An Investigation into the Neural Substrates of Tinnitus

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

By
Faten Mane Aldhafeeri

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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.

Faten M Aldhafeeri

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Publications

Papers

1- **Aldhafeeri F**, Mackenzie I, Kaye T, Alghamdi J and Sluming V (2012) Neuroanatomical correlates of tinnitus revealed by cortical thickness analysis and diffusion tensor imaging. Neuroradiology vol 54 issue 8 pp 883-892.

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3- Abdul-Kareem I, Stancak A, Parkes L, Al-Ameen M, Alghamdi J, **Aldhafeeri F**, Embleton K, Morris D, Sluming V (2011) Plasticity of the superior and middle cerebellar peduncles in musicians revealed by quantitative analysis of volume and number of fibers based on diffusion tensor tractography. Cerebellum vol 10 issue 3 pp 611-62.3

Abstracts

1- **Aldhafeeri F**, Alghamdi J, Sluming V (2011) Functional MRI of regional brain responses to pleasant and unpleasant IAPS pictures: different networks. Proceedings of the European Congress of Radiology. ESR, Vienna pp xx.

2- **Aldhafeeri F**, Mackenzie I, Kaye T, Sluming V (2010) Tinnitus-Related Neuroplasticity in the Limbic System Evidenced by fMRI. Proceeding of the Symposium of International Association of Physicians in Audiology. IAPA, Krakow pp xx

3- **Aldhafeeri F**, Mackenzie I, Kaye T, Sluming V (2010) Neuroanatomical correlates of tinnitus evidenced by cortical thickness alterations: preliminary findings. Proceeding of the Symposium of International Association of Physicians in Audiology. IAPA, Krakow pp xx

4- **Aldhafeeri F** and Sluming V. (2009) Developing Sampling Strategy for Estimating Volume of Prefrontal Cortex in Very Pre-Term Infants. Proceedings of the National Meeting of the British Neuroscience Association. BNA, Liverpool pp 1

5- **Aldhafeeri F** and Sluming V. (2009) The contribution of MR field strength and scanning protocols to estimated volume of the prefrontal cortex subfields. Proceedings of the Scientific Meeting of the European Society of Magnetic Resonance in Medicine and Biology. ESMRMB, Antalya pp 722

Abstract

Aims and Objectives: The overall aim of this thesis was to investigate the neural correlates of tinnitus perception using different magnetic resonance imaging (MRI) techniques. The first objective was to investigate the neuro-functional reorganisation that may be associated with tinnitus-related emotional disturbances using functional MRI (fMRI). The second objective was to investigate the structural brain changes that are hypothesised to be associated with tinnitus perception. This investigation included an analysis of both grey and white matter using diffusion tensor imaging (DTI), a cortical thickness analysis (CTA) and voxel-based morphometry (VBM). The third objective was to investigate any correlations between the neuro-functional and the neuro-structural reorganisation that are associated with tinnitus and behavioural data, such as hearing thresholds, the Newman Handicap Inventory score and age. These analyses were performed for both the experimental and control groups.

Methods: A total of 18 tinnitus sufferers and 15 age- and sex-matched healthy volunteers participated in the work that is presented in this thesis. The functional MRI study in this thesis utilised internationally standardised emotionally evocative pleasant and unpleasant visual and auditory stimuli. The fMRI paradigm consisted of a block design, during which the participants from the two groups (tinnitus and controls) viewed blocks of images and listened to sounds. The structural investigations in this thesis included DTI, CTA and VBM.

Results: In the fMRI study, tinnitus sufferers exhibited significant hyperactivation in the limbic system, the prefrontal cortex (PFC) and the temporal lobe. Correlation analyses between the mean fMRI Blood Oxygen Level-Dependent (BOLD) signal and hearing thresholds revealed a significant positive correlation in the subjects with tinnitus but not the in the controls. This correlation was observed in the following regions: the right cingulate gyrus, the right medial frontal gyrus, the right superior temporal gyrus, the left inferior frontal gyrus and the left superior temporal gyrus. Tinnitus severity as measured using the Newman Tinnitus Handicap Inventory score (Newman THI) was observed to be positively correlated with the mean fMRI BOLD signal in the left superior temporal gyrus and the right cingulate gyrus. Tinnitus-like conditions induced the healthy controls to exhibit hyperactivity in the limbic system, the PFC and the temporal lobe. The DTI study demonstrated disrupted white matter (WM) integrity in the following bundles in the subjects with tinnitus relative to the control group: the left and right inferior fronto-occipital fasciculus, the corpus callosum, the left superior and inferior longitudinal fasciculus, and the left and right thalamic radiations. CTA revealed cortical thickness reductions in the subjects with tinnitus compared to the controls in a priori hypothesised regions of interest (ROI), which included the following regions: the temporal lobe, PFC, anterior cingulate cortex (ACC), cingulate gyrus (CG) and posterior cingulate gyrus (PCG). VBM revealed reduced

grey matter (GM) volume in the left medial PFC in subjects with tinnitus compared to controls.

Conclusion: Tinnitus perception may involve functional and structural changes in the following specific brain regions: the PFC, the temporal lobe (including the auditory cortex) and the limbic system. These structural changes may represent antecedent structural deficits that result in subsequent functional reorganisation, causing the tinnitus signal to arise.

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Abbreviations

ACC	Anterior Cingulate Cortex
ANS	Autonomic Nervous System
ATP	Adenotriphosphate
ATR	Anterior Thalamic Radiation
B_0	Static Magnetic field
BA	Brodmann Area
BOLD	Blood Oxygenation Level-Dependent
CC	Corpus Callosum
CG	Cingulate gyrus
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CTA	Cortical Thickness analysis
DB	Decibels
DBHL	Decibels at Hearing Level
DCN	Dorsal Cochlear Nucleus
DTI	Diffusion Tensor Imaging
EEG	Electroencephalography
FA	Fractional Anisotropy
FDG	Fluorodeoxyglucose
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FWHM	Full Width at Half Maximum
GLM	General Linear Model
GM	Grey Matter
HR	Hemodynamic Response
IADS	International Affecting Digitised Sounds
IAM	Internal Auditory Meatus
IAPS	International affecting Picture System
IC	Inferior Colliculus
IHCs	Inner Hair Cells
LE	Left Ear
MD	Mean Diffusivity
MEG	Magnetoencephalogram
NMDA	N-Methyl-D Aspartate
NMR	Nuclear Magnetic Resonance
NMV	Net Magnet Vector
OHCs	Outer Hair Cells
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PO_2	Oxygen Partial Pressure
PTSD	Post Traumatic Stress disorder
QEE	Quantitative Electroencephalogram

RE	Right Ear
ROS	Reactive Oxygen Species
SD	Standard Deviation
SPECT	Single Photon Emission Computed Tomography
STG	Superior Temporal Gyrus
T1	Longitudinal Relaxation Time
T2	Dephasing Relaxation Time
TE	Echo Time
THI	Tinnitus Handicap Inventory
TR	Repetition Time
TRT	Tinnitus Retraining Therapy
VBM	Voxel-Based Morphometry
WM	White matter

