiou et al., 2003). Insula cortex atrophy and dysfunction has been demonstrated previously in Schizophrenia (Crespo-Facorro et al., 2000), PTSD (Rauch et al., 1996) and post stroke depression (Manes et al., 1999). Our findings of GM and cortical thickness reduction in insula cortex may confirm the involvement of this region in the tinnitus perception. Furthermore, a positive correlation was found between

insula cortex thickness and depression and anxiety scores in patients not controls, which was suggested that insula may play a role in effective reactions specified to tinnitus (Leaver et al., 2012). Recently, insula was found to be associated with an early emotional reaction to tinnitus (Carpenter-Thompson et al., 2015). From these findings, we may hypothesize that the insula cortex might play an important role in tinnitus perception and severity. Further research is required into the neural substrate including insula cortex in order to understand the pathophysiology of tinnitus perception.

6.5.5 Tinnitus perception and orbital frontal cortex

Orbital frontal cortex (OFC) is part of the prefrontal cortex (ventromedial) that is involved in the processing of emotion, memory and decision-making (Rolls, 2004). Different neuroimaging studies have shown the involvement of OFC in tinnitus perception (Seydell-Greenwald et al., 2012a, Leaver et al., 2012, Leaver et al., 2011, Rauschecker et al., 2015, De Ridder et al., 2011, Aldhafeeri et al., 2012a). OFC atrophy and dysfunction have been reported in posttraumatic disorder (PTSD) (Geuze et al., 2008), attention deficits (Makris et al., 2007) and major depression (Li et al., 2007), which share some symptoms with chronic tinnitus. Similarly, our findings revealed a reduction of GM and cortical thickness of OFC in tinnitus patients. It is reported that tinnitus sufferers have depression (Falkenberg and BøWie, 2012), memory impairments and attention deficits (Rossiter et al., 2006).

6.5.6 Tinnitus perception and subcortical structures

Neuroimaging studies have shown brain atrophy in different cortical structures in tinnitus patients; however, the findings of subcortical structures are less straightforward because of the anatomical characteristics of subcortical structures (i.e. small size and close to large arteries and ventricles) that considered as a challenging for data analysis (Elgoyhen et al., 2015). Subcortical structures have complex roles in motor and non-motor functions, which receive massive inputs from the cerebral cortex (Elgoyhen et al., 2015).

Few studies have examined the influence of tinnitus perception on subcortical structures. Cortico-limbic morphology was examined in tinnitus patients, and found that no significant difference of subcortical structures' volumes between tinnitus and control groups (Leaver et al., 2012). However, I found the volume and surface shape of subcortical structures might be related to tinnitus patients as a significant reduction of the left pallidum volume was found in tinnitus patients compared to MH, and a significant negative correlation was found between left hippocampus volume and tinnitus severity (measured by TFI). Also, the surface shape of pallidum, brainstem and hippocampus structures were found significantly different in tinnitus patients compared to controls. In terms of coping and suffering from tinnitus, I found that the surface shape of amygdala and putamen associated with tinnitus severity.

Pallidum and putamen structures are parts of the basal ganglia that play an important role in reward and motivation, and considered as a limbic final common

pathway (Smith et al., 2009). We were able to identify the involvement of pallidum and putamen structures in tinnitus perception.

Hippocampus and amygdala are main parts of limbic system that involved in emotion and memory, and showed the involvement in tinnitus (De Ridder et al., 2006a). Moreover, hippocampus is considered as the major brain site of neurogenesis that was found involved in noise induced hearing loss (Liu et al., 2016) and tinnitus (De Ridder and Vanneste, 2014). In our study, we were able to identify the involvement of basolateral amygdala (BLA) and cornu amonis of hippocampus (CA1) in tinnitus perception. It was stated the projection between BLA and CA1 is disrupted in fear conditions (Sparta et al., 2014). The atrophy found in these subcortical structures might reflect the dysfunction of these structures, and demonstrate the involvement of limbic system in tinnitus perception.

The brainstem plays an important role in the auditory pathway, which connects the acoustic inputs from the cochlea (peripheral auditory system) to auditory cortex (central auditory system). Tinnitus patients showed abnormal patterns of auditory brainstem response (ABR) waves compared to healthy controls (Gabr, 2011, Kehrle et al., 2008). It is believed that the initial tinnitus signal is generated at the brainstem that passes via the midbrain and thalamus to arrive at the auditory cortex for conscious perception (Adjamian et al., 2014). In our study, we were able to identify the significant difference of brainstem structure shape between normal hearing controls and tinnitus patients, which may reflect the influence of tinnitus perception upon hearing sensitivity and integrity of the ascending auditory system (Norrix et al., 2012).

In terms of tinnitus laterality, a significant reduction of right nucleus accumbens (NAc) volume was found in unilateral tinnitus subgroup that showed a higher impact of tinnitus perception on the quality of life (this was discussed in chapter 5) relative to bilateral tinnitus subgroup. In the inhibitor gating tinnitus model, it was reported that the efferents from NAc structure are involved in the cancellation of tinnitus signal at the thalamic level (Rauschecker et al., 2010). This may show the reason of the higher impact of tinnitus perception in unilateral tinnitus compared to the bilateral tinnitus subgroups.

6.5.7 Limitations of the study

There are some limitations of this study. Firstly, the sample size is quite small in the three groups, which could result in lack of the statistical power. In order to counteract type 1 errors, we have corrected the results with multiple comparisons (Bonferroni adjusted).

Second, automatic segmentation performed for cortical thickness analysis may cause some misclassification of some voxels. In other word, given voxels that allocates in GM and classified as WM or CSF because of the limited resolution of MRI scanner. This limitation was solved by visual inspection and manual correction to ensure that voxels are representing the correct brain tissues.

The third limitation in the current study is that structural changes could not be assessed over time as this study was designed as a cross section study not longitudinal that may have higher statistical power because of the smaller within subjects' variability (May et al., 2007). It would be advisable to assess the effect of tinnitus perception in a larger tinnitus population over time in future studies.

Lastly, this study aims to investigate the influence of tinnitus laterality on brain structure. Despite the fact that tinnitus patients were asked which ear was affected, it would be advisable to flip brains to account for possible laterality affected relating to tinnitus laterality findings.

6.6 Conclusion

In conclusion, we found that tinnitus perception might be related to grey matter volume, cortical thickness and subcortical structures changes across different auditory and non-auditory brain areas. We combined three advance MRI techniques: VBM, CTA and subcortical shape appearance in order to assess the influence of tinnitus perception in brain structure.

Auditory, attention and emotional brain networks were found more influenced by tinnitus perception. Hearing loss with tinnitus perception seems to have a significant influence on brain structure. Tinnitus severity was found to be associated with brain atrophy in limbic system, which may reflect the interaction between perception and distress networks in tinnitus.

Chapter 7: Neural Correlates of Auditory Perception in Tinnitus

7.1 Introduction

Auditory perception is the ability of the brain to create a clear interpretation and impression of sounds (Warren, 2008). It is believed that cortical tonotopic maps might be reorganized in the tinnitus population, which might affect the auditory perception in people with tinnitus (Eggermont, 2014). Brain activity was found to be increased significantly in tinnitus models in the auditory cortex (Norena and Eggermont, 2003a). There are some hypotheses of the neural activity changes that correlated to tinnitus perception in animals and humans, for example, increased spontaneous firing rate, increased spontaneous neural synchrony and reorganization of cortical tonotopic maps (Munguia et al., 2013).

Human brain neuroimaging has been used widely to investigate the effect of tinnitus on brain structure and function using different modalities such as single-photon emission computed tomography (SPECT) (Gardner et al., 2002, Farhadi et al., 2010), positron emission tomography (PET) (Mirz et al., 2000a, Mirz et al., 2000b), magnetic resonance imaging (MRI) (Davies et al., 2014, Lanting et al., 2008), electroencephalography (EEG) (Vanneste et al., 2010, Vanneste et al., 2011b) and magneto-encephalography (MEG) (Hoke et al., 1998, Sereda et al., 2013). MRI is considered as one of the most common research instruments used, as it is a non-invasive tool that can combine spatial and temporal resolution features (Husain and

Schmidt, 2014). Functional MRI (fMRI) is a technique that can map brain activity during a specific task (task-based fMRI) or without a task (resting state fMRI).

Functional MRI relies on the Blood-oxygen-level-dependent (BOLD) contrast that measures inhomogeneities in the magnetic field because of the changes in the oxygen level in the blood (Huettel SA et al., 2004). It does not directly measure neural activity (Logothetis and Pfeuffer, 2004) because fMRI uses haemoglobin as an endogenous contrast agent that creates an fMRI signal based on the magnetization difference between oxy- and deoxyhaemoglobin (Arthurs and Boniface, 2002).

Abnormal brain activity was found in the tinnitus population in auditory brain areas (Gardner et al., 2002) and non-brain areas such as the limbic system including amygdala (De Ridder et al., 2006b) and prefrontal cortex (PFC) (Seydell-Greenwald et al., 2012b). Abnormalities of the auditory cortex in the tinnitus population have been reported previously in structural and functional neuroimaging studies. Reduction of auditory cortex grey matter was found in tinnitus patients compared to a normal healthy group (Schneider et al., 2009). Moreover, cortical thickness analysis revealed that tinnitus patients have thinner auditory cortices compared to a healthy group (Aldhafeeri et al., 2012a). Furthermore, the tonotopic map of the auditory cortex was found to be disturbed in tinnitus subjects compared to the normal healthy group (Mühlnickel et al., 1998). In addition, functional connectivity between inferior colliculi and auditory cortices was found to be significantly reduced in tinnitus patients with mild to moderate sensorineural hearing loss compared to the normal healthy group (Boyen et al., 2014b). These structural and

functional abnormalities may reflect the dysfunction of auditory cortex function, which could be the origin or a consequence of tinnitus perception.

In the current study, we aim to investigate the influence of tinnitus on auditory perception using blocked design fMRI stimuli. Three different groups were enrolled in this study: the normal hearing (NH), mild to moderate hearing loss (MH) and tinnitus with hearing loss (TI). We used a pure tone stimulus to evoke auditory brain areas and investigate their responses to acoustic stimuli. The difference between groups and correlation with some variables such as tinnitus severity and onset were investigated with respect to the magnitude of BOLD fMRI signal changes. We hypothesized that the auditory cortex may show increased brain activity, which may reflect an increased spontaneous firing activity rate that was assumed previously in tinnitus generation mechanism.

7.2 Material and methods

7.2.1 Subjects

(See section 6.2.1 Page 116)

7.2.2 Audiological examination

(See section 6.2.2 page 116)

7.2.3 Behaviour assessments

(See section 6.2.3 page 117)

7.2.4 MRI acquisition

A Siemens 3T Trio (Siemens, Erlangen, Germany) with a standard 8 channels head coil was used. In order to control head movement during the scan, we used foam padding and head resistance. Structural images were acquired to perform spatial normalization and localization using a 3D modified driven equilibrium Fourier

transform (MDEFT) sequence, with acquisition parameters of: TR/TE 7.92/2.48 ms, flip angle=16°, 176 volumes, slice thickness 1.00 mm, FOV 256*256 mm² and scan time 12 min 51 s.

Functional MRI data were acquired axially, with slices aligned parallel to each subject's own AC-PC plane. The functional acquisition scan was 6 minutes and 30 seconds in length using BOLD echo-planer imaging sequences with the following acquisition parameters: TR 3000 ms; TE 30 ms; slice thickness 2.5 mm; matrix size 128*128; imaging resolution 2*2*2 mm³; interleaved slice order; slice gape 3mm).

7.2.5 Auditory stimuli

The auditory stimulus used in this study was programmed using E-Prime software (<https://www.pstnet.com/eprime.cfm>). Participants were listening to a pure tone during the task-based fMRI session. This function scan used a blocked design that consists of 10 rest conditions and 9 task conditions, and each of these condition lasted 20 seconds long (figure 7.1). In order to take advantage of the fMRI initial dip, the blocked designs were begun 5 s after the acquisition start, and finished with acquisition run.

In the task conditions, pure tone noises were generated via Audacity Software <http://audacity.sourceforge.net> and delivered by an MR Confon auditory stimulator starter kit that is MR-compatible and provides auditory stimulation from PC via specially adapted headphones. The pure tone stimulus consisted of four different frequencies (440, 880, 1600 and 2000 Hz) with a spectral modulation density of 1 cycle per octave and modulation amplitude of 80%, and each one was run for five seconds and formed in a train of Sine waves. In the rest condition, no stimulus was

presented to the subjects. Participants were instructed to stay awake and listen to the auditory stimuli.

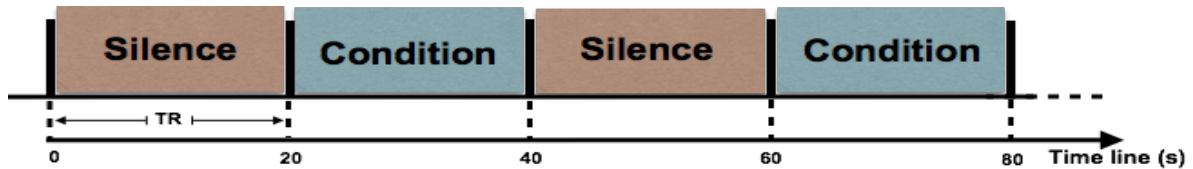


Figure 7.1: Blocked design of fMRI using auditory stimuli, showing rest (silence) and active (condition) epochs.

7.2.6 fMRI data analysis

Functional MRI data was analysed using a FEAT toolbox as part of the FMRIB Software Library (FSL), Oxford, UK. Before running FEAT, multiple 3D images for each subject were merged as a 4D image. A higher-level analysis option was selected to infer across multiple subjects and different groups. Functional data was corrected for motion, and then co-registered to individuals' structural images (T1-weighted images) and finally spatially smoothed to 5 mm. A GLM model was created to inform the software of the number of variables (two groups), conditions (task and rest) and test types (one, two sample t-tests and correlation analysis) using higher level mixed effects analysis. Then, a cluster-based threshold was carried out in this analysis. BOLD signal change (average of Beta value) was estimated using the Featquery tool as part of the FMRIB Software Library (FSL), Oxford, UK. All the results were corrected for multiple comparisons. The FSLView tool (Version 3.2.0) and Mricro software (Version 1.6.0) were used to display the results.

An additional and separate analysis was conducted to examine the association between the composite measure of tinnitus severity, duration and hearing loss and the BOLD activity. A single-group average with additional covariate was applied by

entering an extra EV that is orthogonal with respect to the group mean and demeaned. Two contrasts were set that are positive and negative.

7.3 Results

7.3.1 Demographics

(See section 6.4.1 Page 122)

7.3.2 Audiometry findings

(See section 6.4.2 Page 124)

7.3.3 BOLD whole brain analysis

- Single-group average (One sample T-test):

The significant sound evoked responses of each group are shown in figure 7.2. The mean activation of the NH groups showed significant activations in the right and left superior temporal, and inferior gyri, whereas the mean activation of the MH and TI groups showed significant activation only in the right and left superior temporal gyri.

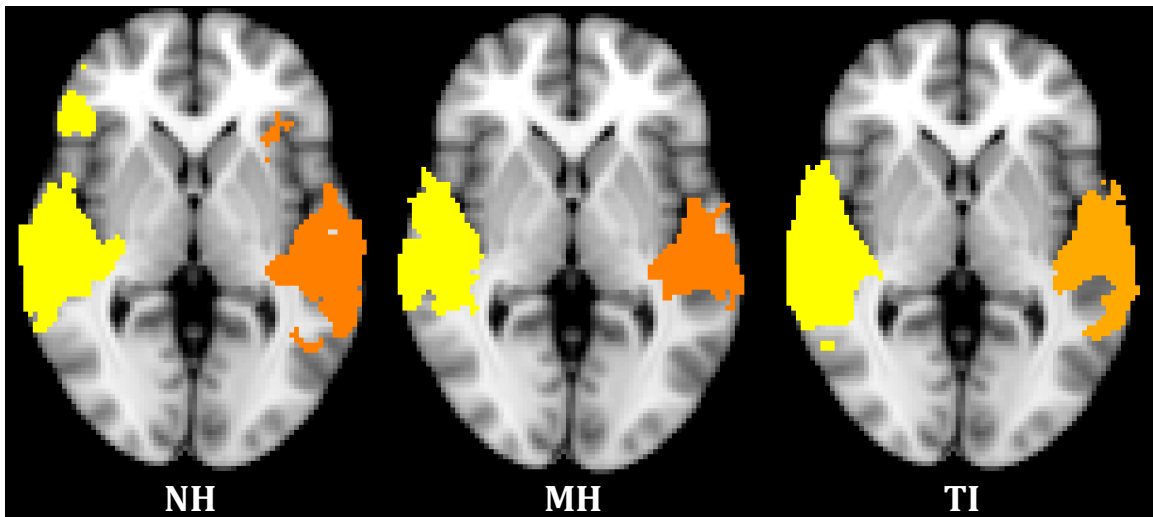


Figure 7.2: Whole group one sample t-test for normal hearing subjects (NH), mild to moderate hearing loss subjects (MH) and tinnitus patients (TI). FSLview tool was used to display the results overlay on the standard template MNI152 T1 2mm brain.

- Unpaired two-group difference (Two-sample Unpaired T-test)

Normal hearers showed significant activation in supramarginal (SMG), inferior (IFG) and middle frontal gyri (MFG) of the right hemisphere compared to mild to moderate hearing loss ($P < 0.05$) (figure 7.3 and table 7.1). On the other hand, the left hemisphere did not show any significant activation differences between normal hearers and mild to moderate hearing loss subjects (FDR, $P < 0.05$).

The tinnitus group showed a significant increased of BOLD signals compared to the MH group in the right hemisphere of the primary and secondary auditory cortex, visual cortex, and middle temporal gyrus (figure 7.4 and table 7.2). No significant difference of BOLD changes was found between tinnitus patients and the normal hearing (NH) group.

Comparison between the auditory perception of tinnitus coping and suffering subgroups revealed that no significant difference was found of the auditory perception pattern in tinnitus subjects who are coping with tinnitus and those who are not. Also, no significant differences of auditory perception were found between unilateral and bilateral tinnitus, and between tinnitus anxious and depressed and tinnitus not anxious and depressed subgroups.

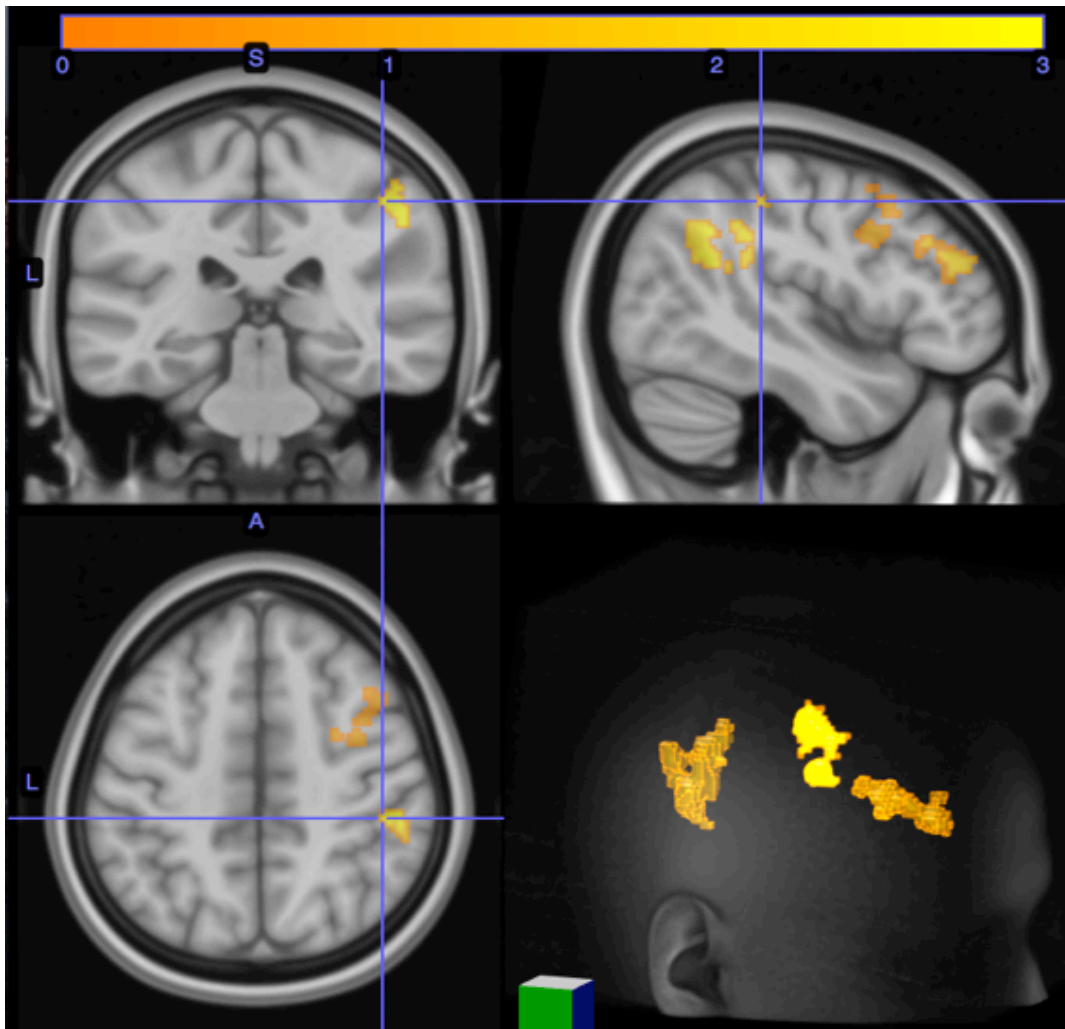


Figure 7.3: T-test maps of fMRI activity between (NH) and (MH) groups. MRIcro software was used to display the results overlay on a standard template MNI152 T1 0.5 mm brain.

Table 7.1: Location of the maxima intensities as in figure 7.3 based on whole brain analysis corrected for multiple comparisons. SMG: supramarginal gyrus, IFG: inferior frontal gyrus, MFG: middle frontal gyrus.

Cluster regions	Voxels	Z score	P	MNI coordinate
Right SMG	630	4.33	0.001	56 -40 -34
Right IFG	502	3.78	0.005	30 26 -10
Right MFG	404	3.55	0.02	38 10 50

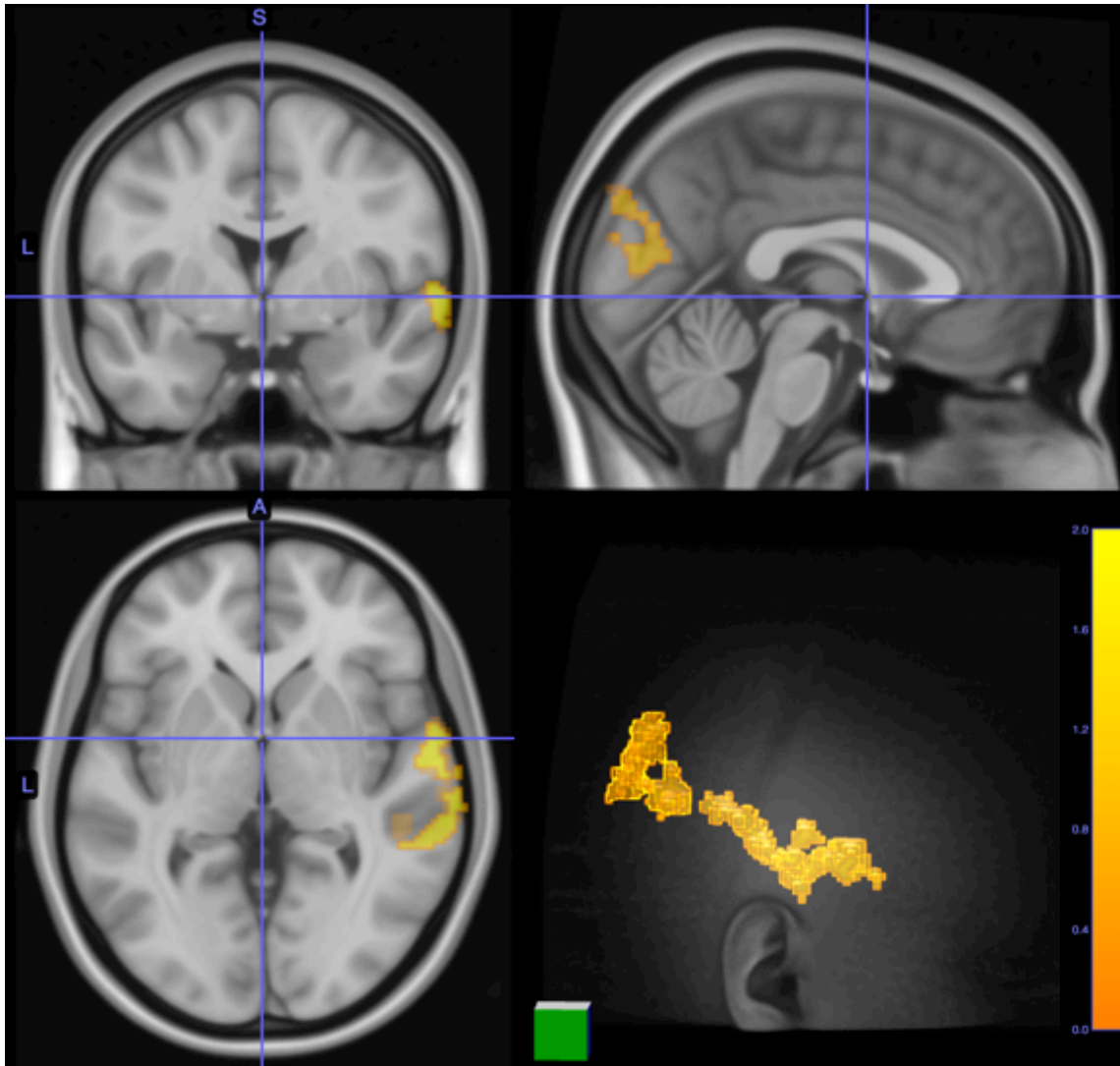


Figure 7.4: T-test maps of fMRI activity between (MH) and (TI) groups. MRIcro software was used to display the results overlay on a standard template MNI152 T1 0.5 mm brain.