1. Chapter 1. Introduction

1.1 Introduction

Tinnitus is a phantom auditory perception (Jastreboff 1990). Although there are many hypotheses (Baguley 2002) that attempt to explain the actual mechanism of tinnitus induction, the pathophysiology of this phantom is still poorly understood. Tinnitus cannot be treated, although its consequences can be reduced to certain extent. A strong association has been demonstrated between ear problems and tinnitus prevalence, as 85% of patients with ear problems experience tinnitus (Jastreboff 1990). In the UK, there are 2.3 million adults (approximately 10% of the adult population) who have experienced tinnitus as an irritating phenomenon that affects their quality of life (Baguley 2002; 2005). There appears to be a significant correlation between tinnitus perception and certain epidemiological factors, such as sex, age and lifestyle. For example, men are believed to experience tinnitus more frequently than women for unknown reasons (Lockwood, Salvi et al. 2002). However, others believe that women are more likely to experience tinnitus (Baguley 2002). Although tinnitus can affect individuals at any age, chronic tinnitus was reported to affect elderly individuals more commonly than adults: 12% of seniors over the age 60 have experienced chronic tinnitus, whereas only 5% of adults who are under 60 years of age have experienced tinnitus (Eggermont and Roberts 2004). Unlike acute tinnitus, which lasts for a few days to several weeks, chronic tinnitus is more persistent and lasts for more than six months (Folmer, Martin et al. 2004). Low socio-economic class and frequent exposure to occupational noise are believed to be associated with a higher prevalence of tinnitus (Baguley 2002).

Tinnitus has been classified into many types according to several criteria, such as sound characteristics (i.e., pulsatile versus continuous), or based on the origin of

the condition. Pulsatile tinnitus resembles the patient's heartbeat and is generally associated with it, whereas continuous tinnitus persists as a continuous signal. Tinnitus is often only perceived by the patient and, in these cases, is referred to as a subjective condition. In other cases, tinnitus can be observed even by the physician; this type of tinnitus is referred to as objective tinnitus. Previous investigations have demonstrated that subjective non-pulsatile tinnitus is more common than objective pulsatile tinnitus (Weissman and Hirsch 2000). Although scientists disagree over the exact mechanism by which tinnitus occurs, the problem is believed to involve pathogenic processes in both the ear and the brain. The question of where tinnitus occurs has therefore been argued for many years. Certain experts (Knobel and Sanchez 2008) have hypothesised that tinnitus can occasionally be attributed to the brain, focusing on background electrical activity, which is a part of normal ear function. Symptoms can also be induced by environmental factors, such as anxiety, depression and fatigue (Eggermont 2003; Eggermont and Roberts 2004).

Tinnitus is not considered to be a disease, but it may be a symptom of many diseases that involve either the peripheral or central auditory system. It is difficult to treat tinnitus given that there are several pathologies that affect the auditory system and do not influence hearing, necessitating the evaluation of each case individually. Given that the underlying neural mechanism has not been clearly identified, there is no method for identifying a common treatment for tinnitus. Another obstacle to the treatment of tinnitus is that it is typically perceived only by the patient; i.e., it is a phantom perception in the vast majority of cases, making the symptoms difficult to measure (Jastreboff 1990; Jastreboff and Hazell 1993).

Functional magnetic resonance imaging (fMRI) has been exploited in the past two decades to better understand the neural substrates of tinnitus and its associated symptoms. Golm et al. (2012) reported the involvement of the limbic system and frontal brain regions in tinnitus sufferers compared to control subjects. These authors suggested that the left middle frontal gyrus may be involved in highly distressing tinnitus compared to mildly distressing tinnitus. Resting-state fMRI studies have revealed increased functional connectivity between the auditory network and both the amygdala and the dorsolateral prefrontal cortex in tinnitus sufferers compared to those who do not suffer from tinnitus (Kim, Kim et al. 2012). Increased activity of the nucleus accumbens has been reported when tinnitus sufferers listened to band-passed white noise stimuli (Leaver, Renier et al. 2011).

The work presented in this thesis investigates the possible neural basis of tinnitus perception using magnetic resonance imaging techniques. This work is important because it increases our understanding of tinnitus pathophysiology and raises possibilities for further research into possible treatments.

1.2 Aim of the thesis

The overall aim of this thesis was to investigate the functional and structural neural changes that are associated with tinnitus perception using magnetic resonance imaging techniques.

1.3 Organisation of the thesis

1.3.1 Chapter 2: Literature review: In this chapter, a brief review of human brain anatomy and physiology is considered. Hypotheses regarding tinnitus induction and theories that relate to tinnitus pathophysiology are also reviewed. The

neurophysiological model of tinnitus proposed by Jastreboff, on which the hypotheses that were tested in this thesis rely, is discussed. Neuroplasticity and similarities among phantom pain, post-traumatic stress disorder and tinnitus are also reviewed. This chapter highlights the role of functional and structural imaging modalities in understanding the neural basis of tinnitus.

1.3.3 Chapter 3: Methods: This chapter explains the experimental methods that were applied in this thesis, although each chapter describes a specific set of methods. This chapter lists the inclusion and the exclusion criteria that were used to recruit participants with tinnitus and the healthy participants. This chapter also describes the audiology tests that were used to assess the hearing status of all the participants and the Newman handicap inventory questionnaires, which was used to assess the severity of the tinnitus in the affected subjects.

1.3.4 Chapter 4: functional MRI: This chapter investigates and discusses the neural substrates of tinnitus on the basis of the fMRI findings of this thesis and the findings of previous studies. This chapter also describes the neural basis of the tinnitus-like condition that was induced in healthy volunteers as a model of tinnitus in humans. The correlations between the fMRI signal change and behavioural characteristics are reported and explained.

1.3.5 Chapter 5: Diffusion tensor imaging: This chapter investigates white matter integrity in patients with tinnitus compared to healthy controls by estimating fractional anisotropy and mean diffusivity. This chapter also discusses the findings of this thesis in the context of other pathologies that cause symptoms that are similar to those of tinnitus.

1.3.6 Chapter 6: Cortical thickness analysis and voxel-based morphometry: This chapter investigates the structural changes that are associated with tinnitus by estimating cortical thickness and grey matter volume. This chapter also correlates these findings with the obtained behavioural data.

1.3.7 Chapter 7: General discussion, Conclusion and future work: This chapter highlights and summarises the major findings of the thesis, and a pathophysiological mechanism for tinnitus is suggested. The conclusion of this chapter highlights the primary findings of this thesis and suggests possible directions for future tinnitus research.

2. Chapter 2. Literature Review

2.1 Introduction

This chapter includes a brief review of human brain anatomy and the cortical areas that are involved in sound perception, emotions, and attention. The selection of these three systems was based on tinnitus and its associations. Tinnitus is an auditory phantom that is characterised by the experience of sound in the ears when no external stimuli are present (Jastreboff 1990). This symptom implicates the role of the auditory cortex in tinnitus. Tinnitus is associated with negative emotions, such as annoyance, depression and anxiety (Jastreboff and Hazell 2004), characterising the emotional aspect of the condition. Human emotions such as depression and anxiety are primarily regulated by the limbic system (Derryberry and Tucker 1992). Therefore, a brief review of the structure and function of the limbic system is presented. The structure and function of the prefrontal cortex (PFC) are briefly reviewed given that this region contributes to attention and emotional tasks in humans (LeDoux 2000; Knudsen 2007).

The aim of this chapter is to identify certain proposed pathophysiological mechanisms of tinnitus that define the research area. The first objective is to identify aspects of tinnitus perception that have not been investigated in previous studies and models of this condition. The second objective is to discuss the role of imaging modalities and their contribution to and validity in better understanding the neural basis of tinnitus. The third objective of the literature review is to formulate the inclusion and exclusion criteria to (1) avoid the limitations of other fMRI studies and (2) take into account the recommendations of previous studies.

2.2 The Human Brain

The central nervous system (CNS) is divided into six primary divisions: (1) the spinal cord, (2) medulla, (3) pons and cerebellum, (4) midbrain, (5) diencephalon, and (6) cerebral hemispheres. Each division performs specific functions and is integrated with the other divisions of the CNS. Because this thesis investigates the neural basis of tinnitus using MRI, the cerebral hemispheres are the focus of this review.

The cerebral hemispheres are separated into two halves by the sagittal fissure. The hemispheres consist of four components: (1) the cerebral cortex, (2) basal ganglia, (3) hippocampal formation, and (4) amygdala. The cerebral cortex is a highly convoluted tissue (approximately 2200 cm^2) that is located on the surface of the cerebral hemispheres. These convolutions are referred to as gyri, which are separated by folds, or sulci. The cerebral cortex is divided into four major lobes: the frontal, temporal, parietal, and occipital lobes.

The basal ganglia, the second component of the cerebral hemispheres, are subcortical nuclei that participate in higher brain functions. The basal ganglia are composed of several structures, including the caudate nucleus, the putamen, the globus pallidus and the amygdaloid nuclear complex (Carpenter 1976; Martin 1989). The third and fourth components of the hemispheres, the hippocampal formation and the amygdala, will be discussed in the following sections and throughout this thesis. The primary components of the human CNS and the four primary lobes of the cerebral cortex are diagrammed in Figure 2.1.

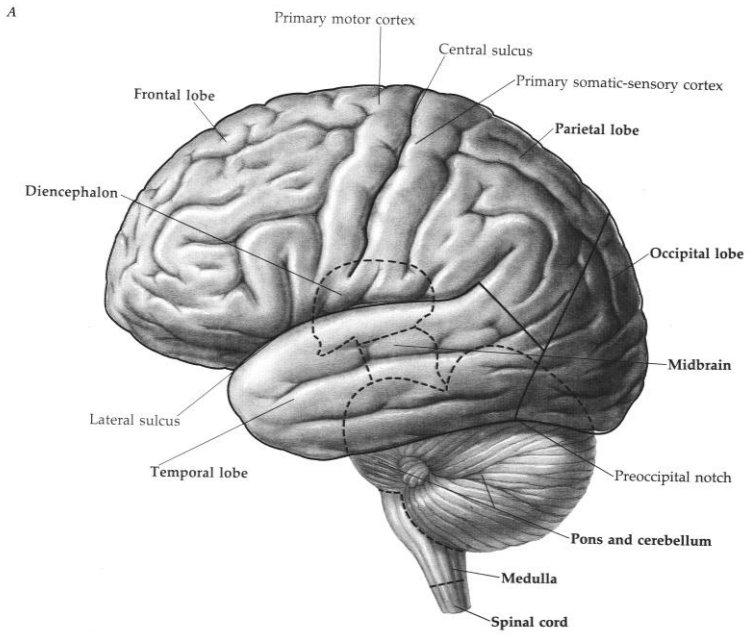


Figure 2.1. Main componsents of the CNS and the four lobes of the human cortex (Martin 1989).

The human cerebrum is composed of a very large number of neurons that generally form six folded layers; all of these neurons function together to form the frontal, parietal, occipital, temporal and insular cortices. The frontal lobe is separated from the parietal lobe by the central sulcus and from the temporal lobe by the lateral sulcus. The lateral sulcus also separates the temporal lobe from the parietal lobe. The occipital lobe is separated from the parietal lobe by the parietal-occipital sulcus. The five lobes work in an integrative manner to perform brain functions.

Each layer of the human cerebrum is distinct in its cytoarchitecture, which is characterised by the layer's constituent cell types, density and arrangement. The cortex varies in thickness in the different brain regions. Moreover, due to variations in the thickness of the individual layers, there are also cortical thickness variations in a given cortex. For example, within the temporal lobe, the superior

temporal gyrus is relatively thicker than the middle and the inferior temporal gyri. Cortical thickness is a representation of the cytoarchitecture of the human cerebrum. That is, cortical thickness reflects cellular density, cell type, cellular organisation, and myelination. In normal human adults, the thickness of the cerebral cortex ranges from 4.5 mm in the precentral gyrus to 1.5 mm in the calcarine sulcus (Economo 1929; Truex and Carpenter 1969; Carpenter 1976; Garey 1999).

The human brain is comprised of three primary components: grey matter (GM), white matter (WM), and the cerebrospinal fluid (CSF). The GM contains neurons, dendrites, and portions of myelinated and unmyelinated nerve fibres. The WM contains far fewer neurons and dendrites but contains myelinated and unmyelinated nerve fibres. These fibres are arranged in bundles that connect the cortical and subcortical brain regions. Scientists have classified cerebral WM fibre bundles into three primary types according to their function. The first type is referred to as projection fibres, which transmit impulses from the cortex to distant locations, such as the spinal cord and the brainstem, and vice versa. The second type of fibre is referred to as association fibres, which connect different cortices within the same hemisphere, such as those that connect the frontal and the temporal lobes within one hemisphere. The commissural fibres, the third type of fibre, connect the right and the left corresponding hemispheric cortices. The corpus callosum is comprised of this type of neural fibre. However, each of these types is subdivided into many groups (Truex and Carpenter 1969; Carpenter 1976; Wakana, Jiang et al. 2004). The integration of the WM is important for proper communication between brain cortices and, therefore, the proper functioning of the brain. WM lesions may affect neuronal communication at cortical and sub-

cortical levels, resulting in neurological disorders. An example of a WM-related disorder is multiple sclerosis (MS). In MS, pathological demyelination of the WM and a loss of neuronal axons occur (Werring, Clark et al. 1999).

2.2.1 Auditory cortex

The human primary auditory cortex (PAC) is located on the superior temporal gyrus, specifically on the transverse Heschel's gyrus (HG). Based on its cytoarchitecture, Brodmann divided the PAC into areas 41 and 42. Area 41 is considered the core auditory cortex and occupies the middle and the posterior segments of the anterior portion of the transverse gyrus. The remaining portions of the posterior segment and the neighbouring portions of the superior temporal gyrus comprise the associative auditory cortex, or area 42 (Carpenter 1976). The core and the associative auditory areas are cytoarchitecturally distinguishable. Area 41 is characterised by its thickness (3 mm) and the perpendicular arrangement of its granular cells. Area 42 is distinguished by the existence of irregularly arranged large pyramidal cells in the external pyramidal layer (Truex and Carpenter 1969). Inter- and intra-subject variations exist in the anatomy and the location of the PAC (Penhune, Zatorre et al. 1996). High-resolution MRI studies have revealed that the left PAC is larger than the right PAC due to the larger volume of the white matter that underlies the HG in the left hemisphere (Penhune, Zatorre et al. 1996). The human auditory cortex does not function in isolation but interacts with other cortical and neocortical structures. The PAC sends inputs to the thalamus (Winer and Schriner 2010), amygdala (LeDoux 2000) and PFC (Fuster 2008).