Table 7.2: Location of the maxima intensities as in figure 7.4 based on whole brain analysis corrected for multiple comparisons, one for each ROI (MNI coordinates) and their P-values and z scores. STG: superior temporal gyrus, MTG: middle temporal gyrus, LOG: lateral occipital gyrus.

Cluster regions	Voxels	Z score	P	MNI coordinate
Right STG	951	5.37	1.53e-05	62 2 0
Right MTG	700	4.82	0.0001	60 -36 2
Right LOG	680	3.97	0.0003	10 -94 24

7.3.4 Correlation analysis

Correlation analysis was carried out to examine the influence of hearing loss, tinnitus duration and severity on the auditory perception. Hearing loss (HL) was found to be significantly positively correlated with the change of BOLD fMRI activity at the bilateral primary auditory cortices and right inferior frontal gyrus (figure 7.5, table 7.3). A significant positive correlation was found between tinnitus duration and the change of BOLD fMRI activity in the left superior temporal and frontal gyri, and right middle temporal and precentral gyri (figure 7.6, table 7.3). Moreover, tinnitus severity (THI and TFI scores) was found to be significantly positively correlated with BOLD signal changes in the right and left superior temporal gyri (STG) (figure 7.7, table 7.3).

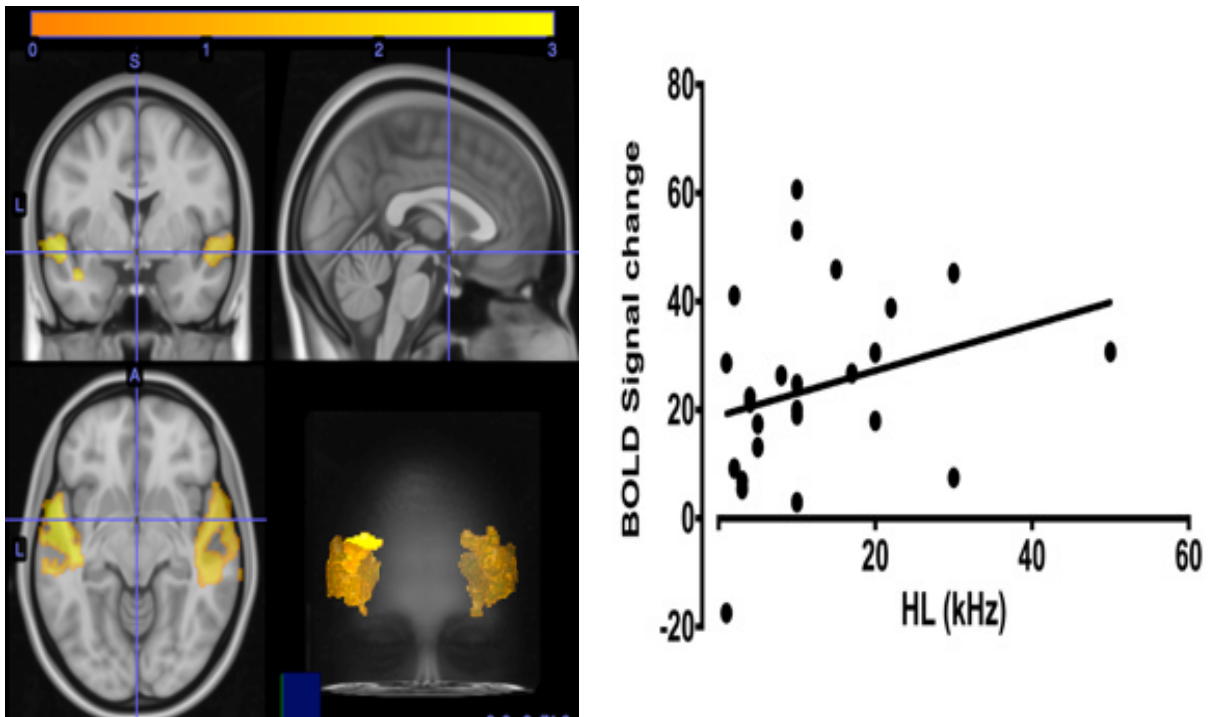


Figure 7.5: T-statistic map of the correlation between hearing loss (HL) and BOLD signal change in tinnitus patients. MRIcro software was used to display the results on a standard template MNI152 T1 0.5 mm brain.

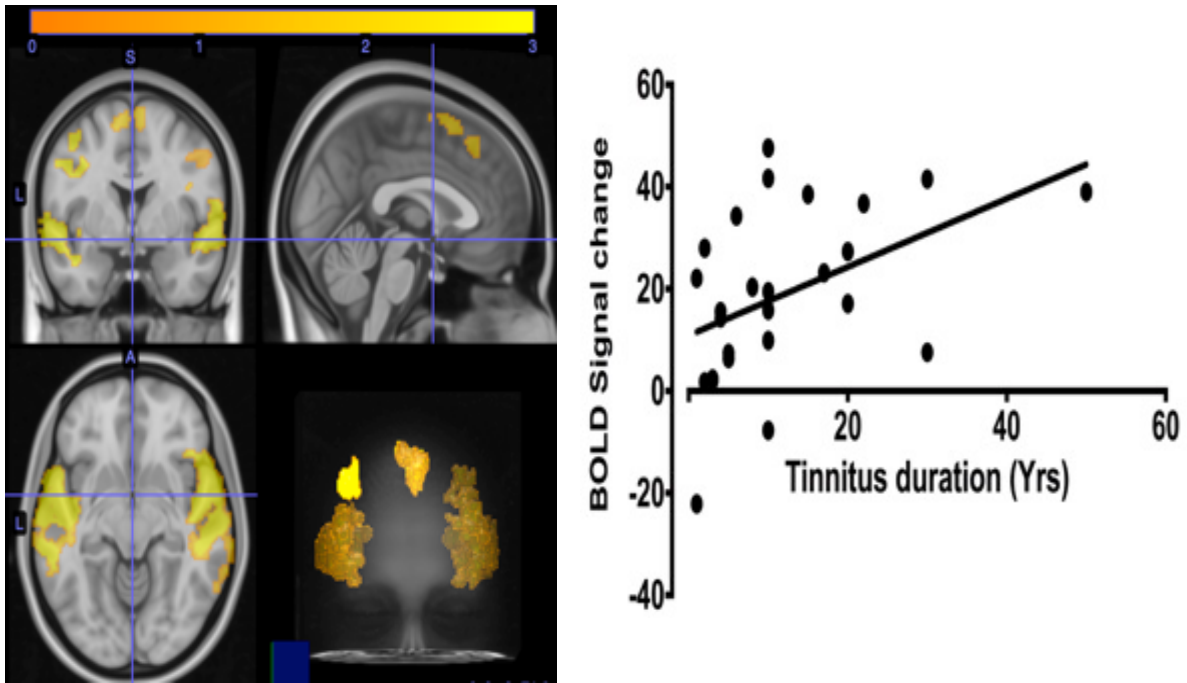


Figure 7.6: T-statistic map of the correlation analysis between tinnitus duration and BOLD signal change. MRIcro software was used to display the results overlay on a standard template MNI152 T1 0.5 mm brain.

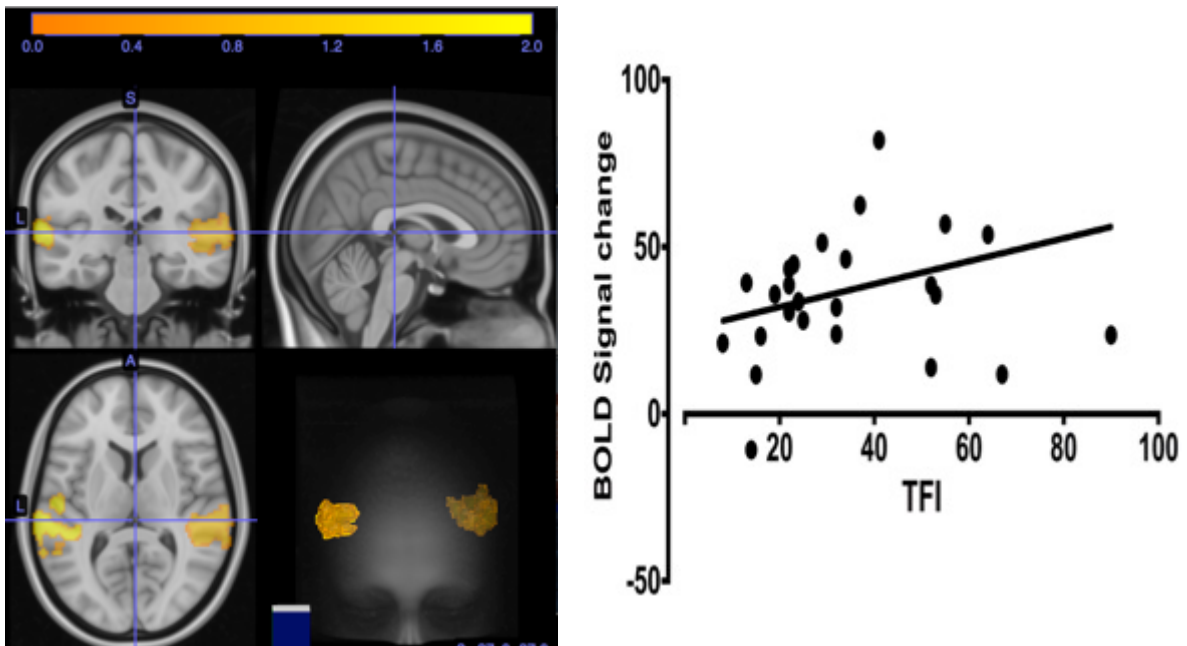


Figure 7.7: T-statistic map of the correlation analysis between tinnitus severity and BOLD signal change. MRIcro software was used to display the results overlay on a standard template MNI152 T1 0.5 mm brain.

Table 7.3: Correlation analysis findings of BOLD activity based on the whole brain analysis corrected for multiple comparisons.

Variables	Brain regions	Voxels	Z score	P	MNI coordinate (x, y, Z)
Hearing loss	Left PAC	4589	5.03	5.9e-17	-50 -6 -8
	Right PAC	4058	4.98	1.6e-15	48 -28 6
	Right IFG	403	4.65	0.0279	56 20 18
Tinnitus duration	Left STG	5961	5.18	1.2e-20	-60 -34 2
	Right MTG	5326	5.81	4.7e-19	46 -34 -4
	Left SFG	665	3.71	0.000948	-6 26 56
	Right PCG	472	4.28	0.0104	52 0 40
Tinnitus severity	Left STG	1016	4.68	1.7e-05	-66 -26 10
	Right STG	965	3.82	3.0e-05	58 -28 8

PAC: primary auditory cortex, STG: superior temporal gyrus, MTG: middle temporal gyrus, IFG: inferior frontal gyrus, SFG: superior frontal gyrus, PCG: precentral gyri.

7.4 Discussion

This study aims to investigate the effect of tinnitus perception on the auditory perception using fMRI-blocked design with acoustic stimuli. Whole brain analysis was carried out to test the influence of tinnitus perception on auditory processing.

7.4.1 Results summary

The findings of the present study revealed the following: 1)- tinnitus patients showed increased brain activity compared to the MH group in the right of the superior, middle temporal and lateral occipital gyri, 2)- mild to moderate hearing loss subjects showed a significant reduction of brain activity compared to normal hearers in the right supermarginal, inferior and middle frontal gyri, 3)- hearing loss was found to be significantly positively correlated with brain activity in the bilateral auditory cortex and right inferior frontal gyrus, 4)- significant positive correlation

between brain activity and tinnitus duration in the superior and middle temporal and superior inferior gyri, 5)- and significant positive correlation between brain activity and tinnitus severity in the superior and middle temporal gyri.

7.4.2 Hyperactivity of auditory cortex in tinnitus patients

In the present study, we focused on auditory perception and how the auditory cortex responds to acoustic stimuli. Primary and association auditory cortices are allocated in the superior temporal gyrus that were reported as strongly associated with tinnitus generation (Leaver et al., 2011, Eggermont and Roberts, 2004). Many tinnitus models have hypothesised that the dysfunction of the auditory cortex could cause tinnitus perception by increasing the spontaneous stochastic fired rate in the auditory pathway (Jastreboff, 1990, Eggermont and Roberts, 2004, Sun et al., 2009). Furthermore, it was demonstrated that tinnitus is related to neural plasticity changes in the auditory cortex that may induced auditory phantom phenomenon (Mühlnickel et al., 1998).

7.4.3 Hemispheric asymmetry and tinnitus

Brain hemispheres are anatomically and functionally asymmetric (Dolcos et al., 2002). The left hemisphere is more involved in the processing of verbal information, whereas the right hemisphere is more involved in the processing of spatial information (Nebes, 1974, Sergent et al., 1992). In our study, the right hemisphere was found to be hyperactive in tinnitus patients compared to the MH group at the superior, middle temporal and lateral occipital gyri. The baseline cortical activity in the right hemisphere, regardless of the lateralization of tinnitus was found to be abnormally high in tinnitus patients (Mirz et al., 1999). In addition, the right

hemisphere shows greater age-decline than the left hemisphere (Dolcos et al., 2002).

7.4.4 The role of inferior frontal gyri in tinnitus

The pattern of BOLD activity was found to be slightly different between the three groups as the frontal and temporal lobe are involved in the NH group, while BOLD activity was only shown at the temporal lobe in the MH and TI groups. Brain regions of the frontal lobe have specific functions such as behaviour (emotion), thinking initiation and memory. The dysfunction of the inferior (orbital) frontal cortex has been demonstrated previously in addiction (Goldstein and Volkow, 2011), clinical depression (George et al., 1994) and schizophrenia (Takei et al., 2013).

7.4.5 The pathophysiology mechanism of hearing loss and tinnitus

Consistent with our results, previous studies have shown the increase of brain activity in the auditory cortex in the tinnitus population (Seydell-Greenwald et al., 2012a). Animal studies have demonstrated increased spontaneous activity of sound evoked stimulus in the auditory pathway (Roberts et al., 2010). In the present study, we could pick up the differences of BOLD oxygenation level in the auditory cortex between the mild to moderate hearing loss group and tinnitus patients who showed a significant increase of brain activity compared to the controls. Interestingly, the TI group did not show significant difference of auditory perception compared to the NH group, whereas the difference was survived in comparison to the MH group.

7.4.6 The effect of tinnitus duration and severity on brain function

Correlation analysis showed that the duration of tinnitus perception is positively correlated with the hemodynamic response to auditory stimuli in the auditory (STG)

and limbic (IFG) brain regions. Also, tinnitus severity measured by THI and TFI showed a significant positive correlation with BOLD signal activity change at only in the auditory brain regions. The dysregulation of auditory and limbic networks in tinnitus has been reported (Leaver et al., 2011). Brain stimulation has been used to reduce brain activity within auditory and non-auditory brain regions (Müller et al., 2013, Plewnia, 2011). However, the efficiency of brain stimulation to reduce tinnitus severity and improve the quality of life was found to be limited (Baguley et al., 2013).

7.4.7 Study limitations

Despite the fact that this study was designed carefully, there are some limitations that need to be considered carefully in future studies. The acoustic stimulus was presented during the noise of MRI acquisition scan that might interpret the measured BOLD signal. Therefore, it would be advisable to use “sparse” temporal sampling that acquires single volume of brain images at the end of stimulus and baseline conditions in order to reduce the effect of the scanner noise (Hall et al., 1999). Although “sparse” temporal sampling has the advantage of reducing the effect of scanner noise, there are some drawbacks of this technique such as less information acquired and longer acquisition time that need to be considered carefully (Peelle, 2014).

7.5 Conclusion

In summary, in order to find a complete cure for tinnitus, it is essential to understand the natural basis of auditory perception in the tinnitus population. The present study reported the differences of auditory perception between three groups: normal hearers, mild to moderate hearing loss participants, and tinnitus sufferers. The auditory cortex was the main target in this study as it is the main station of auditory processing. The dysfunction of the auditory cortex was found as the tinnitus group showed hyperactivity compared to the mild to moderate hearing loss group. We conclude, therefore, that the auditory cortex plays an important role in tinnitus perception as its function was found to be disturbed in tinnitus patients.

Chapter 8: An Investigation the Effect of Tinnitus on the Cerebral Blood Flow (CBF)

8.1 Introduction

Arterial spin labeling (ASL) is a non-invasive technique that has been widely used as a biomarker to diagnose some neurological and psychological disorders such as schizophrenia (Pinkham et al., 2011), dementia (Chao et al., 2010), Alzheimer's (Wolk and Detre, 2012), epilepsy (Pendse et al., 2010) and hallucinating (Steinmann et al., 2014). ASL provides direct quantitative measurements of the cerebral blood flow (CBF), which is associated with brain tissue functioning (Alsop et al., 2015). Resting brain perfusion (ASL) provides quantitative CBF measurements at the baseline, and it does not require the subject to perform a specific task during the scan.

The ASL technique aims to apply radiofrequency (RF) energy to approximately the brain region to be scanned in order to flip the spins in this region from normal alignment with magnetic field B_0 (Deibler et al., 2008). This change in proton spin orientations is the label that is carried by the blood flow downstream, which creates a flow-related signal by subtracting the water from the capillary bed (Alexander et al., 2014). The major benefit of ASL is that it is a non-invasive method of investigating CBF. However, there are some artifacts noted in the use of ASL including label decaying before image acquisitions, tissue misclassification on the subtracted images and other functional MRI common artifacts such as motion and susceptibility artifacts (Deibler et al., 2008).

The influence of tinnitus perception on brain perfusion has been studied previously in order to understand the pathophysiology of the tinnitus mechanism. A significant correlation was reported between brain cortex metabolism and perfusion in subjects with idiopathic tinnitus (Mahmoudian et al., 2013). The effect of tinnitus perception on cerebral perfusion has been explored, and significant regional abnormalities in cerebral perfusion in bilateral of temporal, frontal, parietal and hippocampal amygdala regions in the tinnitus group were found (Shulman et al., 1995). In addition, the pattern of brain perfusion using single-photon emission computer tomography (SPECT) technology using Technetium 99m has been investigated, and the results demonstrated the abnormal pattern of brain perfusion in the tinnitus population in the middle temporal gyrus and temporalparatial cortex (Mahmoudian et al., 2012). In addition, the same technology was used to evaluate the changes in CBF in the tinnitus population, and the results revealed on abnormal CBF status in the parahippocampus gyrus in the tinnitus group (Laureano et al., 2014). Further evidence came from Gardner et al (2002) who reported a significant hypoperfusion in the frontal, parietal and occipital lobes in the tinnitus group compared to in the normal control group, and a positive correlation between the anxiety scale and changes in CBF in the limbic system in tinnitus group (Gardner et al., 2002). To the best of our knowledge, no ASL perfusion MRI study has been conducted in tinnitus research. The purpose of this study was to investigate resting-brain perfusion in the tinnitus population using the whole brain approach. The correlations between the changes in CBF in brain tissue and tinnitus behavior assessments were investigated in this study.

8.2 Materials and methods

8.2.1 Participants and clinical investigations

The characteristics of control and tinnitus patient groups are summarized in Table 8.1. This study-included data collected from 52 participants, who underwent MRI scans: 26 in the normal control group, and 26 tinnitus patients. The tinnitus subjects completed the following assessment questionnaires: Edinburgh Handedness Inventory (EHI), Hospital Anxiety and Depression Scale (HADS), Tinnitus Handicap Inventory (THI) and Tinnitus Functional Index (TFI). A Routine hearing examination was performed for the tinnitus patients using calibrated audiometry, which included seven frequencies (0.5-8.0) kHz used for clinical hearing evaluation (Figure 8.1).

Table 8.1: Participants' characteristics for each group

Characteristics	Control	Tinnitus
N	26	26
Age (yrs)		
Range	(18-60)	(30-65)
M±SD	35±12	44±11
Gender		
M/F	10/16	18/8
Handedness		
R/L	25/1	21/5
H.A.D.S		
Range	---	(0-32)
M±SD		10±6.6
Hearing loss (dB)		
Range	---	(0-60)
M±SD		25±17
Tinnitus duration (yrs)		
Range	---	(1-30)
M±SD		9±8
Tinnitus Type		
BL/UL	---	16/ 10
THI		
Range	---	(6-80)
M±SD		26.2±19.
TFI		
Range	---	(8-90)
M±SD		35.9±21

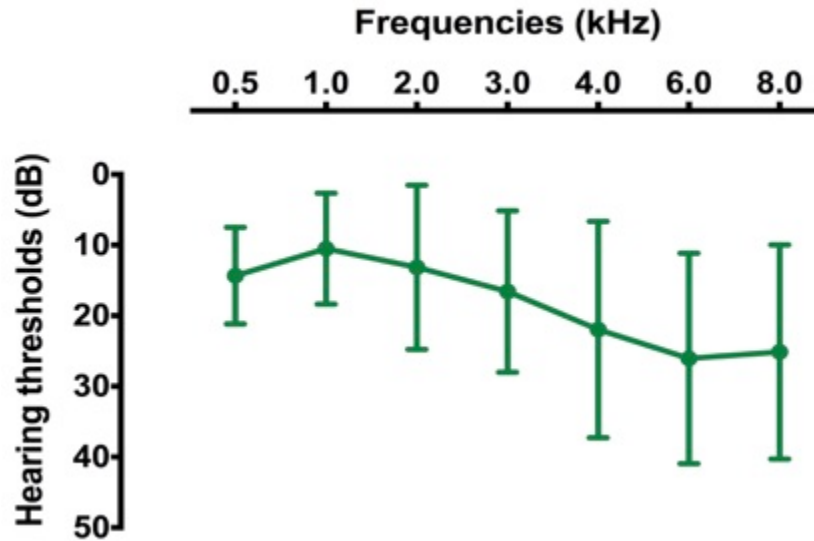


Figure 8.1: Mean and error bar audiogram for tinnitus group (TI).

8.2.2 Image acquisition

ASL raw data were collected on the 3T MRI scanner (Siemens Trio) using a standard eight-channel radiofrequency (RF) head coil. A pulsed ASL technique was used to measure the CBF with the following parameters: TR 2000 ms, TE 19 ms, 150 volumes, flips angle=90°, slice thickness= 5 mm, FOV= 256*256 mm, tag saturation time (TI₁)=0.7 s, tag saturation end-time (TI_{1stop})= 1.3 s, time between the label and readout (TI₂)=1.4 s, tag-width=10 cm, a 10-mm tag-slice gap and crusher gradients with b=5 mms⁻¹. A tag image was acquired at the beginning and this included the inflowing arterial blood water labeled using magnetic inversion (the yellow box, Figure 8.2). Then, a control image was acquired and this included the inflowing blood, which was not labeled (the red box, Figure 8.2). Slice coverage of the ASL sequence was restricted to the frontal, temporal and parietal brain regions. In addition to this scan, Structural images were obtained to perform spatial normalization and localization using a 3D modified driven equilibrium Fourier transform (MDEFT) sequence.