The human auditory cortex is characterised by its functional tonotopic organisation, i.e., regions that respond to specific frequencies. The tonotopic

organisation arises in the cochlea and is maintained throughout the auditory system (Truex and Carpenter 1969). Functional neuroimaging studies of humans have revealed that higher-frequency pure tones trigger mirrored deep structures within the PAC (Pantev, Bertrand et al. 1995). Recent fMRI studies have demonstrated that as pure tone stimuli increase in frequency, responses progress from the lateral HG towards the anterior and posterior sides of the medial HG (Formisano, Kim et al. 2003; Langers and van Dijk 2011). Although there is inconsistency in frequency-specific regions within the human auditory cortex, there is a consensus regarding the presence of a tonotopic map.

2.2.2 Limbic lobe

The limbic lobe forms the grey matter that lies on the medial aspect of the hemisphere and surrounds the rostral portion of the brain stem and the interhemispheric commissure. The primary components of the limbic lobe include the subcallosal, cingulate and parahippocampal gyri and the hippocampal formation. In addition to these regions, the limbic system includes the amygdaloid complex, the olfactory system, the anterior nucleus of the thalamus, the epithalamus and the mammillary bodies of the hypothalamus. The use of "limbic system" rather than "limbic lobe" includes the limbic lobe structures in addition to the associative limbic nuclei, such as those that have been described as comprising the limbic system (Carpenter 1976). With regard to the cytoarchitectural features of the limbic lobe, the limbic lobe structures contain neurons that are organised into identifiable cortical layers (Isaacson 2001). These layers are referred to as the archicortex (the hippocampal formation and the dentate gyrus), palaeocortex and juxtallocortex (cingulate gyrus) (Carpenter 1976).

The amygdala, which is a core structure in emotion and memory processing, consists of a large number of nuclei and is located in the anterior of the temporal lobe in humans. It has been revealed that there are at least 13 distinct nuclei and cortical regions within the amygdala in non-human primates (Amaral, Price et al. 1992). The majority of the amygdala-neocortical interactions occur in the basolateral nuclei group, which includes the lateral nucleus, basal nucleus, and accessory basal nucleus. The key role of the amygdala involves its extensive anatomical and functional connections with other brain regions, including the hypothalamus, basal forebrain, hippocampal formation, and striatum (Amaral 2002). The amygdala receives sensory inputs from all of the sensory modalities, including olfactory, visual, auditory and somatosensory stimuli. This information is processed within the amygdaloid complex, where responses to incoming stimuli are initiated. Thereafter, the amygdala projects either back to the same cortex from which it received the input or forward, and the input informs the response (Price 2003).

Anatomical tracing studies in animals have demonstrated dense projections from the posterior and the ventral temporal cortex to the lateral nucleus of the amygdala (Romanski and LeDoux 1993). Conversely, the injection of a tracer into the lateral nucleus of the amygdala resulted in the labelling of cells in the rostral half of the superior temporal gyrus (rostral to the primary auditory cortex). The labelled cells were located in the pyramidal cells of layers III and V. Both of these studies are believed to characterise the inputs and outputs between the temporal cortex and the amygdala (Aggleton, Burton et al. 1980; Amaral and Insausti 1992). Microelectrode recordings in rats have demonstrated that neurons in the lateral nucleus of the amygdala are responsive to broad-band acoustic stimuli (Bordi and

LeDoux 1992). Based on animal studies, two projections from the auditory region and the lateral nucleus of the amygdala have been suggested to exist. The first pathway, referred to as the thalamo-cortico-amygdala projection, involves the transmission of auditory signals to the medial geniculate body and then to the primary auditory cortex via the ventral nucleus of the medial geniculate body. Subsequently, this pathway terminates in the lateral amygdaloid nucleus via the associative auditory cortex. The second pathway, which is referred to as the thalamo-amygdala projection, transmits signals more rapidly and is shorter than the first pathway. This latter pathway projects directly from the medial division of the medial geniculate body to the lateral amygdaloid nucleus (LeDoux 1995; Li, Stutzmann et al. 1996; LeDoux 2000). The amygdala projects back to the auditory regions via two pathways. The first pathway originates in the lateral basal nucleus and terminates in the auditory regions. The second pathway sends outputs from the lateral and accessory basal nuclei of the amygdala to higher-order auditory association regions (Yukie 2002). The afferent and efferent amygdalar connections with other cortical and subcortical structures are diagrammed in Figure 2.2.

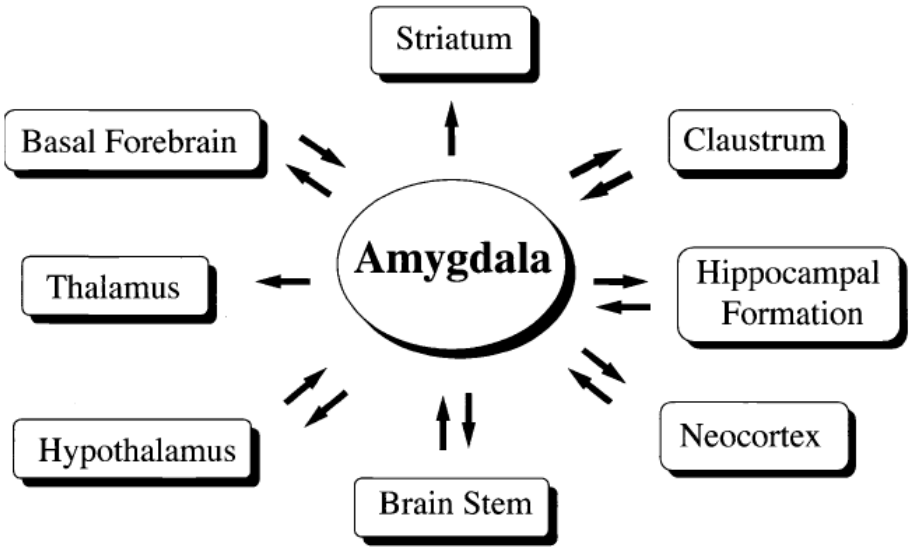


Figure 2.2. Amygdalar connectivity with other cortical and subcortical regions (Amaral 2002).