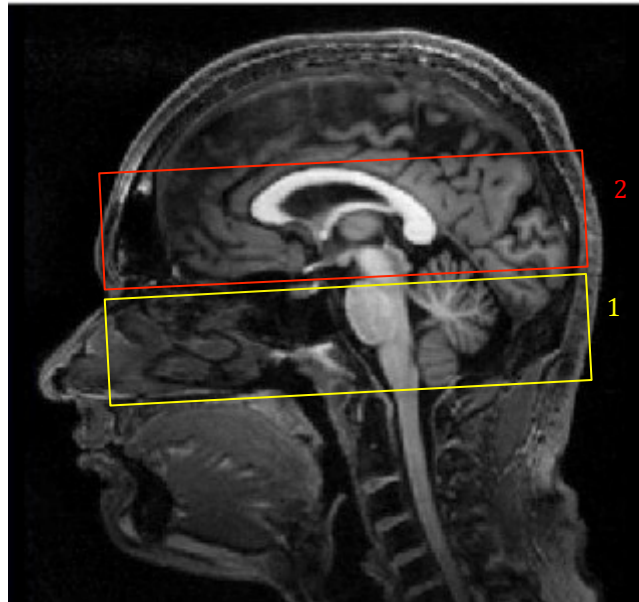


Figure 8.2: Arterial spin labeling (ASL) scan: Box number 1 (**Yellow**) represent the tag images, and box number 2 (**Red**) represents control images.

8.2.3 Image analysis

ASL raw data were corrected for motion, and slice timing. Then, the corrected ASL raw data, including the tag and control images, were subtracted between each others in order to produce perfusion-weighted images. The perfusion images were then normalized and co-registered to a standard space (152 MIN). The normalized images were then smoothed to 10 mm. Two different kinds of designs were applied in this study: the two-sample t-test and correlation analysis. The two-sample t test was applied between two groups: a normal control group, and tinnitus patients. Correlation analysis was applied for different covariates: THI scores, TFI scores, hearing loss thresholds (HLT) and tinnitus duration. Whole brain analysis was conducted in this study using random effect analysis. Perfusion analysis was explained in Chapter 4 (See section 4.8.3, page 89).

8.3 Results

8.3.1 Comparison between healthy controls and tinnitus patients

Global grey matter perfusion analysis carried out on the normal control group and tinnitus sufferers revealed three regions that showed a significant hypoperfusion in the tinnitus group compared to in the control group: the left insula, orbital frontal and visual cortex (Figure 8.3 and Table 8.2).

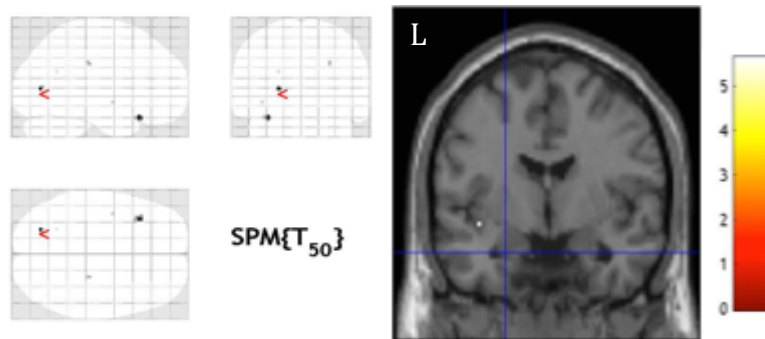


Figure 8.3: Contrast healthy controls > Tinnitus patients
Voxels that were significantly lower perfused in tinnitus patients than in healthy controls. Findings displayed are corrected for multiple comparisons.

8.3.2 Comparison between tinnitus coping and suffering subgroups

In comparing the tinnitus coping (N=13) and tinnitus suffering (N=13) subgroups, we found a significant reduction in CBF in right primary auditory cortex in the subgroup of those suffering with tinnitus compared to in the subgroup of those coping with tinnitus (Figure 8.4 and table 8.2).

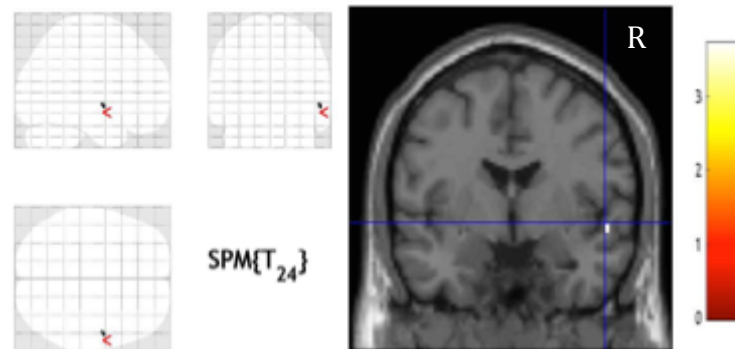


Figure 8.4: Contrast tinnitus coping > Tinnitus sufferers
Voxels that were significantly lower perfused in tinnitus bothersome than in tinnitus non-bothersome.

8.3.3 Comparison between tinnitus patients with high and low level of anxiety and depression

When comparing tinnitus patients with high level in anxiety and depression (N=11) and tinnitus patients with low level in anxiety and depression (N=15) subgroups, we found a significant increase in CBF in right cingulate gyrus in those with tinnitus and high level of anxiety and depression compared to those with tinnitus and low level of anxiety and depression subgroup (Figure 8.5 and table 8.2).

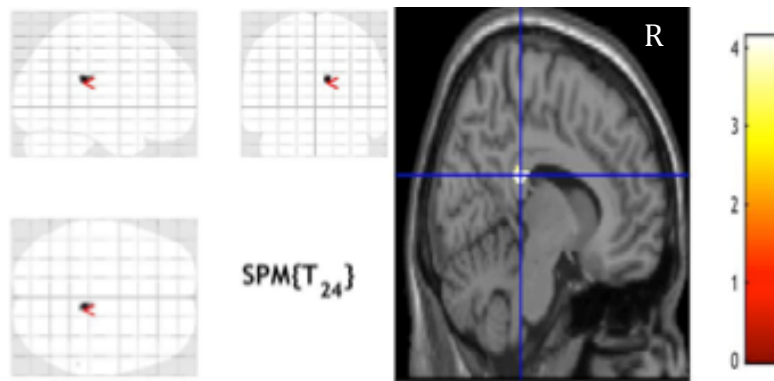


Figure 8.5: Contrast tinnitus with high HADS > tinnitus with low HADS
Voxels are significantly hyperperfused in tinnitus patients with high HADS compared to tinnitus patients with low HADS

Table 8.2: Perfusion analysis findings of cerebral blood flow changes relative to tinnitus.

Contrast	Brain regions	MNI coordinate	t	P
Controls > Patients	Left Insula	-40 -4 -6	4.70	0.045
	Left Orbital frontal	20- 34 12	4.91	0.019
	Left Visual	-24 -74 6	4.93	0.017
Tinnitus copers > Tinnitus sufferers	Right Auditory cortex	54 -4 -6	3.71	0.01
Tinnitus with High HADS> tinnitus with low HADS	Right Posterior	10 -34 22	3.56	0.01
	Cingulate gyrus			

8.3.4 Correlation analysis

Correlation analysis was performed in order to investigate the association between brain perfusion patterns and hearing loss, tinnitus duration and severity in tinnitus patients. Hearing loss in tinnitus patients was found to be significantly negatively correlated with brain perfusion in the bilateral brainstem (midbrain) (Figure 8.6 and Table 8.3). Furthermore, it was found that tinnitus duration is significantly positively correlated with the amount of CBF in the bilateral superior temporal, right middle temporal and left precentral gyri (Figure 8.7 and Table 8.3). Tinnitus severity was estimated by measuring the scores of two different tinnitus self-assessment questionnaires namely the THI and TFI. It was found that tinnitus severity is significantly negative correlated with CBF in left the parahippocampus gyrus (Figure 8.8 and Table 8.3).

Table 8.3: The correlation analysis findings of CBF quantity.

Variables	Correlation	Brain regions	MNI coordinate	t	P
Hearing loss	Negative	Brainstem	14 -34 -4	6.46	0.03
		(Midbrain)	-10 -34 -4	6.86	0.01
Tinnitus duration	Positive	Right MTG	55 -34 4	5.1	1.7365e-05
		Left PCG	-60 -4 20	4.3	2.9410e-04
		Right STG	54 -14 12	4.2	3.4154e-04
		Left STG	-62 -30 10	3.9	3.6509e-04
Tinnitus severity	Negative	Right AC	44 -18 0	6.51	0.0001
		Left PHG	-46 4 -32	6.51	0.0001

MTG: middle temporal gyrus, PCG: precentral gyri, STG: superior temporal gyri, AC: auditory cortex, PHG: parahippocampus gyrus

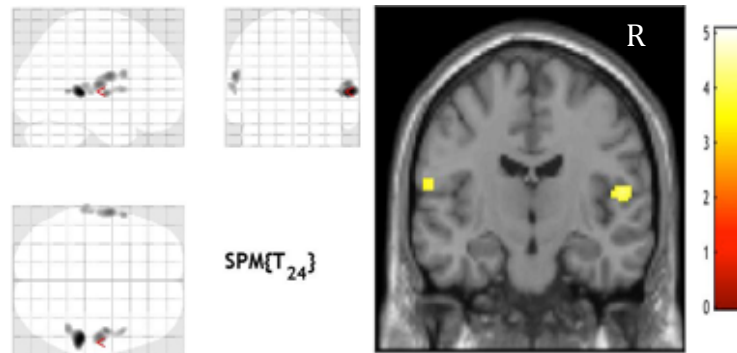


Figure 8.6: The correlation analysis findings between CBF quantity and hearing loss in tinnitus patients.

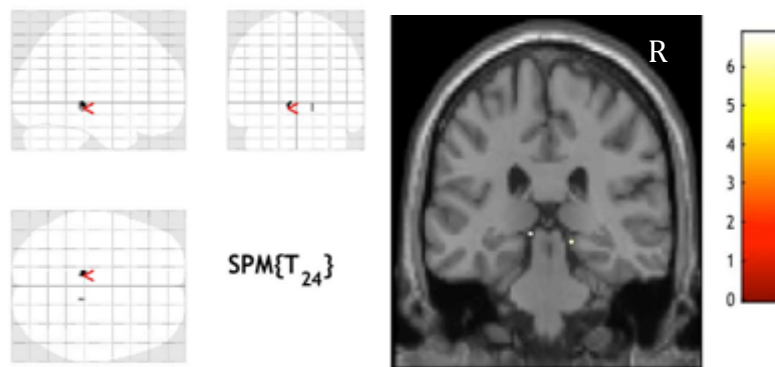


Figure 8.7: The correlation analysis findings between CBF quantity and tinnitus duration.

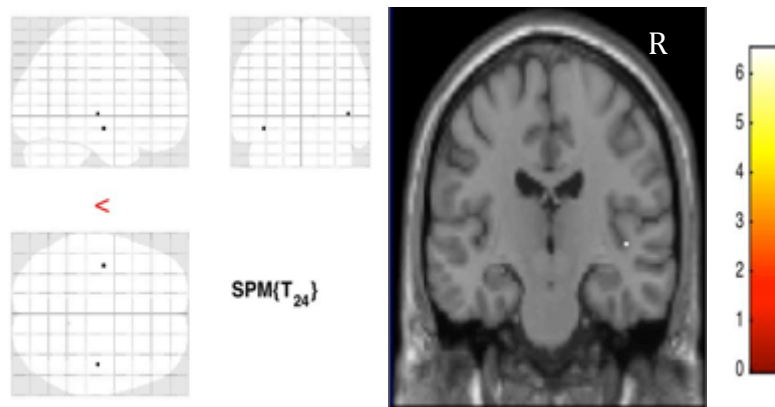


Figure 8.8: The correlation analysis findings between CBF quantity and tinnitus severity.

8.4 Discussion

In the present study, the sample included 52 participants who had been undertaken an MRI scan: 26 in the normal control group and 26 tinnitus patients. An

audiological examination and tinnitus self-assessment inventories were performed for the tinnitus group. Pure tone audiometry analysis revealed that tinnitus group had a mild to moderate rang of hearing loss. Pulsed arterial spin labeling (PASL) was used in this study with the aim of quantifying CBF in the brain.

8.4.1 Summary of the results

We demonstrated that the brain perfusion status is significantly different in the tinnitus population compared to normal control group. Significant hypoperfusion brain regions were found in three brain regions: frontal orbital, insula and visual cortices in the tinnitus group compared to in the control group. Moreover, hearing loss and tinnitus characteristics (duration and severity) were significantly correlated with the brain perfusion status in the tinnitus population. Correlation analysis revealed that brain perfusion in the tinnitus population was significantly positively correlated with tinnitus duration and negatively correlated with tinnitus severity in the auditory and limbic networks respectively, and with hearing loss in the brainstem.

8.4.2 Tinnitus and the orbital frontal cortex

Structural and functional alterations in the frontal orbital cortex have been found previously found in tinnitus and other emotional disorders using other neuroimaging modalities. The orbital frontal cortex plays an important role in the emotional processing of sounds (Damasio et al., 1996, Dias et al., 1996). A connection between the orbital frontal cortex and limbic system has been reported (Beauregard, 2007). It was found that patients with lesions in the orbital frontal cortex have a reduced self-evaluated perception of the unpleasant auditory stimuli

(Angrilli et al., 2008). There is evidence of involvement of the orbital frontal cortex in tinnitus, and it has been reported that highly distressed tinnitus patients showed a significant increased of brain activity in the orbital frontal cortex compared to tinnitus patients with low levels of distress (Song et al., 2015). Additional evidence of the involvement of the orbital frontal cortex in tinnitus has revealed that females with tinnitus showed a more emotional response to tinnitus than males with tinnitus (Dineen et al., 1997). It has been demonstrated that female tinnitus subjects showed a significant brain activity in the orbital frontal cortex compared to male tinnitus subjects (Koch et al., 2007). These findings along with our results may demonstrate the involvement of the orbital frontal cortex in relation to tinnitus severity.

8.4.3 Tinnitus and the parahippocampus gyrus

The involvement of the parahippocampus gyrus in tinnitus perception has been found in previous neuroimaging studies (Laureano et al., 2014, Vanneste and De Ridder, 2012, Gunbey et al., 2015). The posterior part of the parahippocampus is believed to be involved in auditory inhibition (Bickford et al., 1993, Boutros et al., 2008) and this can be prevented by tinnitus perception due to the fact this structure is also involved as well in memory. Unilateral tinnitus subjects showed a higher brain activity in the parahippocampus region compared to the bilateral tinnitus group (Vanneste et al., 2011b). Moreover, this region was found to be involved in tinnitus severity as the highly distressed tinnitus group showed a higher activity compared to the tinnitus group with low level of distress (Vanneste and De Ridder, 2012). Moreover, tinnitus participants who described their tinnitus sound as

narrow band noise showed a significant increased in brain activity in the parahippocampus brain region compared to the pure tone tinnitus group (Vanneste et al., 2010). The parahippocampus has a fundamental function in auditory memory in tinnitus (Shulman, 1995). Our results were found to be in line with previous findings as we could demonstrate that brain perfusion in the parahippocampus is associated with tinnitus severity (the THI and TFI scores). This finding may suggest the role of limbic and memory networks in reaction to tinnitus reaction and explains why some can cope with the symptoms of tinnitus and others cannot.

8.4.4 Tinnitus and the auditory cortex

The auditory cortex plays an important role in auditory perception, and is considered to be the last terminal in the auditory pathway. Structural atrophy and dysfunction of the auditory cortex in tinnitus perception have been identified (Maudoux et al., 2012, Mühlnickel et al., 1998, Schneider et al., 2009, Crippa et al., 2010). In this study, we were able to identify a significant hyperfusion in tinnitus sufferers compared to the subgroup coping with tinnitus in the right auditory cortex, and a positive correlation between the pattern of brain perfusion and tinnitus duration in the bilateral auditory cortex. These significant reductions in brain perfusion in the auditory cortex may reflect the cerebral atrophy in tinnitus sufferers, and explain the reason why they cannot cope with the tinnitus symptoms. As long as tinnitus patients have tinnitus symptoms, the amount of perfusion in the auditory cortex increases, which may suggest the role of habituation to coping with tinnitus symptoms. Therefore, auditory cortex stimulation could be a therapeutic target for tinnitus patients who are not coping and at an early onset of the tinnitus.

8.4.5 Tinnitus and brainstem

The brainstem occurs in the auditory pathway: this contains the cochlear nucleus (CN) and inferior colliculus (IC), which are located in the pons and midbrain respectively. It connects the acoustic inputs from the cochlea (peripheral auditory system) to the auditory cortex (central auditory system). The auditory brainstem response (ABR) has been used to evaluate the cochlea and brainstem auditory pathway, and this estimates hearing sensitivities and assesses the integrity of the ascending auditory system (Norrix et al., 2012). Abnormal patterns in ABR waves were found in tinnitus patients compared to in a healthy control group (Gabr, 2011, Kehrle et al., 2008). An initial tinnitus signal is believed generated at the brainstem and passes via the midbrain and thalamus to arrive at the auditory cortex to achieve conscious perception (Adjajian et al., 2014). In our study, we were able to identify the negative correlation between hearing loss and brain perfusion in the brainstem, which may reflect the influence of tinnitus perception upon hearing sensitivity and the integrity of the ascending auditory system.

8.4.6 Limitations of the study and future studies

There are some limitations in our study. One that must be considered carefully is that the hearing status of normal controls was not examined in this study. This should be examined and compared to that of tinnitus patients in order to eliminate the influence of hearing loss on the results. The sample size is quite small, and this needs to be increased in future studies. More variables can be added in future studies such as, educational, socioeconomic and tinnitus loudness levels. In terms of ASL image acquisition, the subjects' motions during the scan and susceptibility are

common artifacts, and these could be easily misinterpreted as pathology (Pollock et al., 2009). Future studies are required to investigate brain perfusion on a larger population and examine the influence of certain treatments by comparing the brain perfusion status pre-treatment and post-treatment. Tinnitus related to cerebral blood supply alterations could be a vital sign and useful for explaining the tinnitus mechanism and how tinnitus perception is generated.

8.5 Conclusion

In summary, the main aim of this study was to understand the effect of tinnitus perception on brain perfusion in order to understand the pathophysiological mechanism of tinnitus. Despite the fact that many studies have been conducted to gain an understanding of the pathophysiological mechanism of tinnitus perception, there is no globally agreed opinion about how tinnitus perception is generated. This study provides the first evidence for altered brain perfusion in the tinnitus population using non-invasive MRI technology. The findings of this study show that tinnitus perception has a significant effect on brain perfusion. Based on previous neuroimaging studies and our results, we assume there are certain regions of the brain that are involved in tinnitus perception, and that may manage the tinnitus severity. Resting brain perfusion with MRI technology is a non-invasive neuroimaging technique, and is easy to apply; it does not require the subjects to carry out specific tasks during the scan. The perfusion status of three brain networks (auditory, limbic and attention) showed a significant difference and is correlated with tinnitus perception. Measuring resting perfusion with ASL can be a vital clinical tool in tinnitus diagnosis through quantifying cerebral blood flow.

Chapter 9: Neural Correlates of Functional Connectivity (FC) in Tinnitus

9.1 Introduction

In the early stage, blood flow changes could be captured via positron emission tomography (PET), which has some major shortcomings such as poor spatial resolution and radiation exposure (Wineland et al., 2012). Despite the fact that a significant scanner noise is associated with magnetic resonance imaging (MRI), the popularity of this modality has been increasing due to it providing a better spatial resolution without radiation (Wineland et al., 2012). Functional MRI (fMRI) aims to measure cerebral blood flow by measuring changes in blood oxygen level dependent (BOLD) signal (Ogawa et al., 1990). The cerebral blood flow could be measured indirectly during the active (task-based) and silent (resting) states. It has been demonstrated (Biswal et al., 1995) that the correlation of low frequency fluctuations of BOLD activity measured by fMRI could reflect the status of the brain network.

Neuroscientists are studying the correlation between the activities of brain regions to learn about brain FC. The aim is to investigate spontaneous fluctuations in brain activity between two or more anatomically distinct time-series of brain regions (Friston, 2011). It is useful to identify functional alterations in brain activity patterns between two or more groups during rest, which does not require cognitive tasks. These could be assessed using three different methods: independent component analysis (ICA), seed based analysis and graph theory analysis. ICA is whole brain analysis, and does not require a prior hypothesis. It aims to estimate the

time courses of voxels in fMRI scans to produce a specific number of spatial components (Husain and Schmidt, 2014). Seed based analysis is a region of interest (ROI) analysis, and relies on the seeding regions selected that are selected based on the questions being asked by the researchers (Husain and Schmidt, 2014). Graph theory analysis aims to assess the correlation between a set of selected nodes and examines the edges and strengths of the nodes (Husain and Schmidt, 2014).

Brain FC in tinnitus has been investigated using different methods with variable outcomes. FC was found to increase between the auditory cortex and amygdala and dorsomedial prefrontal cortex (Kim et al., 2012). In addition, the parahippocampus was involved in tinnitus where FC increased in the auditory cortex (Maudoux et al., 2012). Another study (Schmidt et al., 2013) assessed the effect of tinnitus on the default mode network (DMN) and found a decrease in the tinnitus group compared to in normal controls

In the current study, we investigated the influence of hearing loss and tinnitus perception on FC using ICA in three groups: NH, MH and TI groups. Furthermore, we investigated the differences in FC between subgroups of those suffering from and coping with tinnitus, those with bilateral and unilateral tinnitus, and those tinnitus patients with high and low levels of anxiety and depression. Hearing loss thresholds, and tinnitus duration and severity were investigated in order to identify the correlation between spontaneous fluctuation of brain activity at rest and these variables.

9.2 Materials and methods

9.2.1 Subjects

(See section 6.2.1 Page 116)

9.2.2 Audiological examination

(See section 6.2.2 Page 116)

9.2.3 Behaviour assessments

(See section 6.2.3 Page 117)

9.2.4 MRI acquisition

Using a Siemens 3T Trio (Siemens, Erlangen, Germany) with a standard 8 channels head coil performed in this study. In order to control head movement during the scan, we used foam padding and head resistance. Structural images were acquired to perform spatial normalization and localization using a 3D modified driven equilibrium Fourier transform (MDEFT) sequence. The acquisition parameters were as follows: TR/TE 7.92/2.48 ms, flip angle=16°, 176 volumes, slice thickness 1.00 mm, FOV 256*256 mm² and scan time 12 min 51 s.

The functional acquisition scan was 6 minutes and 30 seconds in length using T2*-weighted echo planer images (EPI's) collected with the following acquisition parameters (TR 2000 ms; TE 30 ms; slice thickness 3.5 mm; matrix size 64*64; interleaved slice order; slice gape 3mm, 180 functional volumes). Participants were instructed to close their eyes, try not to think about anything and let their minds wonder.

9.2.5 Image analysis

Resting state fMRI data were processed using the MELODIC toolbox in FMRIB Software Library (FSL), Oxford, UK (Beckmann et al., 2005). I first conducted

independent component analysis with dual regression to drive subject-specific spatial maps for the networks of interest. Then, I performed whole brain general linear models (GLMs) to examine groups' differences in functional connectivity between tinnitus patients and healthy controls, tinnitus bilateral and unilateral, tinnitus coping and non-coping, and tinnitus with high HADS and low HADS subgroups. We also performed three GLMs on individuals with tinnitus to examine associations of functional connectivity with disease severity, duration and hearing loss.

Preprocessing included: head motion correction, brain extraction, co-registration to standard space (MNI 152 2mm) and spatial smoothing (5 mm). Head motion was corrected using MCFLIRT tool (Part of FSL software package) for multi-resolution rigid body co-registration of resting state volumes (Jenkinson et al., 2002). Brain extraction was performed using BET tool (as part of FSL) to remove non-brain tissue (Smith, 2002). The volumes of fMRI were co-registered to individuals' 3D MDEFT structural images that were then co-registered to the MNI152 standard space using the FLIRT tool (as part of FSL). The images were resampling to 4mm and then smoothed with 5 mm FWHM.