The hippocampal formation consists of a pair of large heterogeneous cortical masses that are located deep in the medial aspect of the temporal lobe. This formation is composed of a series of primary cortices, including the dentate gyrus, the hippocampus proper, and the subicular complex. Each of these cortices is further divided into sub-regions (Carpenter 1976; Amaral and Witter 1989). The hippocampus receives inputs from cortical structures, including the opposite hippocampus, the entorhinal cortex, and itself, via associative fibres. The hippocampal formation also receives inputs from non-cortical structures, such as the septum and the hypothalamus. Similarly, these cortices receive inputs from the hippocampus (Traub and Miles 1991). The central role of the hippocampus, due to its unique convergence of connectivity with other structures, is the formation of episodic memory. It also plays a major role, together with the perirhinal cortex, in the recognition memory system (Brown and Aggleton 2001). An animal tracing

study revealed that the medial portion of the posterior segment of the parahippocampal gyrus has dense projections that terminate in superficial layers I-III of the posterior auditory association cortex. Injecting the posterior auditory association cortex with a tracer labelled neurons in the anterior-posterior parahippocampal gyrus, specifically in layers V and VI (Tranel, Brady et al. 1988). In addition, reciprocal projections were observed between area TF of the parahippocampal gyrus, which is located laterally in the posterior parahippocampal cortex, and the rostrocaudal portion of the superior temporal gyrus (Lavenex, Suzuki et al. 2002).

2.2.3 The prefrontal cortex (PFC)

The PFC is the anterior pole of the brain that primarily receives projections from the mediodorsal thalamic nucleus. According to the Brodmann map of the brain (Garey 1999), the PFC occupies areas 8-13, 24, 32, 46 and 47. The PFC is divided into three primary regions: orbital, medial and lateral. These regions exhibit functional specialisation. For example, the orbital and the medial segments are involved in emotional behaviour tasks. The lateral region of the PFC supports behavioural and speech functions that are primarily performed by the temporal lobe. In fact, the functions of the PFC are based on its connectivity with other brain structures; it is extensively connected with many cortical and neocortical structures. The reciprocal connectivity between the PFC and the limbic system allows for the transmission of information regarding the status of the internal environment and arousal levels. All of this information is required for the behavioural integrative functions of the PFC. The amygdala and the hypothalamus project ventrally and medially to the PFC. This connectivity is believed to play a major role in the representation and enhancement of emotional behaviour. All the

segments of the PFC receive inputs from the hippocampus; therefore, the PFC contributes to memory, motor learning and behavioural functions. Inputs from the cerebellum to the PFC, most likely the lateral regions, suggest the role of the PFC in the organisation of motor actions (Fuster 2001; Fuster 2008).

With regard to the projections between the auditory cortex and the PFC, a tracing study of rhesus monkeys revealed that the auditory cortex (the primary and associative cortices) and the PFC are topographically and reciprocally connected. The rostral and the orbital segments of the PFC were found to be connected with the rostral segment of the auditory belt and parabelt in these non-human primates. Retrograde-labelled cells in the rostral segment of the superior temporal gyrus were confined to layers III and V, with layer III giving rise to the majority of these projections. The caudal and inferior PFC were demonstrated to connect to the caudal segment of the auditory cortex belt and parabelt. The labelled cells within the caudal auditory cortex belt and parabelt were localised to layers III, IV and VI (Romanski, Bates et al. 1999). Electrophysiological recordings combined with anatomical tracing studies have revealed two pathways that originate from the auditory regions and target different PFC regions in macaques. The first pathway originates from the caudolateral region of the auditory belt cortex and targets the caudal dorsolateral PFC. The second pathway originates from the anterior segment of the lateral auditory belt cortex and targets the rostral and ventrolateral PFC. When rhesus macaques were exposed to pure tones and band-passed noise stimuli, neurons in the PFC were observed to respond. It was revealed that neurons in the DLPFC receive inputs from the caudolateral segment of the auditory belt cortex, whereas the rostral and ventrolateral PFC receive inputs from the anterior regions of the auditory belt cortex (Romanski, Tian et al. 1999).

Several human functional neuroimaging studies have revealed functional connectivity between the auditory cortex and the PFC. Increased activation in distinct PFC regions was observed when participants performed auditory tasks (Humphries, Willard et al. 2001; Maeder, Meuli et al. 2001; Foxe, Wylie et al. 2002). Two functional networks involving the PFC were detected in humans during auditory tasks. The first network involves the anterior segment of the middle temporal gyrus and the ventral portion of the precuneus of the left PFC. This pathway is believed to participate in sound recognition. The second network is believed to participate in sound localisation and involves the lower portion of the inferior parietal lobule and the posterior portions of both the middle and inferior frontal gyri (Maeder, Meuli et al. 2001). PFC connectivity with other cortical and neocortical structures is illustrated in Figure 2.3.

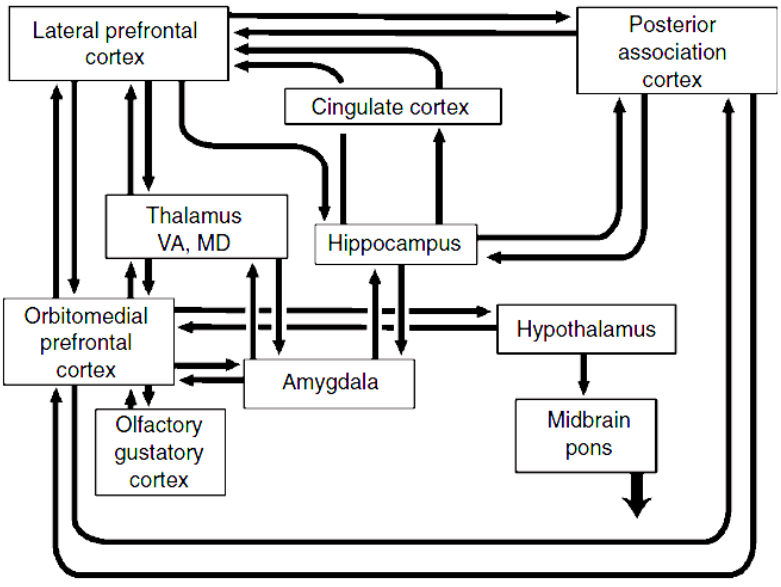


Figure 2.3. The connectivity between the prefrontal cortex (PFC) and other brain structures that are involved in emotion and memory. VA= Anteroventralis Nucleus, MD=Mediodorsalis Nucleus (Amaral 2002).

2.3 The Central Circuitry of Emotion

Emotions are responses to internal or external stimuli and allow for directed and targeted adaptations to changing environmental demands. Our reactions to danger, our sexual interactions, and our escape response during fearful situations are all considered emotions (LeDoux 2000).The neurophysiological process of emotion has three primary components. The first component is the physical sensation that occurs within the autonomic nervous system and is referred to as physiological arousal. The second component of emotion is the expression of the emotion, in which we behave in a manner that reflects what we feel by means of, for example, facial expressions. The third component is the subjective experience that characterises the personal feeling towards our current emotion, such as happiness, sadness or anger (Erdem and Karaismailo lu 2010).

The neural circuit of emotion, which is the focus in the study of tinnitus, involves two key structures in the brain: the PFC and the limbic system. As an example of emotional conditioning, the amygdala receives inputs from each sensory cortex during fear conditioning and relays these inputs back to the cortex. These amygdala-cortical signals convey information regarding the effect of the stimuli on the amygdala, which assesses the significance of incoming sensory stimuli. The amygdala also regulates cortical sensory processing via projections to arousal systems, including the basal forebrain cholinergic system, the brainstem cholinergic system, and the locus coeruleus noradrenergic system, each of which innervates extensive areas of the cortex. For example, when the amygdala is stimulated by the presence of danger, it activates the arousal system in the brain. This physiological arousal affects the sensory processing in the cortical areas, and the body then enacts its response, which includes changes in sensory processing

that are mediated by visceral signals and hormones (LeDoux 2000). A demonstration of the above-mentioned role of the amygdala in conditioned fear stimuli can be explained as follows: (1) the amygdala detects the presence of danger; (2) this information is relayed to the lateral hypothalamus; (3) the sympathetic system is activated via the activity of the lateral hypothalamus; and (4) behavioural responses are enacted in the form of elevated blood pressure, tachycardia and skin paleness (Davis, Rainnie et al. 1994).

Working and long-term memory, judgement and reasoning are all cognitive abilities that are involved in emotion circuits. It is well established that the cognitive functions are organised by the PFC (Chayer and Freedman 2001; Wagner, Maril et al. 2001; Fuster 2008). A conditioned fear stimulus was demonstrated to induce neural plasticity in the PFC, and such plasticity is believed to represent the capability of the conditioned stimulus to predict danger. This representation occurs in the PFC and may characterise the "decision-making" role of the PFC as part of the stimulus-appraising process (Garcia, Vouimba et al. 1999). The amygdala-PFC interaction is necessary for emotion processing, as it has been revealed that stimulating the medial PFC inhibits conditioned fear by inhibiting the neuronal output of the central amygdalar nucleus (Quirk, Likhtik et al. 2003). Alternatively, activating the amygdala by presenting a threatening stimulus inhibits neural spontaneous activity in the PFC, suggesting the neuromodulatory role of these two structures in emotion processing (Garcia, Vouimba et al. 1999). Although the neural circuits that process emotions are still ambiguous with respect to their function and identity, there is a consensus regarding the involvement of the PFC and the amygdala. The primary brain structures that are involved in the emotion circuit are shown in Figure 2.4.

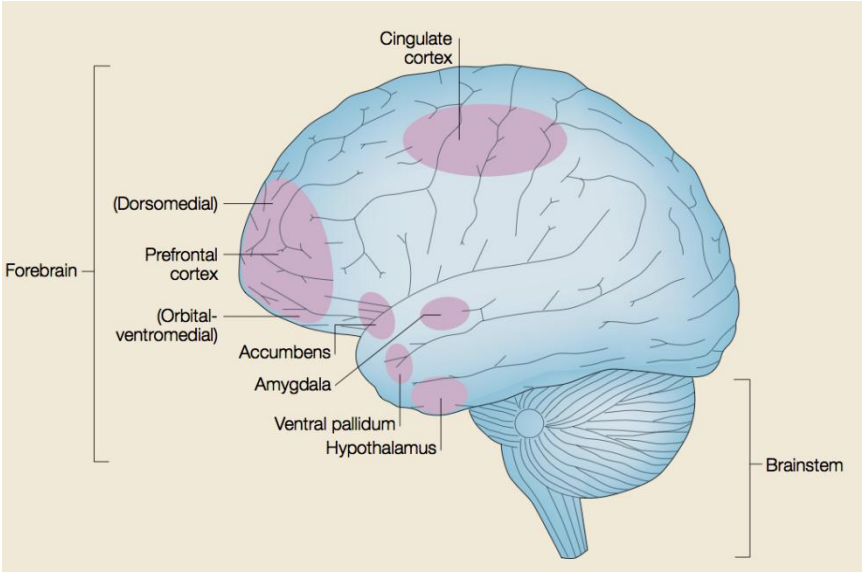


Figure 2.4. Key brain structures that are involved in emotional circuitry (Dalgleish 2004).

2.4 An Overview of Tinnitus Pathophysiology

In 1948, Gold introduced the hypothesis that the normal cochlea produces faint low-intensity or narrow-band sounds in the absence of any external stimulation (Baguley 2002). These sounds are referred to as oto-acoustic emissions (OAEs) and can be classified into two groups: spontaneous and evoked. Unlike spontaneous OAEs, evoked OAEs involve external sound. Kemp later suggested that the active mechanical amplification of sound occurs in the cochlea. As a result of this process, a small outflow of sound energy is re-coded in the external ear canal. Many studies (Penner 1990; Plinkert, Gitter et al. 1990; Ceranic, Prasher et al. 1998) have been performed to understand the relationship between oto-acoustic spontaneous emissions and tinnitus generation. Many researchers believe that spontaneous emissions and tinnitus have the same underlying pathological mechanism (Ceranic, Prasher et al. 2007). Prasher and Ceranic (2001) examined the relationship between spontaneous emissions and the presence of tinnitus.

These authors observed that if the patient has stable spontaneous emissions, he or she is less likely to have tinnitus (<6%) than individuals with non-stable spontaneous emissions. However, it was observed that the spontaneous emissions were unstable in tinnitus patients over repeated tests. Although oto-acoustic emissions can be sensed by patients with tinnitus, this does not mean that such emissions are risk factors of tinnitus. Furthermore, spontaneous oto-acoustic emissions were observed in 50% of individuals with normal hearing systems (Campbell and Mullin 2006).

Although the pathological mechanism of tinnitus is poorly understood, there are several factors that contribute to its occurrence. Generally, it is believed that tinnitus is caused by abnormal neural activity in auditory cortex pathways. These defects result in the subjectively experienced sounds. Based on this belief, scientists' views regarding the induction of tinnitus have been divided into three hypotheses (Norena, Micheyl et al. 2002). First, it has been proposed that tinnitus originates from abnormal neural activity at the peripheral level and primarily results from auditory nerve pathology and cochlear lesions (Jastreboff and Hazell 1993; Norena, Micheyl et al. 2002). The second hypothesis proposes that tinnitus results from abnormal neural activity in the central auditory system in the absence of a peripheral lesion (Muhinickel, Elbert et al. 1998). The third hypothesis is that the perception of tinnitus results from dysfunctional neural activity in the central auditory system as a result of peripheral damage (Norena, Micheyl et al.2002).

Injury to the auditory nerve results in its altered function and in changes in its input to the central auditory system. Damage to the auditory nerve can be characterised by vascular and neoplastic compression, which may cause tinnitus. This pathogenic mechanism results in the thinning of the myelin sheaths of the

nerve fibres, causing a loss of the electrical insulation. Demyelination results in the induction of the transference of nerve impulses at ephapses (i.e., without the use of neurotransmitters), thus interfering in neuronal communication (Lenarz, Schreiner et al. 1993).

Eggermont (1990) suggested that normal inner hair cells (IHCs) are innervated by independently firing auditory nerve fibres with different spontaneous rates and thresholds of firing. The activation of hair cells causes synchronous firings of certain auditory nerve fibres to a degree that is proportional to the stimulus intensity. The synchronisation of the auditory nerve fibres occurs due to neurotransmitter release at the synapse. These neurotransmitters cause influxes of potassium (K^+) or calcium (Ca^{2+}) ions, resulting in the depolarisation of the hair cell. This series of events may explain how tinnitus can result from cochlear lesions that are induced by, for example, noise.