General Linear Model (GLM) for ICA-MELODIC analysis was created, and limited to 20 components. The one sample t-test was used to define the names of brain resting state networks (RSNs). A multisession temporal concatenation tool in MELODIC was applied to perform ICA (figure 9.1). Following the identification of networks of interest, a dual regression approach was applied in order to allow for voxel-wise

comparisons of resting-state networks between groups, which employs the full set of group ICA spatial maps in a linear model fits against each separate subject's original 4D resting state data set. The dual regression approach estimates a version of each of the group-level spatial maps in two main steps: 1)- regresses the group-spatial-maps into each subject's 4D dataset to give a set of timecourses, and 2)- regresses those timecourses into the same 4D dataset to get a subject-specific set of spatial maps (Filippini et al., 2009).

An additional and separate analysis was conducted to examine the association between the composite measure of tinnitus severity, duration and hearing loss and functional connectivity. A single-group average with additional covariate was applied by entering an extra EV that is orthogonal with respect to the group mean and demeaned. Two contrasts were set that are positive and negative.

A randomize nonparametric permutation testing was applied to test group differences between groups and correlation with variables (hearing loss, tinnitus duration and severity) with 5000 permutation tests using a threshold-free cluster enhanced (TFCE) technique that helps to control for multiple comparisons (Nichols and Holmes, 2002). In order to identify functional connectivity pattern differences between groups, we used the Juelich histological and the Harvard-Oxford cortical and subcortical atlases that are provided within FSL software package.

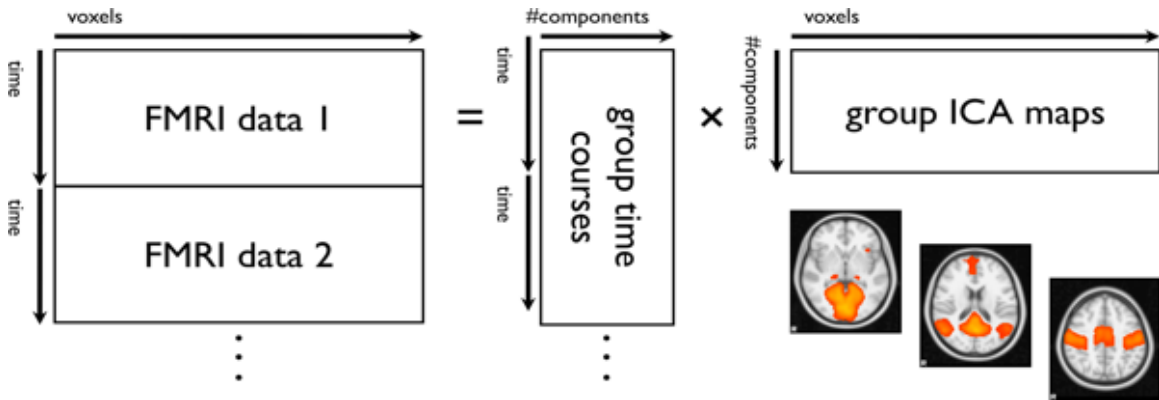


Figure 9.1: The temporal concatenation group ICA. Adapted from (Cole et al., 2010).

9.3 Results

9.3.1 Demographics

(See section 5.3.1 Page 122)

9.3.2 Audiometry findings

(See section 5.3.2 page 124)

9.3.3 Functional connectivity findings:

9.3.4 Visual inspection of brain functional networks

20 brain networks were visual inspected and revealed that seven brain networks are good networks: default mode network (DMN), dorsal attention network (DAN), ventral stream network (VSN), visual network (VN), auditory network (AN), sensorimotor network (SMN) and salience network (SN) (figure 9.2), while 13 artefacts networks (bad networks) resulting from head motion, physiological or scan noise, and cerebrospinal fluid fluctuations that were removed from group comparisons (figure 9.3). Most of the good networks have been reported previously shown stable across participants and over time (Beckmann et al., 2005, Damoiseaux et al., 2006).

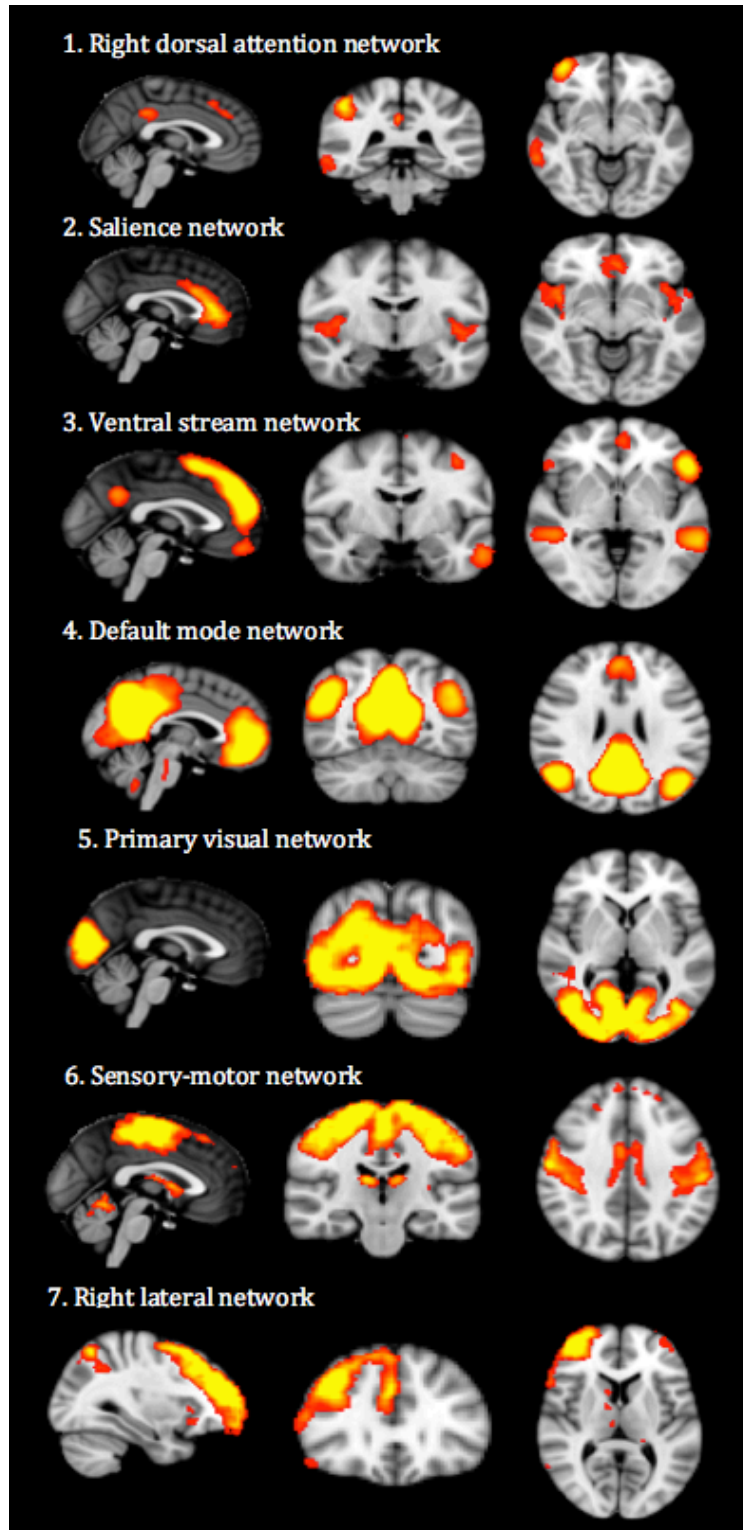


Figure 9.2: A summary of resting state networks' locations

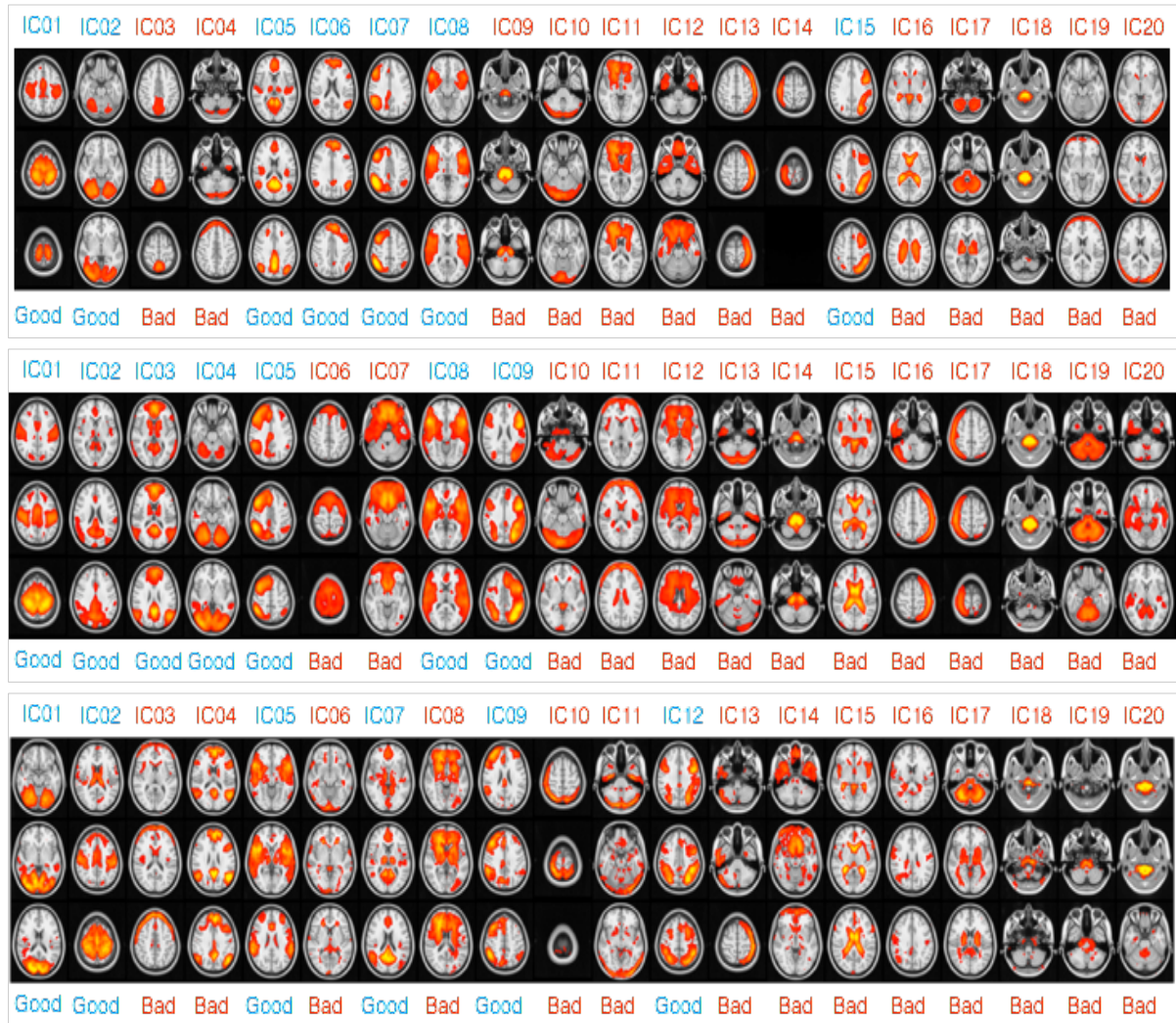


Figure 9.3: Group maps of multi-sessions temporal concentration for independent component analysis (ICA). *Top map:* Group ICA output for the NH group, *middle map:* group ICA output for the MH group and *bottom map:* Group ICA output for the TI group.

9.3.5 Functional connectivity of tinnitus patients and normal controls

It was found that tinnitus patients showed altered (typically increased) of functional connectivity (FC) in VSN and DAN compared to normal hearers (figures 9.4 and table 9.2) and mild to moderate hearing loss groups respectively (figures 9.5 and table 9.2).

Within VSN, temporoparietal junction (TPJ) and the ventral frontal cortex (VFC) shown functional connected. Tinnitus patients showed increased functional connectivity in left anterior cingulate cortex (ACC) compared NH group within this network.

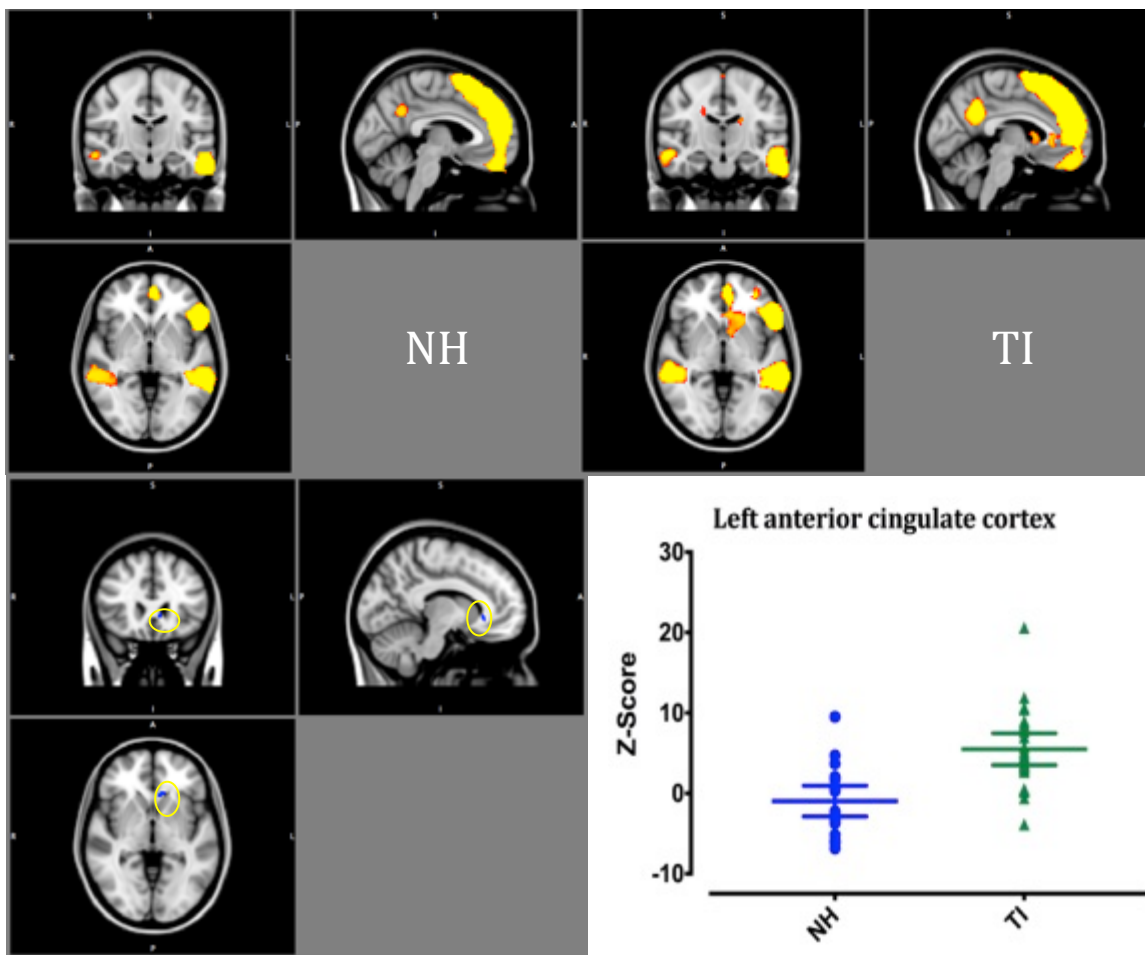


Figure 9.4: The independent component analysis results in a comparison between NH and TIN groups in ventral stream network. Findings are displayed on a template (standard space MNI 152_T1_2mm). Distribution of the mean individual z-scores (beta values) shown within the left anterior cingulate cortex between NH and TIN groups.

Within DAN, the intraparietal sulcus (IPS) and frontal eye field (FEF) shown functional connected. Tinnitus patients showed increased FC in left temporal fusiform cortex compared to MH group within this network.

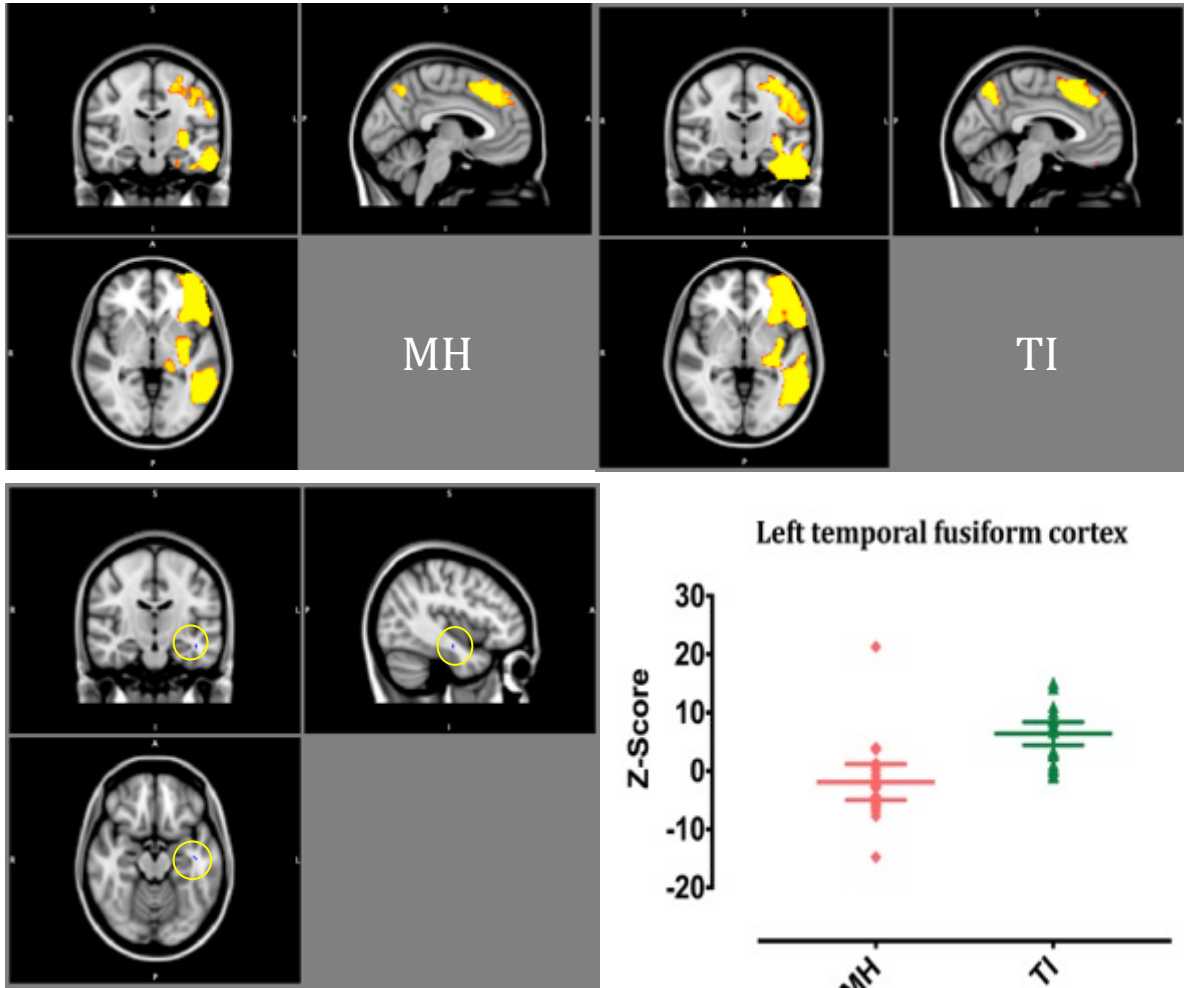


Figure 9.5: The independent component analysis results in a comparison between MH and TIN groups. Findings are displayed on a template (standard space MNI 152_T1_2mm). Distribution of the mean individual z-scores (beta values) shown within the left temporal lobe between MH and TIN groups.

9.3.6 Functional connectivity within tinnitus patient group

In comparison between tinnitus subgroups (tinnitus coping and suffering), (tinnitus bilateral and unilateral) and (tinnitus with anxiety and depression and tinnitus without anxiety and depression).

Ventral pathway network was found the only brain network significantly different between tinnitus bilateral and unilateral subgroups. Within this RSN, temporoparietal junction (TPJ) and the ventral frontal cortex (VFC) shown functional connected. Tinnitus bilateral subgroup has shown increased connectivity of left thalamus compared to unilateral subgroups (figures 9.6 and table 9.2).

Functional connectivity of right DAN was found involved in tinnitus with anxiety and depression patients who showed a significant reduction of FC in anterior division of cingulate gyrus compared to tinnitus patients without anxiety and depression (figures 9.7 and table 9.2). Within this RSN, the intraparietal sulcus (IPS) and frontal eye field (FEF) shown functional connected.

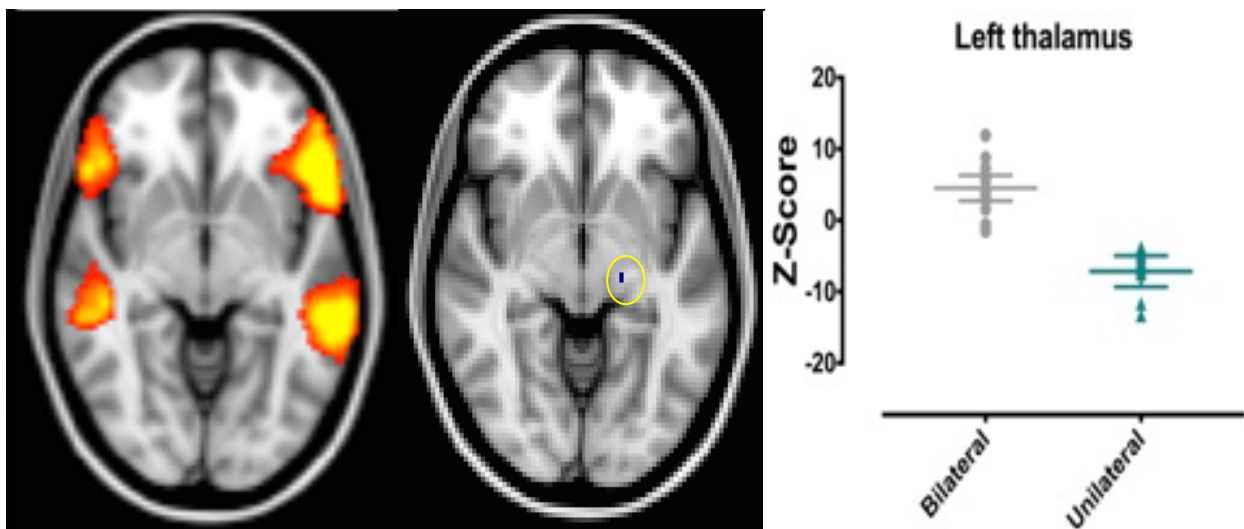


Figure 9.6: The independent component analysis results in a comparison between tinnitus coping and suffering subgroups. Findings are displayed on a template (standard space MNI 152_T1_2mm). Distribution of the mean individual z-scores (beta values) shown within the left thalamus between bilateral and unilateral subgroups.

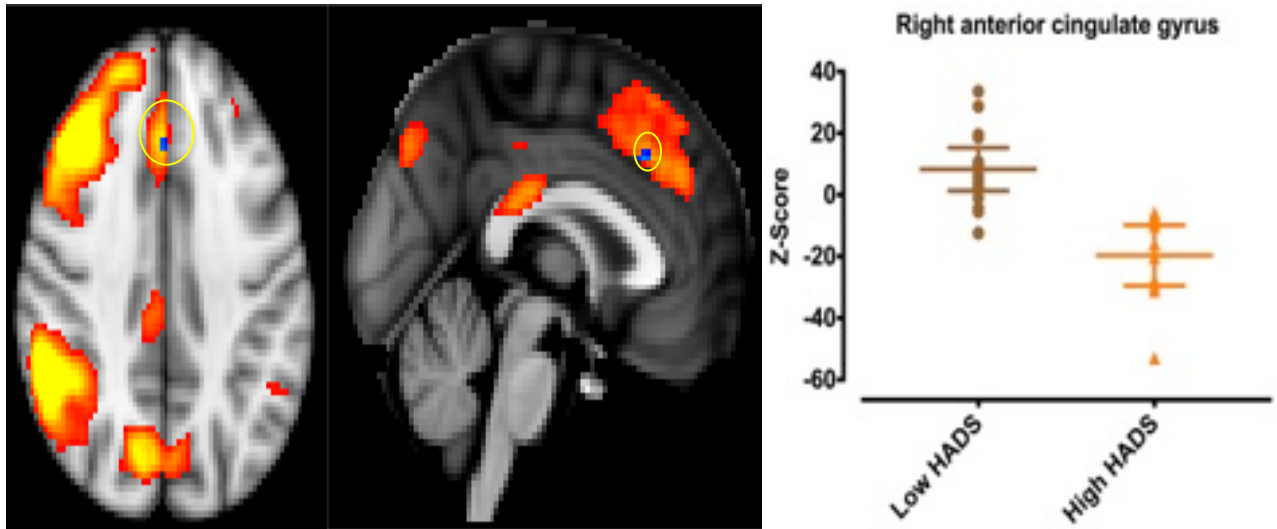


Figure 9.7: Independent analysis component (ICA) results in a comparison between tinnitus with high level of anxiety and depression and tinnitus with low level of anxiety and depression subgroups. Findings (Blue blob) are displayed on a template (standard space MNI152_T1_2mm). Distribution of the mean individual z-scores shown within the anterior division of cingulate gyrus between tinnitus with low anxiety and depression compared to tinnitus with high anxiety and depression subgroups.

9.3.7 Correlation analysis of functional connectivity

No significant correlation was found between FC and tinnitus duration and hearing loss. Correlation analysis revealed that a significant positive correlation was found between tinnitus severity (measured by THI and TFI) and FC at VN, SMN, DMN, AN, DAN and VAN (figure 9.8 and table 9.2).

Within primary VN, bilateral visual cortex regions assembly functional connected that is involved in visual processing. Tinnitus severity was found positively correlated with FC of bilateral visual cortices within this resting state network (RSN). Within SMN, pre and post central gyri shown functional connected. FC of bilateral posrcentral and cingulate gyri were found significantly positive correlation with tinnitus severity. Within DMN, ventral medial prefrontal cortex (VmPFC), posterior cingulate cortex (PCC), inferior parietal lobule (IPI), lateral temporal cortex (LTC), dorsal medial temporal prefrontal cortex (dmPFC) and hippocampus

formation (HF) show functional connected. Tinnitus severity was found positive correlated with FC in right frontal pole and left lateral occipital cortex within this RSN. Within AN, bilateral primary and associated auditory brain regions extended to bilateral insula and temporal poles shown functional connected. FC in left insula and primary auditory cortex were found significantly positive correlated to tinnitus severity. Within DAN, intraparietal sulcus (IPS) and frontal eye field (FEF) were found functional connected that is involved attention processing. FC of left postcentral gyrus showed a significant positive correlation with tinnitus severity within this RSN.

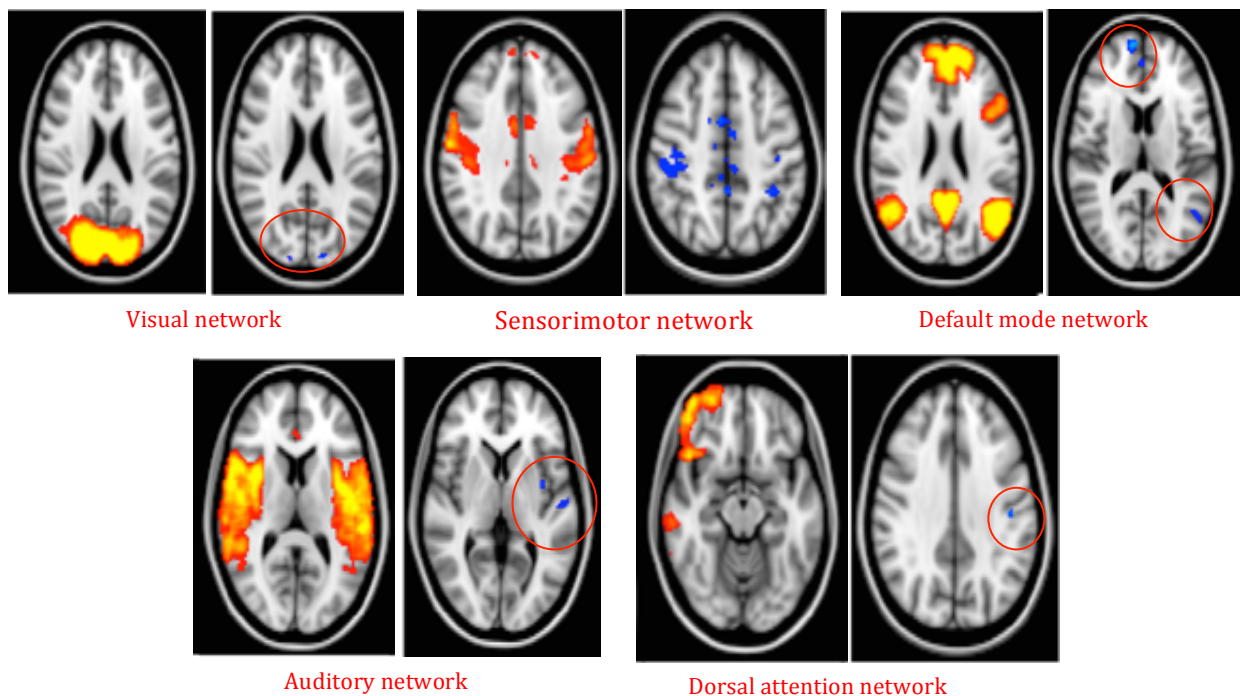


Figure 9.8: Independent component analysis results (extent threshold $P_{corr}=0.05$) showing brain components network with statistically significant correlation of functional connectivity relative to tinnitus severity. Findings are displayed on a template (standard space MNI152_T1_2mm).

Table 9.1: Independent component analysis results (extent threshold $P_{corr}=0.05$) showing brain components network with statistically significant altered of FC relative to tinnitus.

Contrast	Brain networks	Brain regions	MNI coordinate	Z	P
NH < TI	Ventral stream	Left subcallosal cortex	-8 26 -4	4.25	0.04
MH < TI	DAN	Left temporal fusiform cortex	-40 -14 -18	4.67	0.05
Tinnitus coping < tinnitus suffering	Saliency	Left hippocampus	-36 -32 -10	7.82	0.05
Tinnitus unilateral < tinnitus bilateral	Ventral stream	Left thalamus	-18 -22 -6	5.97	0.03
Tinnitus with low level of anxiety and depression > tinnitus with high level of anxiety and depression	DAN	Anterior division of cingulate gyrus	0 26 36	3.56	0.04
Positive correlation (Tinnitus severity)	Visual	Right visual cortex	16 -92 22	5.08	0.03
		Left visual cortex	-14 -92 22	4.88	
	Sensorimotor	Right postcentral gyrus	48 -30 46	4.21	0.04
		Left postcentral gyrus	-38 -24 48	4.39	
		Cingulate gyrus	0 -12 46	4.38	
	DMN	Right Frontal pole	10 60 14	4.96	0.02
Left Lateral occipital cortex		-52 -66 14	4.81		
Auditory	Left Insula cortex	-38 -4 4	4.13	0.01	
	Left Primary auditory cortex	-52 -16 6	4.09		
DAN	Left Postcentral gyrus	-48 -18 34	4.15	0.02	

9.4 Discussion

In the present study, we set out to investigate differences in whole brain FC between tinnitus patients and two control groups: normal hearers and mild to moderate hearing loss subjects. We hypothesized that altered brain FC may be found in resting state networks involved in auditory, emotion and attention processes.

9.4.1 Summary of the results

In this study, whole brain ICA showed an increased in FC in 1)- the ventral stream and dorsal attention networks (DANs) in tinnitus patients compared to in those of the control groups, 2)- the ventral pathway network in the bilateral tinnitus subgroup compared to the unilateral tinnitus subgroup, 3)- the right DAN in tinnitus patients with high level of anxiety and depression patients relatively to tinnitus patients with low level of anxiety and depression, and 4)- positivity correlation with tinnitus severity in the visual, sensorimotor, default mode, temporal, dorsal and ventral attention networks.

9.4.2 Tinnitus and the DMN

The DMN comprises the ventral medial prefrontal cortex (VmPFC), posterior cingulate cortex (PCC), inferior parietal lobule (IPI), lateral temporal cortex (LTC), dorsal medial temporal prefrontal cortex (dmPFC) and hippocampus formation (HF), which are more active during rest compared to in a wide range of active tasks (Raichle, 2015). In our study, the tinnitus patients showed a strong positive correlation between FC in the DMN and tinnitus severity. However, another study (Schmidt et al., 2013) has been examined the effect of tinnitus on FC, and reported a decrease in the coherence of the DMN in tinnitus patients. These mismatched results might be related to differences in the data analysis methods used as we applied the ICA method, which involves whole brain analysis and does not require a prior hypothesis, whereas Schmidt et al (2013) applied a seed-based method, which requires a prior hypothesis involving the examination of brain regions. Moreover, tinnitus severity may play a vital role in FC results as we recruited subjects with a

wide range of tinnitus severity as measured by THI (26 ± 19 , mean \pm SD) and TFI (36 ± 21 , mean \pm SD), but Schmidt et al (2013) only recruited only tinnitus patients with low THI scores (8.33 ± 6.76 , mean \pm SD).

9.4.3 Tinnitus and the DAN

The DAN comprises the intraparietal sulcus (IPS) and frontal eye field (FEF), which are active when attention is oriented in space (Vossel et al., 2014). In our study, the FC of the DAN was found to be significantly higher in tinnitus patients compared to mild to moderate hearing loss subjects, and there was also a significantly positive correlation with tinnitus severity in tinnitus patients. This finding is in line with a previous study (Schmidt et al., 2013), which showed an increased in the FC of the DAN in tinnitus patients compared to in subjects with hearing loss and no tinnitus subjects.

9.4.4 Tinnitus and the VSN

The VAN comprises the temporoparietal junction (TPJ) and the ventral frontal cortex (VFC), which are involved when behaviorally relevant stimuli occur unexpectedly (Vossel et al., 2014). In our study, the FC of the VAN was found to significantly increase in bilateral tinnitus patients compared to in the unilateral subgroups, and positively correlated with tinnitus severity in tinnitus patients. Burton et al (2012) identified a significant negative correlation of FC between the visual cortex and the DAN in patients where the tinnitus was bothersome (Burton et al., 2012). In the best of our knowledge, no previous study on tinnitus has examined the effect of tinnitus laterality on FC.

Despite the fact that the dual-regression results were found in the left thalamus that is allocated outside of the VSN network, it is not necessarily indicative of a problem. It just means that the connectivity of the left thalamus with the VSN is different in the two groups (bilateral and unilateral tinnitus subgroups). Also, the left thalamus might have a weak positive or negative correlation with the main areas of this RSN in the bilateral and unilateral tinnitus subgroups.

9.4.5 Tinnitus and the SMN

The SMN includes the pre- and post- central gyri, which are involved in the processing of somatosensory information (Biswal et al., 1995). For instance, alteration of resting-state brain sensorimotor connectivity was found following spinal cord injury (Min et al., 2015). Moreover, abnormal connectivity of the SMN was found in patients with multiple sclerosis (MS) (Rocca et al., 2009) and epilepsy (Xiao et al., 2015). In our study, the FC of the SMN was found to be significantly correlated with tinnitus severity in tinnitus patients. Recently, it was found that tinnitus severity could be manipulated by stimulation arising from the somatosensory system (Sanchez and Rocha, 2011). This may provide an evidence of the involvement of the somatosensory system in tinnitus perception.

9.4.6 Tinnitus and the AN

The AN comprises bilateral primary and associated auditory brain regions, which are involved in auditory processing (Cordes et al., 2000). Kim et al (2012) reported the a significant reduction in FC between the right and left auditory cortices in tinnitus patients (Kim et al., 2012). In our study, no significant difference in FC was found in the AN between normal controls and tinnitus groups. This finding is in line

with that of Davies et al (2014), who found no significant difference in FC in the AN between tinnitus patients and the controls. We could identify a significant positive correlation between tinnitus severity and FC in the AN in tinnitus patients. In other words, as the tinnitus severity increases, FC increases as well in the AN. Furthermore, tinnitus severity was found to be associated with auditory perception (as discussed in Chapter 7) in the auditory cortex. Abnormality of the structure and function of auditory brain regions has been reported previously in tinnitus patients (Schneider et al., 2009, Muhlnickel et al., 1998). This correlation finding may demonstrate the effect of tinnitus severity in auditory connectivity.

9.4.7 Tinnitus and the VN

The VN includes the spontaneous activity of the visual cortex in the occipital lobe, which is involved in visual processing. Abnormality of the fMRI signal in the primary visual cortex was reported in the tinnitus group compared to in healthy controls during a 1-back task (Amaral and Langers, 2015). In our study, we could detect an association between FC in the VN and tinnitus severity in tinnitus patients. This finding may support previous research that has shown the involvement of the VN in tinnitus perception.

9.4.8 Limitations of the study

This study has some methodological advantages, as well as some limitations, which should be taken into considerations. The main advantage of the present study is the application of whole brain analysis applied (ICA), which does not require a prior hypothesis. However, a number of independent components were selected manually, which can strongly influence the results, and spatially independent

components may overlap (Husain and Schmidt, 2014). Therefore, a number of independent component were optimized to obtain reliable resting state networks. Seed based and graph theory analyses have the advantages of examining selected brain regions, which require a prior hypothesis. Each method has benefits and drawbacks that need to be considered carefully. In addition, two FC findings were found in white matter brain regions in a comparison between the NH, MH and TI groups, which might be because of a problem in the co-registration step.

9.5 Conclusion

The present study has demonstrated the influence of tinnitus perception on the FC of different brain networks, in particular the default mode, dorsal and ventral attention and auditory, visual and somatosensory networks. We used whole brain analysis to determine that brain networks are related to tinnitus perception. The findings of this study are consistent with those of previous research, which showed the involvement of the brain areas (auditory and non-auditory) in the generation, modulation, awareness of and attention to tinnitus (Husain and Schmidt, 2014, Maudoux et al., 2012). The abnormality of FC in the VAN may be the key to understanding tinnitus laterality. The functional abnormality of AN may suggest the dysfunctioning of auditory pathway in tinnitus patients. Further research is required to investigate the influence of tinnitus therapy on FC in subjects with a wide range of tinnitus severity.

Chapter 10: Neural Correlates of White Matter Microstructures Integrity in Tinnitus

10.1 Introduction:

Diffusion tensor imaging (DTI) is an MR neuroimaging modality that gives an insight into brain structural connectivity and white matter (WM) microstructure. Molecular diffusion refers to the random translational motion of molecules that is called Brownian motion, which results from the thermal energy carried by these molecules (Le Bihan and Johansen-Berg, 2012). In a free medium and during a given time, molecular displacements follow a three dimensional Gaussian distribution. Molecular travel randomly in space over distance, which is statistically well described by a “diffusion coefficient” (D) that depends only on the size of the molecules, the temperature and the nature of the medium (Le Bihan and Johansen-Berg, 2012).

In biological tissues, the actual diffusion distance is reduced compared to in free water, and the displacement distribution is no longer Gaussian, as water molecules move in tissues bouncing, crossing, contouring or interacting with many tissue components, such as cell membranes, fibers or macromolecules (Le Bihan and Johansen-Berg, 2012). In the grey matter (GM) and cerebrospinal fluid (CSF), the diffusion-weighted signal is independent of the direction in which the gradients are applied, and the diffusion appears to be *isotropic*, on the other hand, in white matter, water molecules diffuse more freely along the dominant fiber orientation than

across GM and CSF. This *anisotropy* of diffusion provides insights into the microstructural organization of the white matter (Jones and Leemans, 2011).

The most common DTI parameter used is fractional anisotropy (FA) that reflect the shape of the diffusion tensor ellipsoid, and correspond to the underlying white matter microstructure, and reflects white matter integrity and brain network reorganization. Also, mean diffusivity (MD) has been widely used to investigate the total diffusivity of WM tract in the brain structural connectivity by quantifying the magnitude of water diffusion in a given tissue (Basser, 1995). FA and MD have been investigated widely in clinical medicine including the diagnostic ischemic stroke (Adluru et al., 2014), brain trauma (Dinkel et al., 2014) and multiple sclerosis (MS) (Sbardella et al., 2013).

White matter integrity in tinnitus subjects has been investigated using different analysis methods with mixed results (Lee et al., 2007, Aldhafeeri et al., 2012a, Benson et al., 2014, Crippa et al., 2010, Husain et al., 2011a). A significant reduction of FA was found in a tinnitus group compared to normal healthy controls at left frontal and right parietal arcuate fasciculus (Lee et al., 2007), right prefrontal, auditory cortex and corpus callosum (Aldhafeeri et al., 2012a), and left superior longitudinal fasciculus (Benson et al., 2014). On the other hand, others white matter tracts showed a significant increase of FA values in tinnitus groups compared to normal healthy controls at thalamic radiation and the inferior and superior longitudinal fasciculus (Benson et al., 2014). In addition, it was demonstrated that white matter integrity in chronic tinnitus patients was both directly affected by age and mediated by hearing loss (Yoo et al., 2016).

Tract-based spatial statistics (TBSS) is a FSL approach aims to investigate the anatomical connection in the brain between groups of diffusion tensor imaging. It projects DTI data into group-mean tract skeleton, which allows voxel-wise analysis. It addresses alignment issues unsolved by nonlinear registration (Smith et al., 2006). Few tinnitus studies have used this technique to investigate the influence of tinnitus perception on WM. TBSS technique was used to investigate the association between WM integrity with clinical symptoms in tinnitus patients, which showed that depression symptom score is significantly correlated to the mean diffusivity (MD) and the axial diffusivity (AD) in WM tracts under the auditory cortex and limbic system (Ryu et al., 2016).

In this study, we aim to investigate the influence of tinnitus perception on white matter microstructure integrity using whole brain tract based spatial statistics (TBSS) by comparing tinnitus suffers to normal healthy controls. Also, we investigate the influence of tinnitus severity, laterality, and anxiety and depression in patients with tinnitus on white matter microstructure integrity. We hypothesize that a significant reduction of WM integrity will be found relatively to tinnitus perception in WM tracts around auditory, limbic and attention brain regions.

10.2 Materials and methods

10.2.1 Subjects

(See section 6.2.1 Page 116)

10.2.2 Audiological examination

(See section 6.2.2 Page 116)

10.2.3 Behaviour assessments

(See section 6.2.3 Page 117)

10.2.4 MRI acquisition

Using a Siemens 3T Trio (Siemens, Erlangen, Germany) with a standard 8 channels head coil performed in this study. In order to control head movement during the scan, we used foam padding and head resistance. Earplugs and headphone was used attenuate the scanner noise. Structural images were acquired to perform spatial normalization and localization using 3D MDEFT sequence. The acquisition parameters were as follows: TR/TE 7.92/2.48 ms, flip angle=16°, 176 volumes, slice thickness 1.00 mm, FOV 256*256 mm² and scan time 12 min 51 s. Diffusion images were acquired using a spin echo echo-planar imaging sequence with 60 directions and b-value 1200 (TR 10100 ms; TE 106 ms; spatial resolution 2.1*2.1*2.1 mm³). Five references images were acquired with no diffusion gradients (b_0 scans).

10.2.5 Image analysis

Diffusion MRI data were processed using FDT and TBSS toolboxes as part of the FSL Software Library (FMRIB, University of Oxford, UK) (Figure 10.1). Non-brain tissues were extraction from structural images using BET toolbox. DTI-volumes with a b-value 1200 s/mm² was affine registered to the b_0 volumes after eddy current distortion and head motion correction. Every FA image was registered to the FA target image in a standard space (MNI 152). All alignment FA images were averaged, and then white matter skeleton was generated. Each subject's FA data was projected into the mean white matter tract skeleton. Group comparisons were carried out between normal hearers (NH) and mild-to moderate hearing loss subjects (MH), and

between MH and tinnitus (TI) groups.

Non-parametric permutation testing with Randomise software was applied to test group differences between groups and correlation with variable (hearing level, tinnitus onset and severity) with 5000 permutation tests using a threshold-free cluster enhanced (TFCE) technique that helps to control for multiple comparisons (Nichols and Holmes, 2002). Also, it was applied fsstats and fsmaths tools in order to identify the cluster voxel size and their t-score and p values.

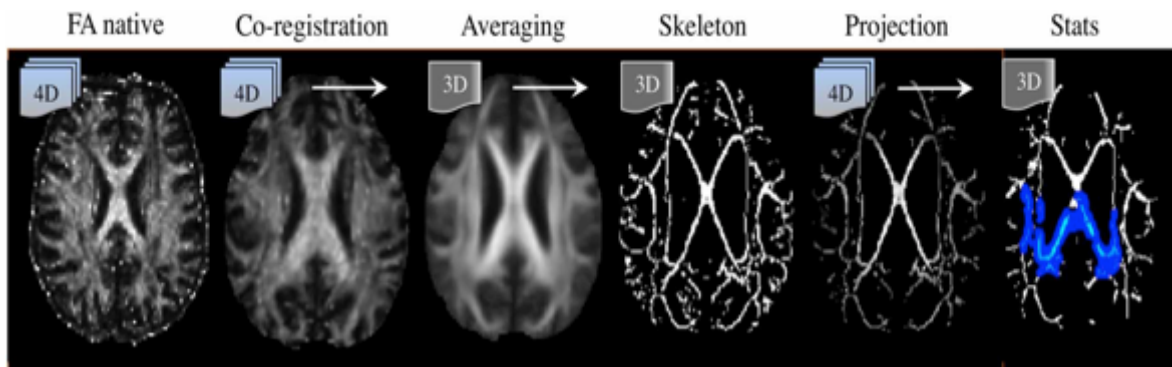


Figure 10.1: Tract-based spatial statistics (TBSS) processing pipeline. Adapted from (Acosta-Cabronero and Nestor, 2014)

10.3 Results

10.3.1 Demographics

(See section 6.4.1 Page 122)

10.3.2 Audiometry findings

(See section 6.4.2 Page 124)

10.3.3 White matter integrity findings

All results reported in this study have been corrected for multiple comparisons (Bonferroni adjusted). ANOVA test revealed that the means of FA values are significantly different ($F(2,63)=4.768$, $P=0.012$) between three groups (figure 10.2 and table 10.1), while mean MD was not significantly different ($F(2,63)=1.998$,

$P=0.144$) between three groups (figure 10.3 and table 10.1). A Tukey post-hoc test revealed that the FA was significantly lower ($t=-2.81$, $P=0.02$) in subjects with tinnitus group (0.417 ± 0.013) Compared to NH group (0.429 ± 0.011). No significant difference ($t=-2.35$, $P=0.065$) was found of FA values between mild to moderate hearing loss group (MH) (0.427 ± 0.017) and tinnitus group (TI) (0.417 ± 0.013).

Table 10.1: the mean and standard deviation of mean FA and MD of normal hearers (NH), mild to moderate hearing loss (MH) and tinnitus group (TI). Asterisks indicate a significant difference between groups.

	NH	MH	TI	F	P
Mean_FA	0.429 ± 0.011	0.427 ± 0.017	0.417 ± 0.013	4.969	0.012*
Mean_MD	0.000765 ± 0.00001	0.000763 ± 0.00002	0.000769 ± 0.00002	1.998	0.144

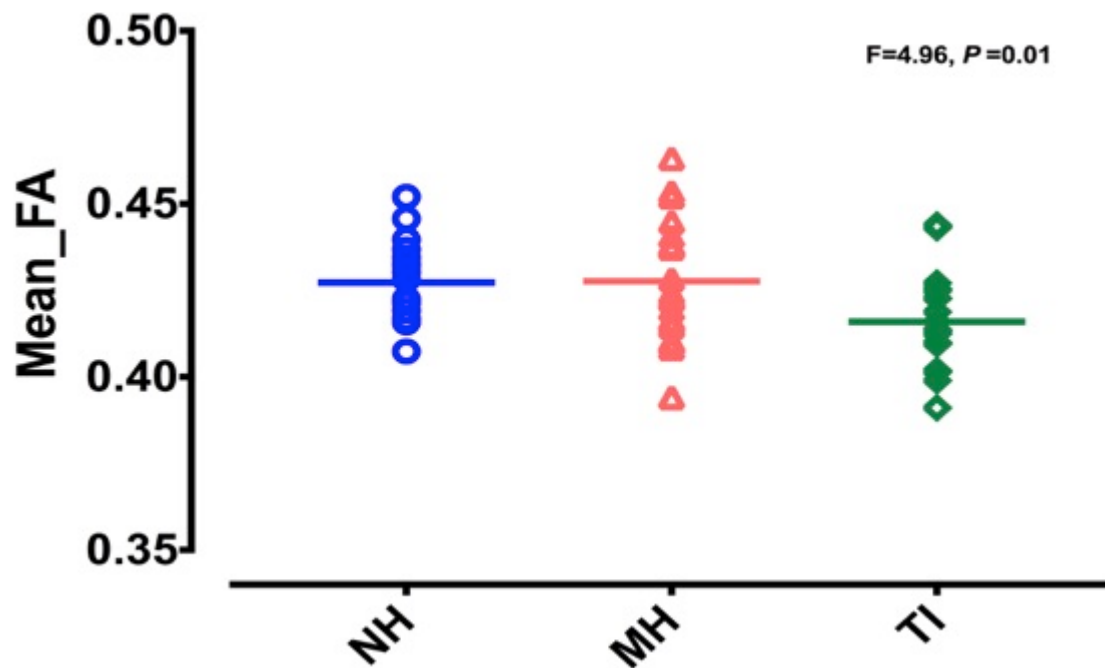


Figure 10.2: : Plots show the mean of fractional anisotropy (FA) values between normal hearers (NH), mild to moderate hearing loss (MH) and Tinnitus (TI) groups.