As noted above, tinnitus perception is commonly associated with hearing loss, regardless of its cause; however, noise-induced hearing loss (NIHL) has been commonly reported to be associated with tinnitus. Tinnitus-associated NIHL was observed in 24% of patients with tinnitus (Eggermont 2006). Based on this result, it is important to explain the possible mechanisms by which hearing loss is related to tinnitus perception. Noise exposure was reported as the most common cause of hearing loss, and the severity of such hearing loss is dependent on the degree of ear damage (Brookhouser, Worthington et al. 1992). Frequent exposure to intense sounds damages the outer hair cells (OHCs), although these cells are more resistant to damage than are inner hair cells (IHCs). However, long periods of exposure to noise may damage IHCs and inner ear structures, including cells in the organ of Corti and several cell types in intracochlear structures, such as the stria

vascularis and the spiral ligament (Jastreboff 1990; Brookhouser, Worthington et al. 1992). As a consequence of this damage, the mechanical properties of the organ of Corti are affected, and abnormal basilar membrane movement can result. It has been proposed that the position of the basilar membrane gives the organ of Corti optimum transduction by changing the length of the OHCs via efferent inputs. The afferents that are sent by the OHCs to the brainstem provide information that describes their nature. This information is fed back through efferent fibres following processing by the brainstem. This feedback then acts to adjust the length of the OHCs. Accordingly, any reduction in inputs from OHCs on the basilar membrane would result in decreased activity in the efferent fibres and decreased inhibition on afferents from the IHCs. Abnormal activity of the IHCs would result and would be perceived as tinnitus (Cody and Russell 1985; Zenner, Zimmermann et al. 1988; Jastreboff 1990). Functional changes in OHCs and IHCs have been reported in previous investigations of animals that were exposed to intense tones. These changes were characterised by a reduction in the amplitude and increased symmetry of the HC's evoked responses. Furthermore, the OHCs exhibited continuous depolarisation of the membrane potential, which could result in abnormal motion of the adjacent hair bundles and the reticular lamina, raising the possibility of tinnitus perception (Cody and Russell 1985; Kennedy, Evans et al. 2006). Normal and damaged organs of Corti are shown in Figure 2.5.

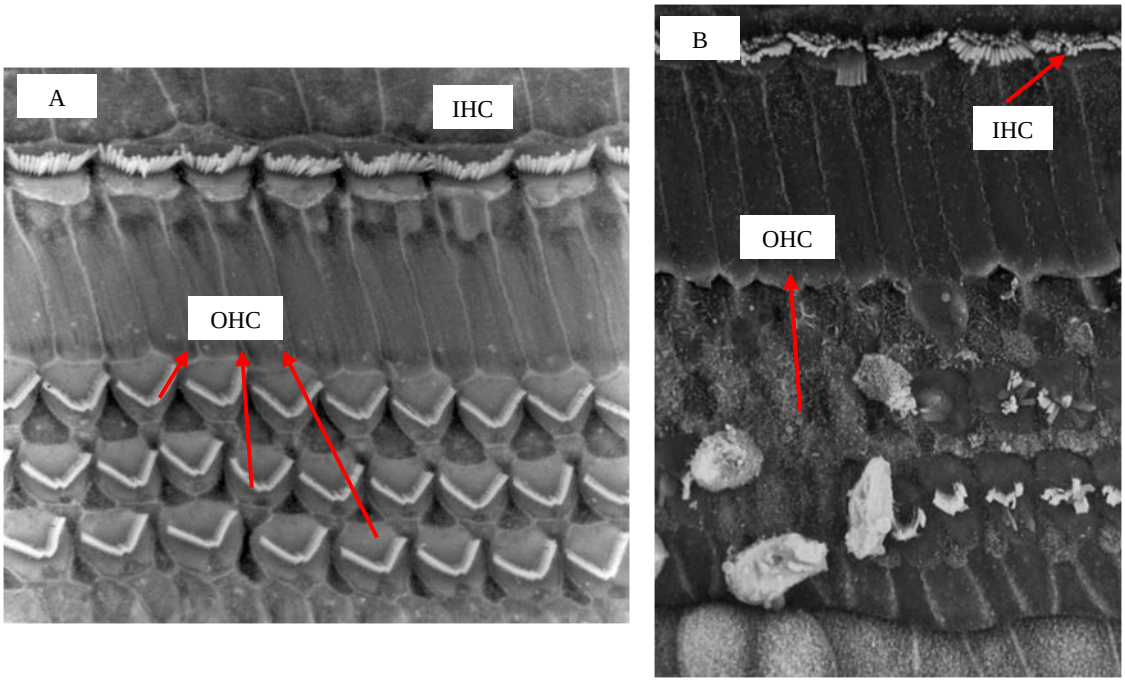


Figure 2.5. A normal organ of Corti. Three rows of outer hair cells (OHC) and one row of inner hair cells (IHC) can be observed. (B) A severely damaged organ of Corti exhibiting hair cell damage that resulted from either noise trauma or ototoxic drugs. The images were taken by the Auditory Science Lab (lab 2010).

It has been reported that the dorsal cochlear nucleus (DCN) is involved in tinnitus-signal generation following noise exposure. Recently, Kaltenbach and McCaslin demonstrated that the neurons in the DCN become hyperactive following exposure to high levels of sound. In 1999, Kaltenbach and Afman (2000) used adult Syrian golden hamsters to test their hypothesis that high noise-induced tinnitus results from the generation of a tonotopic distribution of chronic DCN hyperactivity. The authors of this previous study divided the animals into three groups: high-tone-exposed animals, normal-tone-exposed animals and unexposed animals. Electrophysiological recordings of neural activity in the DCN in unexposed and tone-exposed animals were obtained following 30 days of exposure. Based on

their results, the authors concluded that neural activity in the DCN is similar following intense sound or normal stimuli (Kaltenbach, Zhang et al. 2005). Brozoski et al. (2002) reported elevated spontaneous activity in the fusiform cells in the DCN in intense-noise-exposed animals. In addition, these animals exhibited chronic psychophysical behaviour that is characteristic of tinnitus. Hence, it was proposed that increased spontaneous activity in the fusiform cell layer of the DCN may underlie the perception of tinnitus. Middleton et al. (2011) linked increased activity in the DCN to reduced gamma-aminobutyric acid (GABA) levels. GABA is an inhibitory neurotransmitter, and the authors proposed that reduced inhibition might be an underlying cause of tinnitus.

Another view suggested that exposure to intense noise causes a drop in Oxygen partial pressure (pO_2) in the perilymph and cochlear blood flow. Continuous noise exposure resulted in hearing loss and cochlear hypoxia in guinea pigs (Lamm and Arnold 1996). The actual mechanism by which noise affects cochlear blood flow may be related to sound-induced stress of both the organ of Corti and cochlear circulation. Another possibility is that intense noise damages the sensory epithelium, which could cause pathology of the blood vessels. The effect of this would be insufficient blood supply to the cochlea and, ultimately, hypoxia (Nuttall 1999). Morphological studies demonstrated that intense noise exposure, regardless of whether it is continuous, reduces the amount of blood flow in the auditory system and the morphology of blood cells. This effect is believed to be due to the transference of sound energy to blood vessels. Hypoxia has been noted as a pathogenic factor of hearing impairment and tinnitus pathogenesis. Exposure to intense sound causes a reduction in cochlear oxygen partial pressure (pO_2) and therefore increases the risk of hypoxia. Ischemia, in which the blood supply to a

tissue is abolished, also has a role in tinnitus induction. The roles of hypoxia and ischemia in the development of hearing disturbances, including tinnitus, have been well demonstrated.