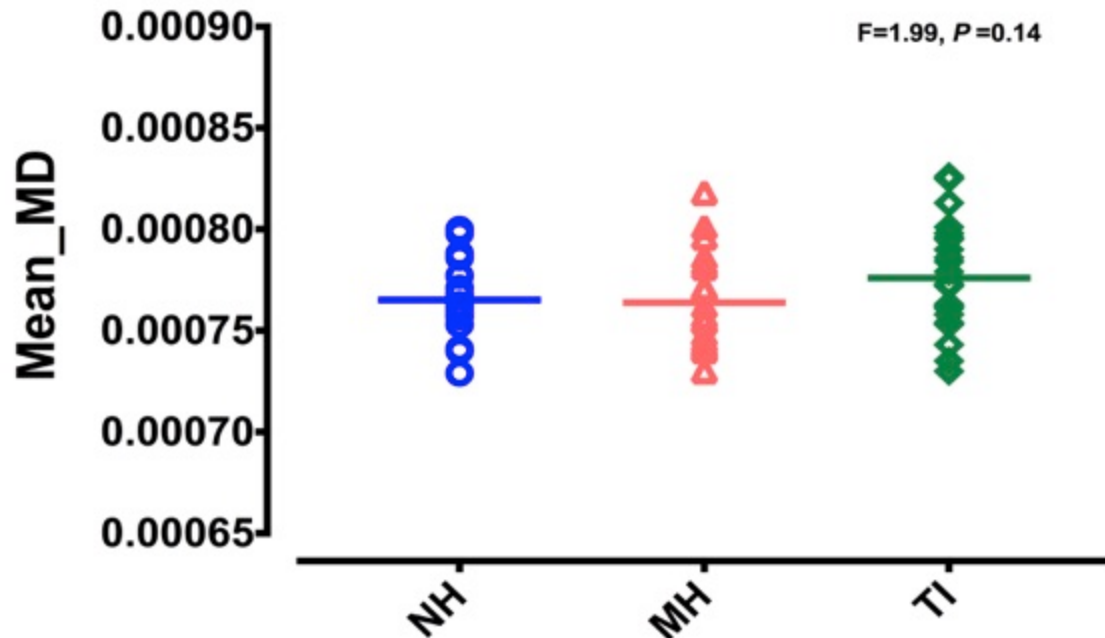


Figure 10.3: Plots show the mean of mean diffusivity (MD) values between normal hearers (NH), mild to moderate hearing loss (MH) and Tinnitus (TI) groups.

10.3.4 Whole brain voxel-wise analysis

Whole brain analysis revealed that no significant different of white matter integrity (FA) between normal hearers (NH) and mild to moderate hearing loss (MH) group. On the other hand, tinnitus group showed a significant reduction of white matter integrity compared to the healthy controls groups at corpus callosum (body and splenium), bilateral inferior longitudinal fasciculus (auditory cortex) and corticospinal tract, inferior-frontal occipital fasciculus (prefrontal and insula cortex), left anterior thalamic radiation (thalamus), and superior longitudinal fasciculus (middle temporal gyrus) (figures 10.4 and 10.5, table 10.2). In comparisons of mean diffusivity (MD) between groups, no significant different of MD was found between NH and MH, and between TI and MH. However, tinnitus patients showed a significantly increased of mean diffusivity compared to normal hearers at right inferior-frontal occipital fasciculus (figure 10.4, table 10.2).

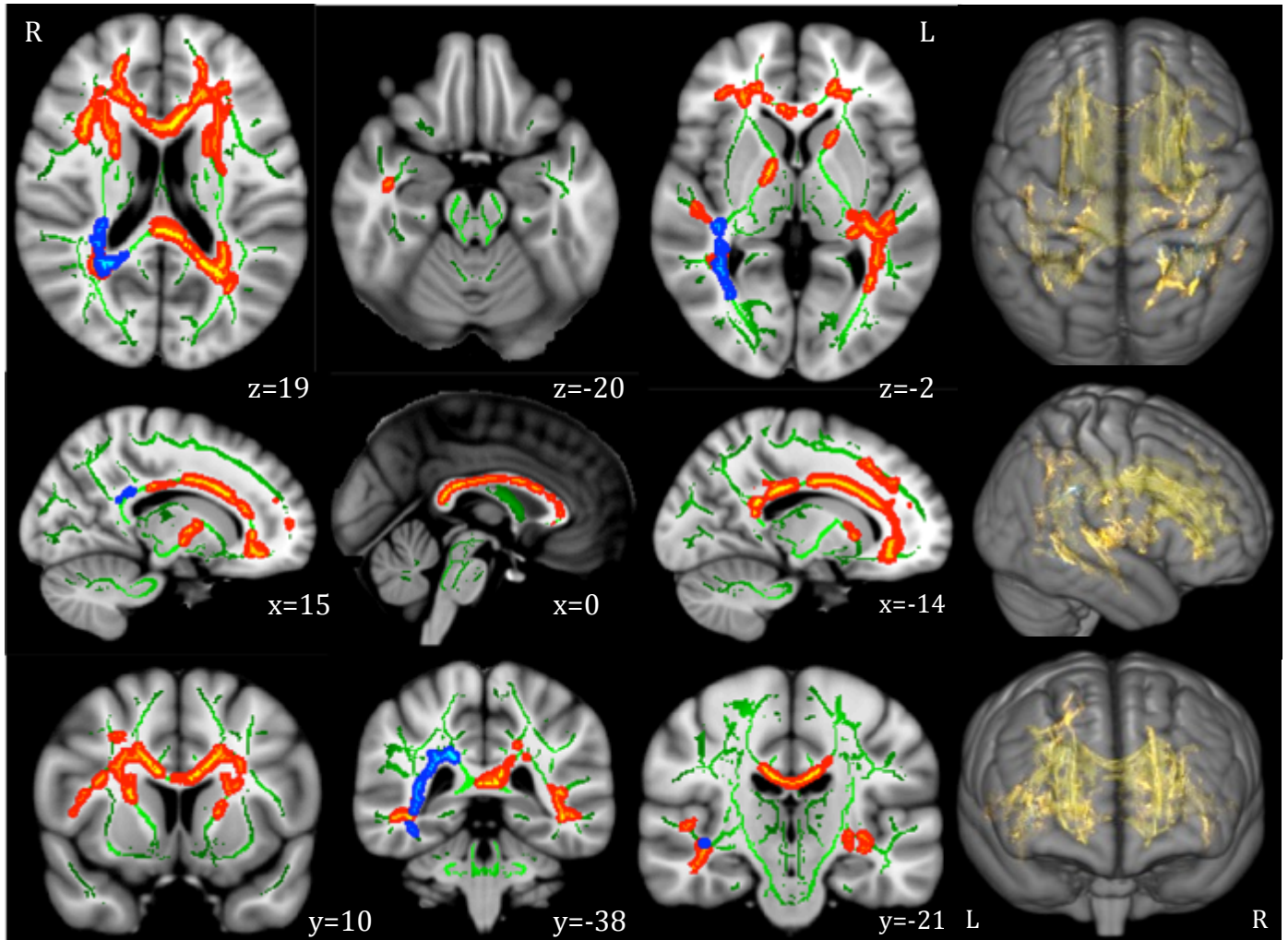


Figure 10.4: Whole-brain group comparison between NH and TI of DTI data obtained from TBSS analysis. The statistically significant clusters are shown in red-yellow color (reduction of FA) and blue-light blue (increase of MD) over a FA skeleton map in green color

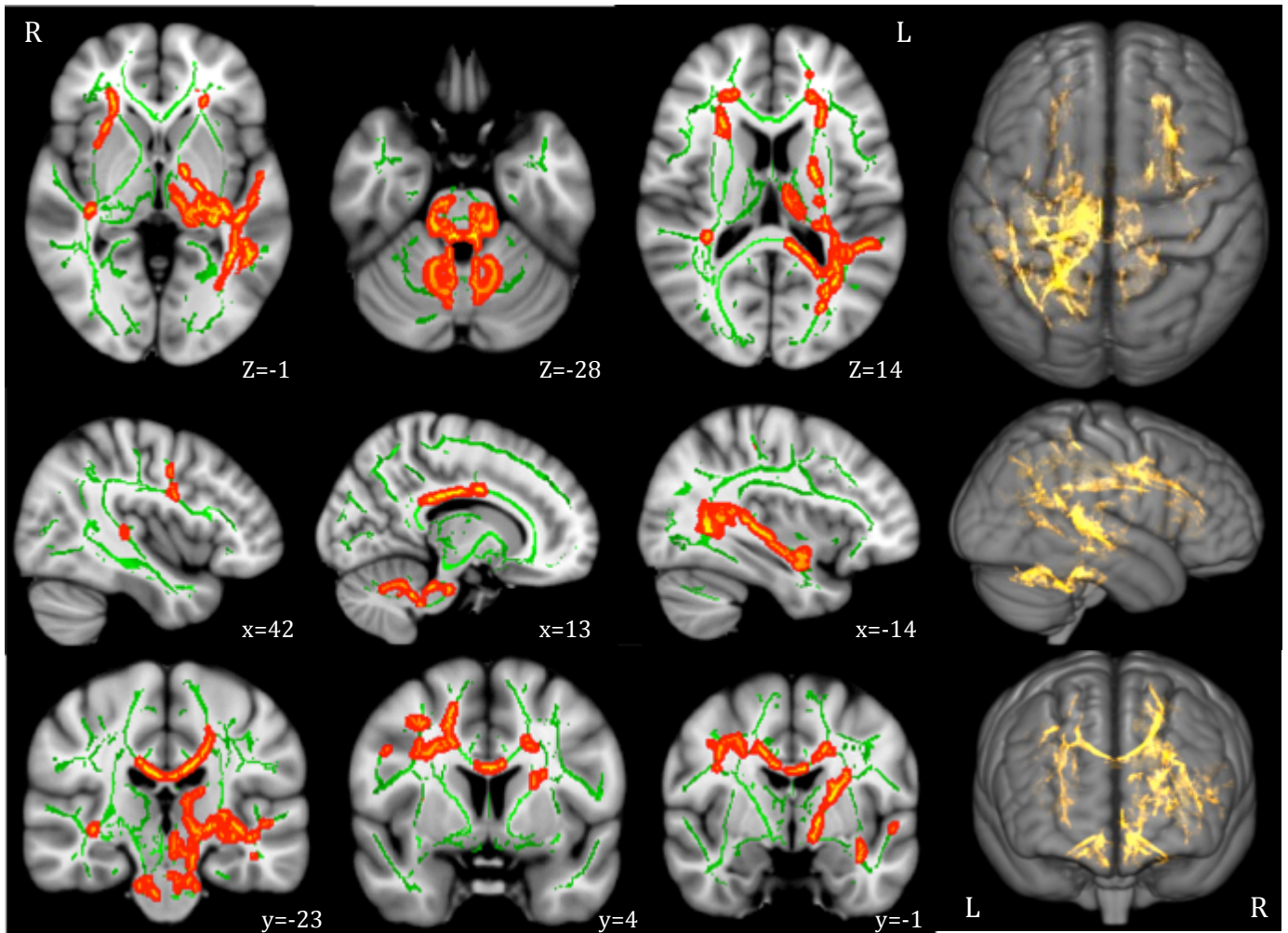


Figure 10.5: Whole-brain group comparison between MH and TI of DTI data obtained from TBSS analysis. The statistically significant clusters are shown in red-yellow color (reduction of FA) over a FA skeleton map in green color

Table 10.2: Whole-brain group comparison between NH and TI and between MH and TI of DTI data obtained from TBSS analysis.

Contrast	WM tract	Brain hemisphere	MNI coordinate x, y, z	<i>P</i>
NH>TI (FA)	Anterior thalamic radiation	R	19 7 13	0.006
		L	-17 11 7	0.006
	Inferior-frontal occipital fasciculus	R	29 -35 -7	0.008
		L	-25 29 14	0.02
	Corticospinal tract	R	29 -7 28	0.002
		L	-26 -13 27	0.02
	Corpus callosum	R	12 22 19	0.002
		L	-1 15 19 -1 26 19	0.004 0.006
	Superior longitudinal fasciculus	R	41 10 16 45 -24 3	0.02 0.02
		L	-41 -41 5 -42 -26 3	0.008 0.008
NH<TI (MD)	Inferior-frontal occipital fasciculus	R	37 -49 -1	0.04
	Corpus callosum	R	17 -40 26	0.045
	Inferior longitudinal fasciculus	R	37 -43 -15	0.045
MH>TI (FA)	Anterior thalamic radiation	L	-8 -22 12	0.03
	Superior longitudinal fasciculus	L	-42 -45 5 -34 -32 6	0.03 0.04
		R	26 -37 43 37 -37 13	0.04 0.05
	Corpus callosum	L	-13 -8 33	0.04
		R	10 -8 29	0.04
	Inferior-frontal occipital fasciculus	L	-24 21 12	0.05
		R	37 -23 -4	0.04
	Corticospinal tract	L	-14 -25 -29	0.03
		R	11 -32 -27	0.04
				-

Within the tinnitus group, the influence of tinnitus severity, lateralization, and anxiety and depression on white matter integrity was investigated by dividing tinnitus participants into the tinnitus coping and suffering subgroups, the tinnitus bilateral and unilateral subgroups, and the tinnitus with high level of anxiety and depression and tinnitus with low level of anxiety and depression respectively. We also assessed the relationship between anxiety and depression and microstructure WM integrity (measured by FA) using the HADS questionnaire.

The cingulum bundle showed a significant reduction of white matter integrity in tinnitus subjects with high level of anxiety and depression (0.37 ± 0.01 ; mean \pm SD) (HADS > 10, n = 11) compared to tinnitus participants with low level of anxiety and depression (0.43 ± 0.03 ; mean \pm SD) (HADS > 10, n = 15) in the anterior and posterior cingulate gyri (figure 10.6, table 10.3). A significant negative correlation ($r^2 = 0.47$, $P = 0.0001$) was found between WM integrity of left cingulum bundle and HADS scores in tinnitus patients. However, no significant correlation was found between WM integrity (FA) in the cingulum bundle and HADS scores in the high and low HADS tinnitus subgroups.

Furthermore, tinnitus lateralization was assessed, which showed that the bilateral tinnitus subgroup (0.415 ± 0.04 ; mean \pm SD) has a significant lower FA value compared to the unilateral tinnitus participants (0.474 ± 0.04 ; mean \pm SD) in the left corpus callosum ($P = 0.002$) (figure 10.7, table 10.3). No significant correlation was found between WM integrity (FA) in the left corpus callosum and TFI scores in the bilateral and unilateral tinnitus subgroups. No significant difference was found of white matter integrity between the tinnitus coping and suffering subgroups.

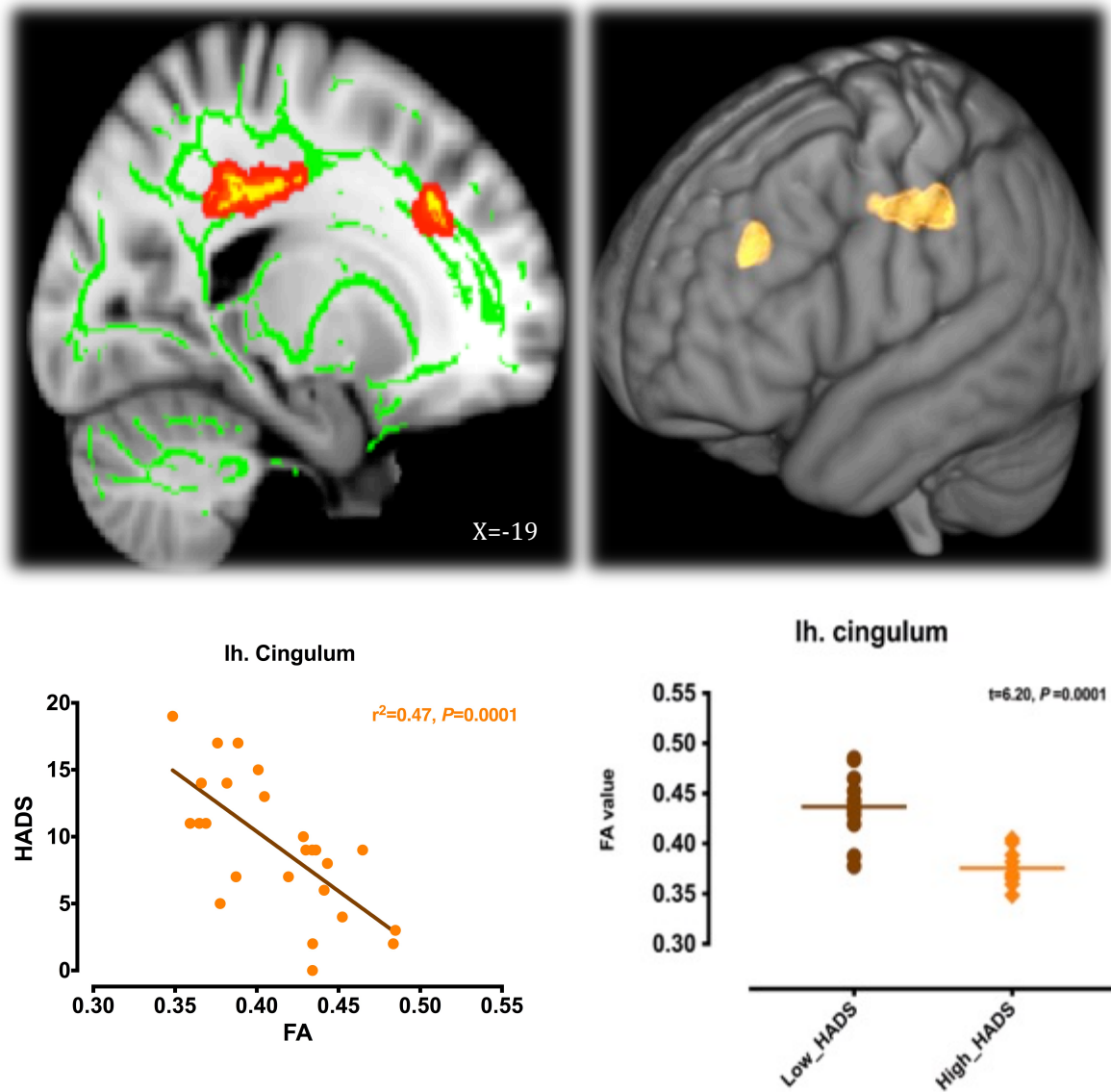


Figure 10.6: Whole-brain group comparison between tinnitus anxiety and depression subgroups of DTI data obtained from TBSS analysis. The statistically significant clusters are shown in red-yellow color (reduction of FA) over a FA skeleton map in green color. The negative correlation between HADS scores and FA values in cingulum was shown in tinnitus patients. Tinnitus participant (P25) was removed in this analysis due to the HADS score (HADS=33) of this participant is an outlier (HADS > mean \pm 3*SD).

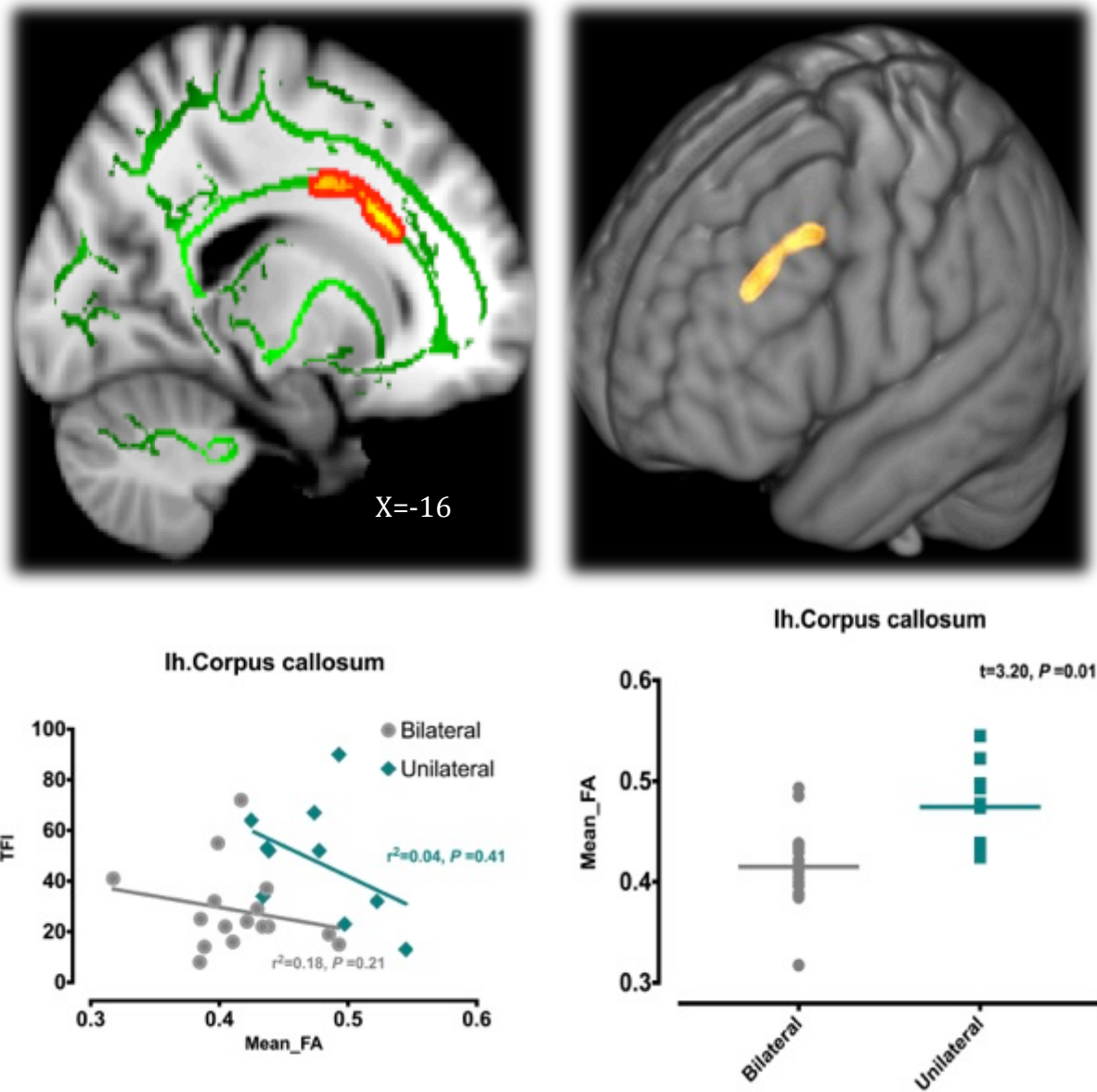


Figure 10.7 Whole-brain group comparison between tinnitus laterality subgroups of DTI data obtained from TBSS analysis. The statistically significant clusters are shown in red-yellow color (reduction of FA) over a FA skeleton map in green color. The negative correlation between TFI scores and FA values in corpus callosum was shown in tinnitus patients.

Table 10.3: Whole-brain group comparison between tinnitus anxiety and depression subgroups, and between tinnitus lateralization subgroups of DTI data obtained from TBSS analysis.

Contrast	WM tracts	Brain hemisphere	Brain regions	MNI coordinate X, y, z	P
Tinnitus with low HADS > tinnitus with high HADS	Cingulum	L	Anterior and posterior cingulate gyri	-19 26 35 -19 -37 35	0.04
Tinnitus bilateral < tinnitus unilateral	Corpus callosum	L	Body of corpus callosum	-16 16 28	0.05

10.4 Discussion

In this study, the white matter microstructural integrity was assessed between the normal hearers (NH), mild to moderate hearing loss (MH) and tinnitus patients using two different methods: 1)- calculation of the global mean of white matter FA and MD values, and 2)- whole brain voxel-based analysis,

10.4.1 Results summary

The global mean of FA value was found significant lower in tinnitus patients compared to normal hearers (NH). This reduction may be a biomarker predictor of the status of white matter tract myelination in tinnitus patients. In the whole-brain voxel based analysis, widespread reductions of white matter integrity were found in tinnitus patients compared to the NH and MH groups in different white mater tracts: at corpus callosum (body and splenium), bilateral inferior longitudinal fasciculus (auditory cortex) and corticospinal tract, inferior-frontal occipital fasciculus (prefrontal and insula cortex), left anterior thalamic radiation (thalamus), and superior longitudinal fasciculus (middle temporal gyrus).

10.4.2 Tinnitus and hearing loss

Most of tinnitus causes result from hearing loss that has been considered as one of the potential cause of tinnitus (Crummer and Hassan, 2004). However, few people with severe tinnitus have normal hearing, and some people with hearing loss does not report tinnitus (Baguley et al., 2013). It was reported that tinnitus with a normal audiogram might have a hidden hearing loss that could not be detected by the clinical audiometry (Schaette and McAlpine, 2011). So, the link between tinnitus and hearing loss is unclear yet.

The effect of tinnitus with hearing loss on WM integrity has been revealed with different results. WM integrity was found affected by age and mediated by the hearing loss in tinnitus patients (Yoo et al., 2016). The influence of hearing loss was found greater than tinnitus on WM alterations (Husain et al., 2011a).

In the current study, we found that tinnitus with hearing loss has an influence on WM integrity (FA) and mean diffusivity (MD) relative to normal hearing controls. In comparison between tinnitus patients and healthy control with mild to moderate hearing loss, no significant differences of MD maps between the groups, and significant differences were found only in the FA map, which is more in the left compared to the right hemisphere. These differences of FA and MD maps between the groups show the influence of hearing loss in tinnitus patients.

10.4.3 Tinnitus and thalamocortical radiations

Thalamocortical radiations (TRN) are the WM tracts connecting the thalamus and cerebral cortex. The medial geniculate nuclei (MGN) are part of the thalamus, which play an important role in the auditory system to process the sound from the cochlea to the auditory cortex (Yu et al., 2009). Anterior thalamus radiation (ATR) includes a specific

proportion called the acoustic radiation that connects the MGN and the primary auditory cortex (Husain et al., 2011a). It was hypothesized that the unwanted noise (tinnitus signal) is identified by the limbic system and eliminated from perception in the auditory cortex by feeding it back to the TRN (Rauschecker et al., 2010). In the current study, we could identify a significant reduction of WM integrity in tinnitus patients compared to the controls in the ATR tracts. Thus, it can be said that this reduction of WM integrity in anterior thalamus radiation might be the cause of tinnitus perception, as the brain was unable to cancel the unwanted noise (tinnitus signals) that perceived in the auditory cortex.

10.4.4 Tinnitus and brain laterality

In this study, a reduction of white matter integrity was found mainly in the left hemisphere in tinnitus subjects compared to mild to moderate hearing loss, whereas both hemispheres showed a decrease in white matter integrity and mean diffusivity in tinnitus group compared to normal hearers. Left hemisphere dominated of WM integrity is on line with prior studies (Ryu et al., 2015, Benson et al., 2014, Seydell-Greenwald et al., 2014) that identified WM integrity changes in left cerebral WM. This finding may explain the role of language processing to cause this dominant left laterality. On the other hand, comparison of WM integrity between normal hearers and tinnitus patients with hearing loss demonstrated that both hemispheres were dominated by tinnitus perception with hearing loss. Therefore, we may assume that there might be an association between language processing and hearing loss in tinnitus perception, which could give insight to understand the pathophysiology of tinnitus mechanism.

The corpus callosum (CC) is the largest WM tract fibers that connect cerebral hemisphere, which was found in the current study be affected by tinnitus perception compared to controls. Reduced of WM integrity at CC has been reported previously in tinnitus studies (Aldhafeeri et al., 2012a). Corpus callosotomy was found an effective surgical treatment in many cases of intractable epilepsy (Sunaga et al., 2009). Furthermore, we found that tinnitus laterality is associated with WM integrity as the bilateral tinnitus subgroup showed a significant reduction of FA value at CC compared to the unilateral tinnitus subgroups. It was proposed that the reduction of white matter integrity at CC might cause imbalance between cerebral hemispheres regarding to the simultaneous of excitation and inhibition (Kitterle, 1995). Therefore, we argue that the significant reduction of WM integrity in Corpus Callosum in bilateral tinnitus subgroups, compared with unilateral tinnitus subgroups, contributes to the imbalance of tinnitus sound perception. Future work is required to confirm this finding in a larger tinnitus population over time.

10.4.5 Tinnitus and the auditory system

Abnormalities of WM integrity within auditory system have been reported in tinnitus studies with variable results (Benson et al., 2014, Aldhafeeri et al., 2012a, Seydell-Greenwald et al., 2014, Crippa et al., 2010, Gunbey et al., 2015). A negative correlation was found between hearing loss threshold and FA values at left primary auditory cortex (Seydell-Greenwald et al., 2014), inferior colliculus (IC) and medial geniculate body (MGB) (Gunbey et al., 2015) in tinnitus group. In the current study, a reduction of WM integrity was found in tinnitus group compared to NH and MH groups at auditory cortex. The structural atrophy and dysfunction of auditory cortex

have been reported previously in tinnitus patients (Boyen et al., 2014a, Schneider et al., 2009). Thus, tinnitus with hearing loss seems to be associated with the structural alteration of the auditory system connectivity.

10.4.6 Tinnitus and the limbic network

Limbic system plays an important role in tinnitus perception as can be seen clearly in tinnitus severity as some can cope with tinnitus symptoms while others could not. Structural atrophy and dysfunction of limbic system brain structural have been reported in tinnitus patients (Aldhafeeri et al., 2012b, Leaver et al., 2011, Seydell-Greenwald et al., 2012a, Schmidt et al., 2013). In the current study, prefrontal and insula cortex were found involved in the abnormalities of WM integrity in tinnitus patients. The association between the structural atrophy of prefrontal cortex and hyperactivity of auditory system was proposed in “noise cancellation” model of tinnitus, where prefrontal cortex might reduce the ability to cancel tinnitus noise that could cause hyperactivity in the auditory cortex (Seydell-Greenwald et al., 2014). Therefore, abnormalities of WM integrity between auditory and limbic network may drive the generation of tinnitus noise.

10.4.7 Tinnitus and the salience network

Salience system is another brain network has been paid attention in tinnitus research. The role of anterior insula (AI) and anterior cingulate cortex (ACC) have been demonstrated in pain perception (Singer, 2006). Insula cortex is a brain structure connected with different brain regions: prefrontal, auditory cortices, amygdala, thalamus, orbital frontal cortex and cerebrum (Lenhardt et al., 2008). Increased activation of insula and cingulate cortices was found in recent onset

tinnitus patients compared to late onset tinnitus patients (Carpenter-Thompson et al., 2015). The authors conclude that insula and cingulate cortices may be involved in early detection of tinnitus signals. Furthermore, insula cortex has been targeted to treat severe disabling tinnitus patients using ultra-high-frequency therapy, which found that the severity of tinnitus symptoms decreases (Lenhardt et al., 2008). In the current study, WM integrity from or to insula and cingulate cortices was significantly lower in tinnitus patients compared to mild to moderate hearing loss participants. This reduction of WM integrity in insula cortex and cingulate gyrus may be caused by the pain perception of tinnitus symptoms.

10.4.8 Tinnitus and the anxiety and depression

The association between psychological disorders and tinnitus perception has been reported (Reynolds et al., 2004, Adoga et al., 2008). The prevalence of anxiety and depression was reported higher in tinnitus suffering patients (Zoger et al., 2006, Krog et al., 2010). In our study, no significant difference in the level of anxiety and depression was found between tinnitus sufferers and controls that might be due to we recruited wide range of tinnitus severity from slight to claustrophobic (THI=26±19, TFI=36±21). Eleven participants with tinnitus showed high level of anxiety and depression (HADS>10), while fifteen participants with tinnitus showed low level (HADS<10). We found a significant reduction of white matter integrity in tinnitus-depressed subjects (HADS>10) compared to tinnitus not depressed participants (HADS<10) at cingulum bundle white matter that is considered as the communication pathway between limbic system components. In addition, HADS scores of tinnitus patients showed a significant negative correlation with WM

integrity at left cingulum and corpus callosum. Alterations of white matter integrity at cingulum was reported in chronic depression (Taylor et al., 2014), dementia (Demey et al., 2015) and epilepsy (Nazem-Zadeh et al., 2014). Also, FA values of cingulum bundle were found negatively correlated to the reaction time in patients with attention impairments following traumatic brain injury (Leech and Sharp, 2014). Furthermore, a negative correlation was found between combined anxiety and depression score and cortical thickness at posterior cingulate gyri in tinnitus patients (Leaver et al., 2012). Thus, abnormalities of WM integrity in cingulum bundle might be considered as an indication of anxiety and depression in tinnitus patients.

10.4.9 Limitations of the study

There are some limitations of this study. Three groups were recruited in this: normal hearers, mild to moderate hearing loss and tinnitus patients with hearing loss. It would be advisable to include another study group that includes tinnitus patients with normal hearing thresholds in order to distinguish between hearing loss and tinnitus effects.

Another issue is raised regarding to diffusion data analysis method was chosen in this study, a voxel-based analysis was applied in this study which does not required a prior hypotheses. It would be useful to investigate the influence of tinnitus perception on WM integrity between specific brain networks (tractography) such as auditory and limbic networks.

Furthermore, the number of tinnitus coping and suffering patients might not be sufficient to identify the WM integrity changes. Further research is required to

investigate the influence of tinnitus severity on WM integrity in a larger population.

10.5 Conclusion

In general, microstructure WM integrity was found to be changed in tinnitus population compared to normal hearers and mild to moderate hearing loss participants near of auditory, attention, salience and limbic networks. Brain structural connectivity seems to be associated with tinnitus perception and mild to moderate hearing loss rather than hearing loss alone. This study provides an evidence of the involvement of corpus callosum in tinnitus lateralization, which may explain the reason of some are suffering from tinnitus in one ear while others both ears. Furthermore, our results suggested that anxiety and depression might have an effect on WM integrity in chronic tinnitus. These findings indicate that there are different brain networks involved on tinnitus perception that need to be considered carefully for treatment purposes. Future studies are required to exploit structural connectivity on larger tinnitus population including functional connectivity as well to give more insight of the tinnitus pathophysiology.

Chapter 11: General Discussion, Conclusion and Future work

11.1 Introduction

In this thesis, the main aim was to investigate the effect of tinnitus perception on the underlying neural process, cortical structure integrity and auditory resting state network using advance MRI techniques (structural MRI, functional MRI, diffusion MRI and arterial spin labeling). I also explored neuroanatomical and functional features in brain regions that are associated with hearing loss and tinnitus severity, onset and laterality. In addition, I assessed the effect of tinnitus perception on the quality of life.

11.2 Summary of findings

The results obtained from each study were described and interpreted separately within each chapter. The following subsections will summarize the findings for each study.

11.2.1 Investigating the effect of tinnitus perception on the quality of life.

The impact of tinnitus perception on the quality of life was found to be higher in the suffering group compared to the coping group. A significant positive correlation was found between the age of the tinnitus subjects and their hearing loss thresholds. The TFI score was found to be significantly higher in the unilateral tinnitus group compared to the bilateral tinnitus group. In TFI subscales, Intrusiveness scored the highest, while Quality of Life scored the lowest. A significant positive correlation

was found between hearing loss and tinnitus duration. Also, the correlation between tinnitus severity and anxiety and depression was identified in this study.

11.2.2 Investigating the neuroanatomical correlates of tinnitus perception.

Total intracranial and grey matter volumes were found to be significantly lower in subjects in the tinnitus group compared to normal hearers without tinnitus (NH). Tinnitus subjects showed a significant reduction of grey matter volume in the left inferior temporal, bilateral orbital frontal, right insula, supramarginal and occipital fusiform gyri compared with the NH. Tinnitus subjects showed a significant reduction of the left pallidum volume compared to the NH. The volume of left hippocampus was significantly negatively correlated to the total score of TFI (this correlation did not survive correction for multiple comparisons).

The mean cortical thickness was significantly thinner in subjects with tinnitus compared to the NH and MH groups. The tinnitus group showed a significant reduction of cortical thickness compared to the NH group at bilateral fusiform, ITG, left lateral occipital, transverse temporal, insula, and right cuneus, orbital frontal, lingual, parahippocampal lateral occipital, and insula. In comparison with the moderate hearing loss (MH) group, tinnitus subjects showed thinner cortices at bilateral fusiform, ITG, parahippocampal, left temporal pole and right MTG. A significant negative correlation was identified between THI scores and two cortical thickness measurements at the right temporal pole and the rostral anterior cingulate gyri. Also, tinnitus severity was found significantly positive correlated to cortical thickness at the left caudal anterior cingulate gyri.

11.2.3 Investigating the neural correlates of auditory perception in tinnitus.

The tinnitus group showed a significant increase of BOLD signals compared to the MH group in the right hemisphere of primary and secondary auditory cortex, visual cortex, and middle temporal gyrus. A significant difference of lateralization index was found between tinnitus coping and suffering subgroups. A significant positive correlation was found between tinnitus onsite and the change of BOLD fMRI activity at left superior temporal and frontal gyri, and right middle temporal and precentral gyri. Moreover, tinnitus severity was found to be significantly positively correlated to BOLD signal changes in bilateral superior temporal gyri.

11.2.4 Investigating the effect of tinnitus perception on cerebral blood flow.

Global gray matter perfusion analysis between normal controls and tinnitus sufferers revealed three regions that showed a significant hypoperfusion in the tinnitus group compared to controls in left prefrontal cortex (PFC), visual cortex (VC) and insula cortex. A significant reduction of CBF was found in the tinnitus suffering subgroup compared to the tinnitus coping subgroup in the right primary auditory cortex. Significant hyperperfused was found in anxious and depressed tinnitus sufferers compared to non-anxious and depressed tinnitus sufferers in the right cingulate gyrus. Correlation analysis revealed that tinnitus duration is significantly positively correlated to CBF quantity in the bilateral auditory cortex. Tinnitus severity was found significantly negative correlated to CBF in the left parahippocampus gyrus.

11.2.5 Investigating the effect of tinnitus on brain functional connectivity

Seven functional RSNs were found to be significantly different or correlated to tinnitus perception that were visually inspected as follows: default mode network (DMN), dorsal attention network (DAN), ventral attention network (VAN), visual network (VN), auditory network (AN), sensorimotor network (SMN) and salience network (SN).

11.2.6 Investigating the brain cortical structure integrity in tinnitus

The global mean FA was significantly lower in subjects in the tinnitus group compared to the NH group. The tinnitus group showed a significant reduction of white matter integrity compared to the NH and MH groups at corpus callosum (body and splenium), bilateral inferior longitudinal fasciculus (auditory cortex) and corticospinal tract, inferior-frontal occipital fasciculus (prefrontal and insula cortex), left anterior thalamic radiation (thalamus), and superior longitudinal fasciculus (middle temporal gyrus). Comparing mean diffusivity (MD) between groups, tinnitus patients showed a significant increase of mean diffusion compared to normal hearers at right Inferior-frontal occipital fasciculus. Within the tinnitus group, the cingulum WM tract showed a significant reduction of white matter integrity in the tinnitus subgroup with high level of anxiety and depression compared to the tinnitus subgroup with low level of anxiety and depression at anterior and posterior cingulate gyri. Also, the bilateral tinnitus subgroup showed a significantly lower FA value compared to unilateral tinnitus participants at the left corpus callosum.

11.2.7 The consistency of results across the studies

Three findings were found consistence across the studies (tables 11.1, 11.2 and figure 11.1). The right cingulate gyrus was found to be involved in high level of anxiety and depression in tinnitus patients compared to low level, which were seen in resting state fMRI and ASL perfusion studies. The volumetric and perfusion findings of the left parahippocampus were found to be associated with tinnitus severity. Also, the BOLD activity and perfusion of left superior temporal gyrus were found to be associated with tinnitus duration.

The findings of this thesis are supporting the integrative model of tinnitus phantom perception, which proposes a minimum set of brain regions called a 'tinnitus core' subnetwork, including auditory cortex, inferior parietal area, ventromedial prefrontal and parahippocampus cortices that are jointly activate to cause the tinnitus perception (De Ridder et al., 2014).

Chapter 11: General Discussion, Conclusion and Future work

Table 11.1: Results summary in comparisons between tinnitus patients and healthy controls.

Analysis	Comparison	Hemisphere	Location
VBM	NH>TI	Right	FOC, insula
		Left	FOC, ITC, OFG
CTA	NH>TI	Right	postcentral
		Left	PAC, SFG, Parstriangularis, Pericalcarine
	NH<TI	Right	SPG, IPG, SFG, mOFC
		Left	Precuneus, STG, IPG, Postcentral, ITG
	MH>TI	Right	Bankssts, MTG, SFG, mOFC, STG
	MH<TI	Right	Fusiform, ITG, PCG, Paracentral, IPG, LOG
		Left	Postcentral, Precuneus, SPG
	Coping < suffering	Right	Cingulate gyrus
		Left	Cingulate gyrus
	Unilateral > Bilateral	Left	Cuneus, Temporalpole, cingulate gyrus
Subcortical volumetric and shape	NH > TI	Right	Pallidum,
		Left	Pallidum
		N/A	Brainstem
	MH > TI	Right	Hippocampus
		Left	Pallidum
	Coping > suffering	Right	Putamen, amygdala
	Unilateral < Bilateral	Right	NAc
fMRI (Task)	MH<TI	Right	STG, MTG, LOG
fMRI (Rest)	NH<TI	Left	Anterior cingulate cortex
	MH<TI	Left	Temporal fusiform cortex
	Unilateral < Bilateral	Left	Thalamus
	Low HADS > high HADS	Right	Cingulate gyrus
Perfusion	Control > TI	Left	Insula, FOC, visual cortex
	Coping > suffering	Right	Auditory cortex
	Low HADS < high HADS	Right	Cingulate gyrus
Diffusion	NH>TI	Right	ATR, IFOF, ILF, CST, CC, SLF
		Left	
	MH>TI	Right	SLF, CC, IFOF,CST, ILF
		Left	ATR, SLF, CC, IFOF,CST, ILF
	Unilateral < Bilateral	Left	CC
Low HADS > high HADS	Left	Cingulum	

Chapter 11: General Discussion, Conclusion and Future work

Table 11.2: Results summary of correlation analysis in tinnitus patients.

Analysis	Variables	Correlation	Hemisphere	Location
Subcortical volumetric	Tinnitus severity	Negative	Left	Hippocampus
fMRI (Task)	Tinnitus duration	Positive	Right	PCG and MTG
			Left	STG and SFG
	Tinnitus severity	Positive	Right	STG
			Left	STG
	Hearing loss	Positive	Right	PAC and IFG
			Left	PAC
fMRI (Rest)	Tinnitus severity	Positive	Right	Visual cortex, PCG, Frontal pole
			Left	Visual cortex, PCG, Cingulate gyrus, LOG, insula, PAC
Perfusion	Tinnitus duration	Positive	Right	MTG and STG
			Left	PCG and STG
	Tinnitus severity	Negative	Right	PAC
			Left	Parahippocampus
	Hearing loss	Negative	N/A	Brainstem

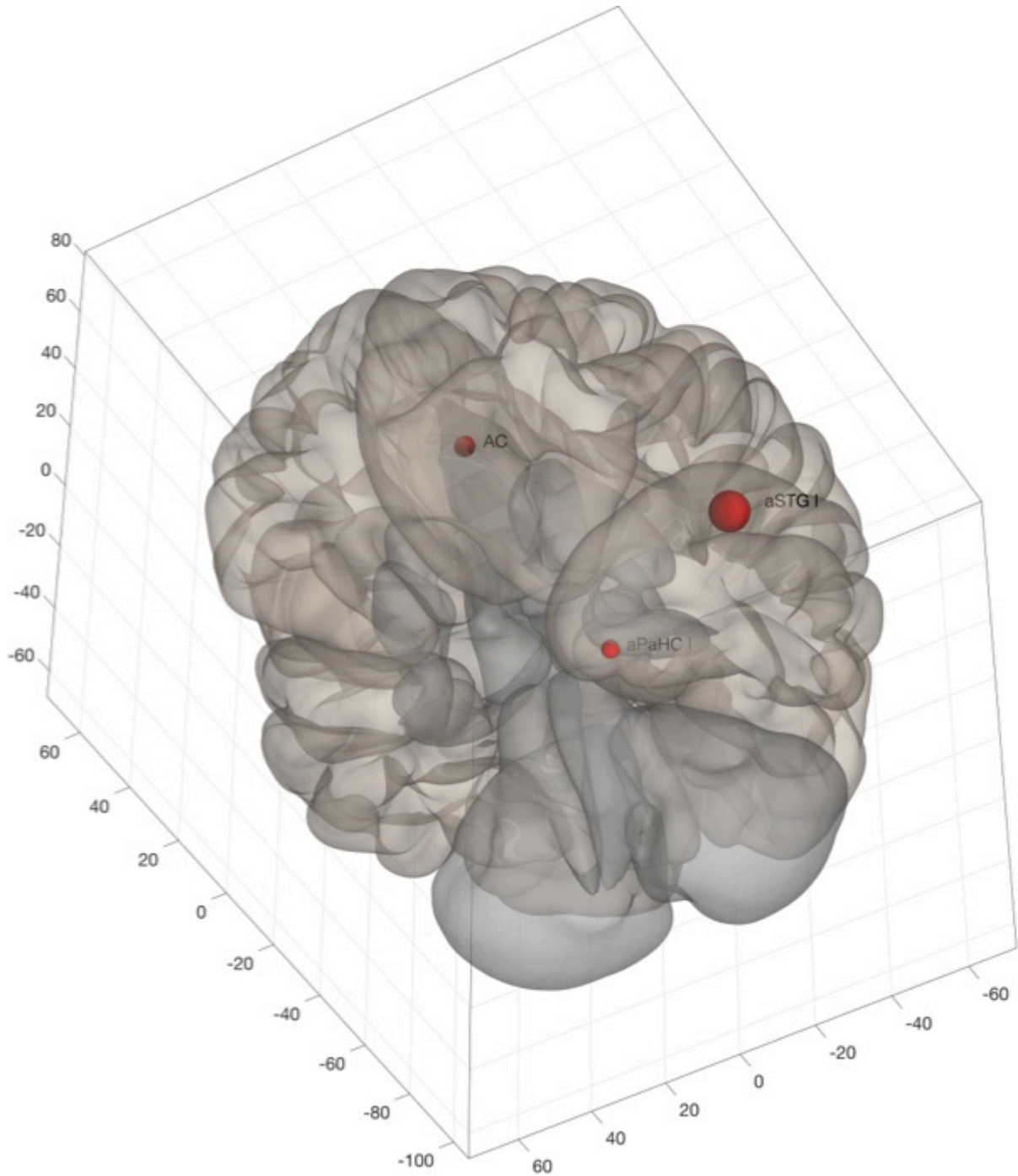


Figure 11.1: Consistent results are displayed in surface view of the human brain. Right anterior cingulate cortex (AC) were found to be involved in tinnitus patients with high level of anxiety and depression, left anterior parahippocampus cortex (aPaHC) was found to be involved in tinnitus severity, and left anterior superior temporal gyrus (aSTG) was found to be involved in tinnitus duration. 3D display reconstructed for illustration purpose using CONN Functional Connectivity toolbox (16.a).

11.3 General discussion

11.3.1 Association between tinnitus perception and brain structure and function

The association between tinnitus perception and brain structural atrophy and dysfunction has been reported widely in the tinnitus population (Adjajian et al., 2014, Aldhafeeri et al., 2012a, Amaral and Langers, 2015, Benson et al., 2014, Boyen et al., 2013, Hinkley et al., 2015). This thesis adds to these studies by showing the association between tinnitus perception and brain structure and function. I evaluated the influence of tinnitus perception on grey matter volume, cortical thickness, cortical structural integrity, cerebral blood flow, auditory perception and functional connectivity. I found that tinnitus perception has an effect on these brain structure features and function, which suggests an association between tinnitus perception and brain structural and function.

Our results support the hypothesis that grey matter reduction and cortical thinning in limbic regions, including the insula, prefrontal cortex, cingulate gyrus and hippocampus, are associated with tinnitus perception. In VBM analysis, hearing loss was seen to be an important factor in identifying grey matter alteration in tinnitus patients, as we could not find a significant difference in grey matter volume with mild to moderate hearing loss group. However, CTA could identify the effect of tinnitus perception on cortical thickness with the hearing loss-matched group.

The cortical thickness of parahippocampal gyrus showed consistent results in the tinnitus group compared to the two healthy groups (NH and MH). We also found the atrophy and dysfunction of parahippocampal gyrus that showed a correlation of grey matter volume and CBF with tinnitus severity respectively. Human and animal

studies have shown that stress changes neuronal morphology suppresses neuronal proliferation, and reduce hippocampal volume (Kim et al., 2015). The hippocampus is densely concentrated with receptors for cortisol in human (Kim et al., 2015). These findings may demonstrate the involvement of limbic and memory networks in tinnitus perception, which could be a target for future treatments.

Brain structural atrophy and dysfunction of prefrontal cortex have been reported in this thesis as we found a reduction of GM volume, cortical thickness, white matter integrity, and increase of functional connectivity in this brain region that has been reported widely in tinnitus literature studies. The PFC is involved in the processing of emotion, memory and decision-making (Rolls, 2004). It was reported that tinnitus suffers have depression (Falkenberg and BøWie, 2012), memory impairments and attention deficits (Rossiter et al., 2006).

Insula cortex atrophy and dysfunction has been reported in this thesis as we found grey matter reduction, cortical thinning, WM integrity reduction, hypoperfusion and increased functional connectivity in this brain region. Insula cortex is a multi-complex brain region that is involved in different processes, such as emotion, auditory and pain. Also, it is part of the salience network that contributes to a wide range of complex brain functions such as communication, behaviour and awareness. From these findings, we suggest that insula cortex might play an important role in effective reactions specific to tinnitus.

Hyperactivity of auditory cortex in tinnitus patients was observed in our task-based fMRI study. Also, functional connectivity of auditory network was found significantly positive correlated to tinnitus severity. I found cortical thickness of auditory cortex

to be significantly thinner in tinnitus patients, which may suggest structural atrophy of this brain region. Many tinnitus models have hypothesized the dysfunction of auditory cortex that could cause tinnitus perception by increasing spontaneous stochastic fired rate at auditory pathway (Jastreboff, 1990, Eggermont and Roberts, 2004, Sun et al., 2009).

11.3.2 Association between tinnitus severity with brain structure and function

Our results may be representative of neural substrate in tinnitus coping and suffering patients as we asked tinnitus patients whether they are seeking medical assistance due to being unable to cope with tinnitus symptoms. Thirteen tinnitus subjects showed they are bothered and cannot cope with tinnitus, while 13 tinnitus subjects showed they could cope and were not seeking medical assistance. I investigated the correlation between tinnitus severity (THI and TFI) and the change of brain structure and function. One of the challenges in tinnitus research is why some tinnitus patients can cope with tinnitus symptoms, while others cannot. Thus, I aimed to investigate how tinnitus severity affects brain structure and function, and whether there are brain regions that are involved in coping with tinnitus severity.

In general, not all MRI techniques have shown a significant difference between tinnitus coping and suffering subgroups. This might be because of the sample size of tinnitus patients. Hippocampus appeared to be an important part of the brain in tinnitus severity as the GM volume of left hippocampus showed a significant negative correlation with the TFI score (this correlation did not survive correction for multiple comparisons), and the cerebral blood flow of left parahippocampus gyrus showed a significant negative correlation with tinnitus severity (THI and TFI).

These structural and functional findings may demonstrate the important role of hippocampus and parahippocampus gyrus in tinnitus coping strategy, and suggest the involvement of limbic and memory networks in tinnitus severity.

11.3.3 Association between tinnitus laterality and brain structure and function

An individual with tinnitus may localize tinnitus perception in one ear, or both that might reflect an important aspect of tinnitus mechanism. Tinnitus laterality has not received attention deeply in neuroimaging studies. One EEG study assessed the neural correlates of tinnitus laterality, which suggests the association between the activities of parahippocampus gyrus and tinnitus laterality (Vanneste et al., 2011a). Also, It was observed that there was an alteration of brain activity pattern in unilateral tinnitus group in the auditory pathway contralateral to where the tinnitus was perceived (Melcher et al., 2000, Smits et al., 2007, Lanting et al., 2008).

In this thesis, I investigated the neural substrates of tinnitus laterality using a wide range of different MRI techniques. I found a significant correlation between tinnitus laterality and severity as the unilateral tinnitus subgroup showed a higher impact of tinnitus perception on the quality of life compared to the bilateral tinnitus subgroup. Also, I found that bilateral tinnitus subgroup showed a significant lower FA value compared to unilateral tinnitus participants at left corpus callosum that connects right and left hemispheres. Moreover, I identified the effect of tinnitus laterality on functional connectivity as the tinnitus bilateral subgroup showed a significant increase of functional connectivity compared to the tinnitus unilateral subgroup in the ventral attention network that is involved when behaviourally relevant stimuli occur unexpectedly.

These findings suggest that tinnitus laterality may play an important role in neural basis caused by tinnitus perception. Corpus callosum and ventral attention network showed the involvement in tinnitus laterality. It could be argued that tinnitus laterality subgroups should be considered carefully in tinnitus treatment, as structural and functional connectivity was found significant difference between tinnitus laterality subgroups. Further research is required to investigate the tinnitus laterality on brain structure and function in a larger population over time that might lead to identification of different tinnitus pathophysiological models between the subgroups.

11.3.4 Association between anxiety and depression and brain structure and function in tinnitus patients

A close relationship between tinnitus perception and a high prevalence of anxiety and depression has been reported in tinnitus patients (Zoger et al., 2006, Krog et al., 2010, Falkenberg and BøWie, 2012). Anxiety and depression are the most common mental health concerns that are often experienced as a complex set of emotional and functional challenges. The association between the neuroanatomical and functional alteration in tinnitus patients and anxiety and depression has not been examined deeply in tinnitus research.

The cortical thickness of the anterior cingulate cortex was found significantly negative correlated to combined anxiety and depression scores (Leaver et al., 2012). Our results are in line with the Leaver et al (2012) findings as I found a significant increased of CBF in cingulate gyrus in tinnitus patients with high level of anxiety and depression compared to tinnitus patients with low level of anxiety and depression.

Furthermore, I found a significant reduction of WM integrity in cingulum WM tract (anterior and posterior cingulate gyri) in tinnitus patients with high level of anxiety and depression compared to tinnitus patients with low level of anxiety and depression. Screening and treating anxiety in tinnitus patients could potentially improve perceived levels of tinnitus (Pattyn et al., 2016). These findings may demonstrate the important role of the cingulate cortex and cingulum WM tract in the anxiety and depression of tinnitus patients that could be a therapy target to reduce the severity of tinnitus.

11.4 General limitations

Although this thesis was carefully prepared and has achieved its aims, there are some limitations and shortcomings that should be considered carefully in future studies.

First of all, the sample size of tinnitus patients was quite small due to time consuming. Twenty-six tinnitus patients (total of 34 subjects) who met the strict inclusion and exclusion criteria took part in MRI studies. Therefore, to generalize the results for a larger tinnitus population, the study should have recruited more tinnitus patients with wide range of tinnitus severity levels.

Second, the task-based fMRI experiment has some limitations due to the scanner noise while auditory stimuli were presented, even though earplugs and headphones were used to eliminate the scanner noise. It would be advisable to use “Sparse” temporal sampling technique in future studies to eliminate the effect of scanner noise by acquiring functional brain images at the end of auditory stimulus and baseline conditions (Hall et al., 1999). Moreover, the resting state fMRI assesses the

brain during rest; therefore, participants were kindly requested to not think about anything in particular and let their mind wander. There is no guarantee that participants were not thinking during the scan.

Third, brain structural analysis is based on automatic tissue segmentation and spatial normalisation. Despite the fact that this automated technique has been validated, it is possible to find a misalignment that could be caused by subject motion artifacts, abnormal ventricles size and image acquisition sequence. Therefore, I visually inspected the images during preprocessing steps and manually post-corrected the automatic segmentation results.

Last but not least, diffusion images are very sensitive to any physiological motion that may affect the accuracy of the results. Therefore, the scan time was reduced to approximately 11 minutes in order to eliminate the effect of the physiological motion of diffusion findings. Another limitation of DTI studies, including the present study, is that interpretation of WM changes at crossing tracts or tract junction is complicated and difficult due to tensor (DTI) having trouble distinguishing voxels with crossing fibers from isotropic region (Smith et al., 2006).

11.5 Clinical applications of the findings

In the majority of tinnitus cases, patients are experiencing tinnitus subjectively, and there is no way to diagnose tinnitus objectively. The left parahippocampus gyrus and hippocampus could be therapeutic targets to reduce tinnitus severity. In addition, the right cingulate gyrus could be a therapeutic target to reduce the level of anxiety and depression in tinnitus patients. Furthermore, behaviour assessments, such as THI and TFI with audiological examination, could be utilized to evaluate the

ongoing progression of tinnitus symptoms or the effectiveness of therapeutic trials in tinnitus patients.

11.6 Future directions

In this thesis, I investigated the neural substrates of tinnitus patients with a wide range of tinnitus severity, onset, laterality and hearing loss. Future studies should conduct the research in a larger tinnitus population with a wider range of tinnitus characteristics in order to investigate the effect of each tinnitus subgroups on brain structure and function.

Furthermore, most tinnitus patients recruited in this thesis had normal-to-moderate hearing loss. It would be advisable to examine the neural substrates between tinnitus patients with normal hearing and tinnitus patients with hearing deficiencies.

In addition, exposing to a loud noise is considered as one of major causes of tinnitus (Møller, 2011b). It would be advisable to compare tinnitus patients, who are working or used to work in very loud environments such as military army and heavy industries and tinnitus patients who are not.

Other future works should examine the effect of early and late diagnosed tinnitus on brain structure and function. The neural substrates of recent tinnitus diagnosed may differ from late tinnitus diagnosed, which may help in the development of tinnitus treatment.

Moreover, longitudinal neuroimaging studies could improve understanding of the tinnitus mechanism, and assess the effectiveness of tinnitus treatments such as

tinnitus retraining therapy (TRI), repetitive transcranial magnetic stimulation (rTMS) and pitch discrimination.

11.7 Concluding comments

To conclude, this thesis employed new advanced MRI techniques with a wide range of behavior assessments to bring a fresh approach to brain imaging of tinnitus patients. Tinnitus subgroups were considered carefully in this thesis, as tinnitus is a heterogeneous disorder, which is considered to be one of the challenges in tinnitus research. Tinnitus severity, laterality and association with anxiety and depression were examined carefully in order to give a new insight into the tinnitus pathophysiology mechanism. The same groups of participants have been scanned in all modalities, which is a potential strength of this research.

Novel findings of the thesis include the presence of cortical and subcortical morphology, changes of structural and functional connectivity, and hyperactivity of auditory perception and alterations of blood perfusion in tinnitus patients. The thesis expands upon previous neuroimaging findings in tinnitus patients and suggests new area for future work; for instance, the neural correlate of early and late diagnosed tinnitus. The findings of this thesis give new insight into neural correlate alterations in chronic tinnitus. It is hoped that future research will continue to be undertaken to enhance our understanding of the pathophysiology of tinnitus mechanism that will aid in the identification of an effective tinnitus cure and lead to improvement in the quality of life in tinnitus patients.

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Appendix I: Study announcement



Institute of Translational Medicine & MARIARC

People with Tinnitus Needed for Brain Research

In order to understand how experiencing tinnitus, for more than 6 months, influences brain structure and function we are inviting tinnitus experiencing volunteers between the ages of 30 and 65 years of age to participate in our research study.

Taking part in this study will involve:

1. Being screened by the radiographer at MARIARC to ensure that it is safe for you to be scanned in the MRI scanner (taking approximately 10 minutes).
2. Completing two questionnaires to understand how tinnitus impacts your daily living.
3. Having hearing tests in order to measure the acuity of your hearing, such as loudness and hearing thresholds. This takes around 15 minutes and will be performed on the day of the scan, outside the scanner room.
4. Having a Magnetic resonance (MRI) scan of your brain, normally of no more than 46 minutes duration, at MARIARC.

No adverse effects are known to result from magnetic resonance imaging. If you would like more information about this study to allow you to decide whether to participate, please contact the researchers for an Information Leaflet.

Thank you!

Researchers:

Fahad Alhazmi , MARIARC, Pembroke Place, University of Liverpool, L69 3GE
Contact: Tel: 01517954627 Mob: 07402226888 Email: F.alhazmi@liv.ac.uk

Dr Vanessa Sluming, Institute of Translational Medicine, Whelan Building, The Quadrangle,
University of Liverpool, Liverpool L69 3GB

Appendix II: Participants Information Sheet

PIS v02_TrAccepted 12th January 2015



Participant Information Sheet

An Investigation of the Effect of Tinnitus on underlying Neural Processes, Cortical Structural integrity and Auditory Resting State Networks

Researchers: Fahad Alhazmi, Vanessa Sluming

- **Invitation paragraph**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, discuss it with others (e.g. friends, relatives and GP) if you wish and take time to decide whether or not you wish to take part. If you would like more information or have any questions, please contact us. We would like to stress that you do not have to accept this invitation and should only agree to take part if you want to. Thank you for reading this.

- **What is the purpose of the study?**

The aim of this study is to better understand the mechanism of tinnitus perception and to identify the structural, functional and connectivity changes of the brain in bothersome and non-bothersome tinnitus sufferers.

- **Why have I been invited?**

We are inviting you to take part in the study due to you fit certain criteria, specifically, that you have experienced tinnitus for more than 6 months and are aged between 30-65 years

- **Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this Information Sheet to keep and be asked to sign a consent form. We are very grateful for your participation, and hope you will enjoy taking part. However, even if you sign the consent form you are still free to withdraw at any time and without giving a reason.

- **What will happen to me if I take part?**

The study consists of five parts:

- i) safety screening for entry into the MRI scanner room;
- ii) answering any questions arising from this information sheet and signing the study consent form;

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PIS v02_TrAccepted 12th January 2015

- iii) an assessment of your hearing acuity or ability,
- iv) you will be asked to complete questionnaires regarding your experience of tinnitus
- v) you will be invited to have MRI scans of the brain.

i) When you attend the reception of MARIARC, you will be asked to fill in and sign a short safety screening form in order to make sure there are no reasons which would prevent you entering the MRI scanner room where there is a strong magnetic field. Such reasons could include having a cardiac pacemaker, having metal splinters in your eye or having a metal implant which is not compatible with MRI scanners.

ii) The researcher will answer any questions you may have. If you are satisfied with their answers and wish to participate in the study, you will be talked through the consent form and then asked to sign it.

iii) You will undergo a hearing test (exactly the same as you might have in a hospital audiology department). The hearing test will take 15 minute. This will be done by a consultant physician in audiological medicine

iv) You will be asked to complete some questionnaires – these are standardised questionnaires and help us to understand how tinnitus affects your daily life.

v) Then, you will be asked to change into a gown (changing rooms are available) in order to remove any material that can be affected by the magnetic field such as hearing aids, mobile phones, credit cards, keys, coins, spectacles and pens (a secured locker will be provided). You will be provided with ear protection – ear plugs and noise reducing headphones – as the scanner is a noisy environment. During the scan, you will be asked to lie on the scanner table and a number of different scans will be performed. Between each scan we will talk to you through the intercom, to check that everything is fine. The total scan time of this investigation is 46 minutes approximately.

You are free to withdraw from the study at any time and without giving any reason.



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What is it?	How long will it take?	When will it take place?	What will it involve?
Hearing test	15 minutes	Before the MRI Scan	Assess your hearing level in order to make sure your hearing level fits within the inclusion criteria
Screening check & Tinnitus Questionnaire	10-15 minutes	Before the MRI scan	You will be asked to fill a form that is for Safety screening check purposes and tinnitus questionnaire
Brain imaging	46 minutes	This will be arranged whenever is convenient for you	MRI brain scan using structural and functional protocols and DTI protocol.

- **Expenses and payments**

We will pay £30 for reimbursement of travel and expenses.

- **What are the possible benefits of taking part?**

There are no direct benefit to participants in taking part in this study. However, your involvement might help us to better understand how living with tinnitus is reflected in brain structure and function.

- **What are the possible disadvantages and risks of taking part?**

The MRI scanner is noisy and claustrophobic, but it causes no harm or long term effects. You will be given high-quality disposable earplugs to protect your hearing. Some people may have slight experience of anxiety and claustrophobic in the scanner. If you do feel uncomfortable, you will be able to notify us via the hand held buzzer you will be given before being moved into the scanner. If you press the buzzer at any stage, we will stop scanning and remove you from the scanner immediately without delay.

- **What if something goes wrong?**

If you are unhappy or there is something wrong in the study, please feel free to let us know by contacting Dr Vanessa Sluming and we will try to help you. If you remain unhappy or have a complaint that you feel you cannot come to us with, then you should contact the Research Governance Officer on 0151-794-8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

- **Will my taking part in this study be kept confidential?**

Yes, I can confirm that all the information collected on you during the study will be kept strictly confidential. If you agree to participate to this study, you have no objection to personal data relating to yourself (as defined by the Data Protection Act, 1998), being used for research purposes. Your personal information will be kept for up to fifteen years and then will be confidentially destroyed. You have a legal right to view your personal information stored with us. If you wish to view your personal data, please write to the University of Liverpool Data Protection Officer, Computing Services Department, Chadwick Building, Peach Street, Liverpool, L69 7ZF, for more information on how to do this.

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- **Will my taking part be covered by an insurance scheme?**
Participants taking part in the University of Liverpool ethically approved study have insurance cover.
- **What will happen to the results of the study?**
The findings of the study will be presented at research meetings and published in scientific journals. Therefore, other researchers can also benefit by sharing this information. The study will take at least one year to conduct and another year to analyse fully. We would be happy to supply you with the final results after conducting and analyze the study fully.
- **Will I hear feedback about the study?**
Yes, your GP will be informed if there are any incidental findings, your GP will discuss these with you.
- **What will happen if I want to stop taking part of the study?**
Without any explanation, you are free to withdraw from the study and even during the course of study. However, the results obtained up to the withdrawal time can be used. If you are not happy for this to be done, you can request that this data is destroyed and we will provide you with written confirmation that this has been done.
- **Who is organising and funding the research?**
This research project is funded by the University of Liverpool through Research Support Fees. No researchers will benefit financially from this research.
- **A person who is independent of the study:**
Ms Val Adams, Superintendent Research Radiographer, MARIARC
- **Contact for further information**
please do not hesitate to contact:

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Appendix III: Consent form

Version 01 28 October 2014

Consent Form



Research title: An Investigation of the Effect of Tinnitus on underlying Neural Processes, Cortical Structural integrity and Auditory Resting State Networks

Volunteer Identification Number (ID):

Please Initial...

1. I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and I am free to withdraw at any time without giving any reason.
3. I understand that My GP will be informed about my participation in this study.
4. I agree that personal data relating to myself (as defining by the Data Protection Act, 1998), being used for research purposes only.
5. I agree to take part in the above study.

Name of the volunteer Date Signature

Name of the person taking
this consent form Date Signature

Name of the researcher Date Signature

Appendix IV: A favorable ethical opinion



03 February 2015

Dr Vanessa A Sluming
Senior Lecturer
University of Liverpool
Whelan Building
The Quadrangle
Brownlow Hill
Liverpool
L69 3GB

Dear Dr Sluming

Study title: An Investigation of the Effect of Tinnitus on underlying Neural Processes, Cortical Structural integrity and Auditory Resting State Networks
REC reference: 14/NW/1473
IRAS project ID: 139931

Thank you for your e-mail responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Mrs Carol Ebenezer, nrescommittee.northwest-liverpoolcentral@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

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Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra_studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Appendices

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Copies of advertisement materials for research participants [NCoT Advert]	01	28 October 2014
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [UoL Insurance Letter]	01	01 August 2014
Letter from sponsor [NCoT Sponsor Approval]	01	19 November 2014
Letters of invitation to participant [NCoT Advert]	02_TrAccepted	19 January 2015
Participant consent form [NCoT Consent Form]	02_TrAccepted	19 January 2015
Participant information sheet (PIS) [PIS]	02_TrAccepted	19 January 2015
REC Application Form [REC_Form_20112014]		20 November 2014
Referee's report or other scientific critique report [NCoT Referee GM]	01	27 October 2014
Referee's report or other scientific critique report [NCoT Referee JJD]	01	29 October 2014
Research protocol or project proposal [Project Proposal]	01	28 October 2014
Summary CV for Chief Investigator (CI) [CV V A Sluming]	01	14 November 2014
Summary CV for student [CV F Alhazmi]	01	14 November 2014
Summary CV for supervisor (student research) [CV G J Kemp]	01	14 November 2014
Validated questionnaire [NCoT Beck Depression Inventory]	01	28 October 2014
Validated questionnaire [NCoT Tinnitus Functionality Index]	01	28 October 2014
Validated questionnaire [NCoT Tinnitus Handicap Index]	01	28 October 2014

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "*After ethical review – guidance for researchers*" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Appendices

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: <http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/>

HRA Training

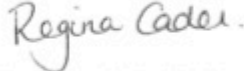
We are pleased to welcome researchers and R&D staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

14/NW/1473

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely



**Signed on behalf of:
Mrs Julie Brake
Chair**

Email: nrescommittee.northwest-liverpoolcentral@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mr Alex Astor, University of Liverpool*

Ms Michelle Mossa, Aintree University Hospitals NHS Foundation Trust

Mr Fahad Alhazmi, University of Liverpool

Professor Graham Kemp, University of Liverpool

Appendix V: Tinnitus Assessment Questionnaire

Tinnitus Assessment Questionnaire V1



Tinnitus Assessment Questionnaire

An Investigation of the Effect of Tinnitus on underlying Neural Processes,
Cortical Structural integrity and Auditory Resting State Networks

Name:..... Gender:..... DoB:.....

- Tinnitus features

- How long have you had tinnitus?
- Was there a particular incident you think caused tinnitus?
.....
- Has your tinnitus changed since it first started?
- What does tinnitus sound like?
 - Hissing
 - Whistling
 - Roaring
 - Chirping
 - Ringing
 - Voices
 - Pulsating
 - Clanging
 - Other ...
- Where do you have tinnitus?
 - Right ear only
 - Left ear only
 - Both ear equally
 - Both ear but no equally
 - In head
- Does your tinnitus remain constant or fluctuate?
 - Remains fairly constant
 - Fluctuates hourly or daily
- Rate the severity of your tinnitus:
 1 2 3 4 5 6 7 8 9 10
(Mild) (Sever)
- Estimate the pitch of your tinnitus:
 1 2 3 4 5 6 7 8 9 10
(Mild) (Sever)
- How annoying is your tinnitus?
 1 2 3 4 5 6 7 8 9 10
(Not annoying) (Very annoying)

Tinnitus Assessment Questionnaire V1

- **Medical history:**

- Do you have anything from the followings:
 - Ear infections
 - Sudden hearing loss
 - Other ear problems
 - Other illnesses
 - Possible TMJ
 - Allergies
 - Dizziness
 - Stress
 - Family history
- If you have dizziness, does it change with your tinnitus?
 - Yes
 - No

- **Social factors:**

- Do/did you work in a loud environment?
 - Yes
 - No
- Do you have hearing problem on one of the following situation:
 - Noise
 - One-to-one
 - Groups
 - Non
- Do you wear hearing protection for noise exposure or at other times?
 - Yes
 - No
- Have you been exposed to loud sounds (loud enough that you have to shout for someone to hear you at arm's length)?
 - Yes
 - No

- **Other factors:**

- Have you had any trauma in the past?
 - Yes
 - No
- Have you ever had a head or neck injury?
 - Yes
 - No
- Are you currently taking any prescription or over-the counter medications?
 - Yes
 - No

- If yes, describe all your medications including aspirin
.....
.....
.....

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