As in all cells, cochlear cells use glucose as a primary source of energy to generate adenotriphosphate (ATP), which is then used to perform various cell functions. However, in pathogenic contexts in which Oxygen levels are significantly reduced, e.g., hypoxia and ischemia, glycolytic ATP generation is also reduced. Sensory-type hearing loss and hair cell degeneration develop in the context of such pathologies. Mazurek et al.(2006) reported that hypoxia and ischemia may alter Ca^{2+} homeostasis, causing reactive oxygen species (ROS) to form. ROS are produced naturally during metabolic processes and are considered to be powerful reactive oxidisers, particularly for lipids. This alteration is represented by an increase in Na^{+} and Ca^{2+} entry into neurons, causing cell swelling. Furthermore, both hypoxia and ischemia cause permanent cell depolarisation, which is associated with N-methyl-D aspartate (NMDA) receptor stimulation and high rates of glutamate release. Subsequently, apoptotic and necrotic cell death occurs. An imbalance between ROS and antioxidant defences have been observed in many patients with tinnitus, conclusively demonstrating the hypotheses that hypoxia and ischemia participate in tinnitus pathophysiology (Yamasoba, Nuttall et al. 1998; Mazurek, Haupt et al. 2006).

The exposure to intense sounds for long periods was observed to damage sensory cells, nerve endings and the vascular supply. Intense sound exposure first damages the sensory cells in the cochlea, which cannot be regenerated. In later stages, the stereocilia, which are essential to the mechanical transduction of sound, are damaged (Cody and Russell 1985; Holme and Steel 2004). This damage can result

in an increase in the thermal noise of the hearing system by 30 dB above the threshold of the sound perception. This resultant noise is believed to be perceived as tinnitus (Jastreboff 1990).

2.5 Jastreboff's neurophysiological model of tinnitus and tinnitus retraining therapy

In the late 1980s, Jastreboff suggested a tinnitus retraining therapy (TRT) that was based on his neurophysiological model as a treatment programme to treat chronic tinnitus and reduced sound tolerance. Jastreboff's neurophysiological model postulates that tinnitus perception involves the contribution of several brain regions other than the auditory system, such as the limbic system, the prefrontal cortex and the autonomic nervous system. The neurophysiological model of tinnitus also hypothesises that tinnitus signals evoke improper neural activity in the limbic and sympathetic autonomic nervous systems. Consequently, tinnitus sufferers have been observed to exhibit certain behavioural reactions that relate to abnormal neural activity, such as anxiety and depression, and are controlled by the limbic system.

The tinnitus signal may originate via several mechanisms, one of which is proposed by the discordant dysfunction theory. This theory proposes that the abnormal neural activity that is associated with tinnitus results from an imbalance between the neural activity coming from cochlear IHCs and the OHCs. This imbalance is due to reduced signalling from the dysfunctional OHCs in combination with normal IHCs signals. The first appearance of the tinnitus signal is in the dorsal cochlear nucleus; however, this signal is processed within auditory pathways and reaches higher cortical areas (Jastreboff and Hazell 2004).

In tinnitus conditions in which the tinnitus signals are associated with emotional distress, "conditioned reflexes" are created. In this case, the neural activity that is evoked by tinnitus signals may hyperactivate the limbic system. Jastreboff considers that activation of the limbic system is responsible for tinnitus-associated negative emotions because of (1) the presence of feedback loops between the auditory and the limbic system and (2) brain plasticity. Within this feedback loop, the tinnitus signal is continuous (in clinically significant tinnitus), causing the continuous activation of both the limbic system and the autonomic nervous system. Consequently, a negative self-reinforcement condition occurs, the continuation of which strengthens conditioned reflexes, which are characterised by increased limbic system activity. This hypothesis is supported by the symptoms of tinnitus sufferers, whose complaints include a loss of attention and anxiety (Jastreboff 1990; Jastreboff and Hazell 2004).

The primary concept of TRT is based on modifying the neural networks that are responsible for producing the tinnitus signals. The modification occurs by preventing the tinnitus signal from activating the limbic and the autonomic nervous systems. The effect will be habituation to the inappropriate neuronal interactions that lead to the production of tinnitus signals. Once significant habituation has been achieved, the tinnitus signals will be gradually ignored by the sufferer. TRT consists of two stages: counselling and sound therapy. During the first stage, therapists attempt to eliminate the negative associations that are associated with tinnitus, such as the thought of tinnitus as being a serious problem, through several individualised teaching sessions. By the end of this stage, it is expected that the tinnitus sufferer has sufficient knowledge regarding the actual

mechanism of tinnitus and the fact that the annoyance is not caused by the tinnitus signal itself.

Understanding the neuro-functional connections between the tinnitus signal and one's reaction to such signals are the keys to controlling the experience of unpleasant emotions. Similarly, these connections are considered the starting points of achieving tinnitus habituation. Sound therapy, which is the second part of TRT, aims to weaken the tinnitus signals within the brain by enhancing the processing of environmental sounds. Sound therapy is based on the fact that the tinnitus signal is highly perceived in a silent environment due to the high contrast between the signal and background sound. Tinnitus tends to cause a high degree of annoyance in a silent environment, an effect that is due to tinnitus-signal-induced hyperexcitation of certain cortical areas. During sound therapy sessions, patients are advised not to remain in silent rooms; however, they are instructed to enrich environmental sounds by listening to their favourite music, for example. Together with counselling sessions, sound therapy aims to distract the sufferer's attention from the tinnitus signal, causing the continuous activation of the limbic and autonomic nervous systems to be gradually reduced. The conclusive TRT model is characterised by the creation of a neuro-functional mechanism that opposes the negative reflexes that are created by tinnitus signals (Jastreboff and Hazell 1993; Jastreboff and Hazell 2004; Jastreboff and Jastreboff 2006).

Although TRT exhibits improvement rates exceeding 80%, it has been criticised in some studies. A group of psychologists (Kroener-Herwig, Biesinger et al. 2000) emphasised the role of directive counselling as part of the therapy; however, they criticised Jastreboff's method, which relies on a therapist. The lack of information, the nature of the counselling sessions and the techniques that should be followed

during these sessions have all been considered to be limitations of TRT. Another alleged drawback of Jastreboff's TRT approach is its neglect of the importance of psychologists in specifying the counselling techniques and the types of emerging cognitive-emotional therapy that should be used to reduce tinnitus-related stress (Kroener-Herwig, Biesinger et al. 2000). However, many clinical studies have demonstrated the efficacy of TRT in improving tinnitus and its negative associations. Henry and others (2007) demonstrated that tinnitus was significantly improved in patients who were involved in educational counselling sessions as a part of TRT compared to those who did not undergo TRT. A higher degree of improvement was even observed over those who attended a traditional support group. TRT has evolved and has been improved to become clinically valid. Henry et al. (2003) standardised the interview forms that are used in the TRT counselling sessions to achieve consistency in the methods and outcomes across clinics worldwide. A schematic diagram of Jastreboff's neurophysiological model of tinnitus is shown in Figure 2.6.

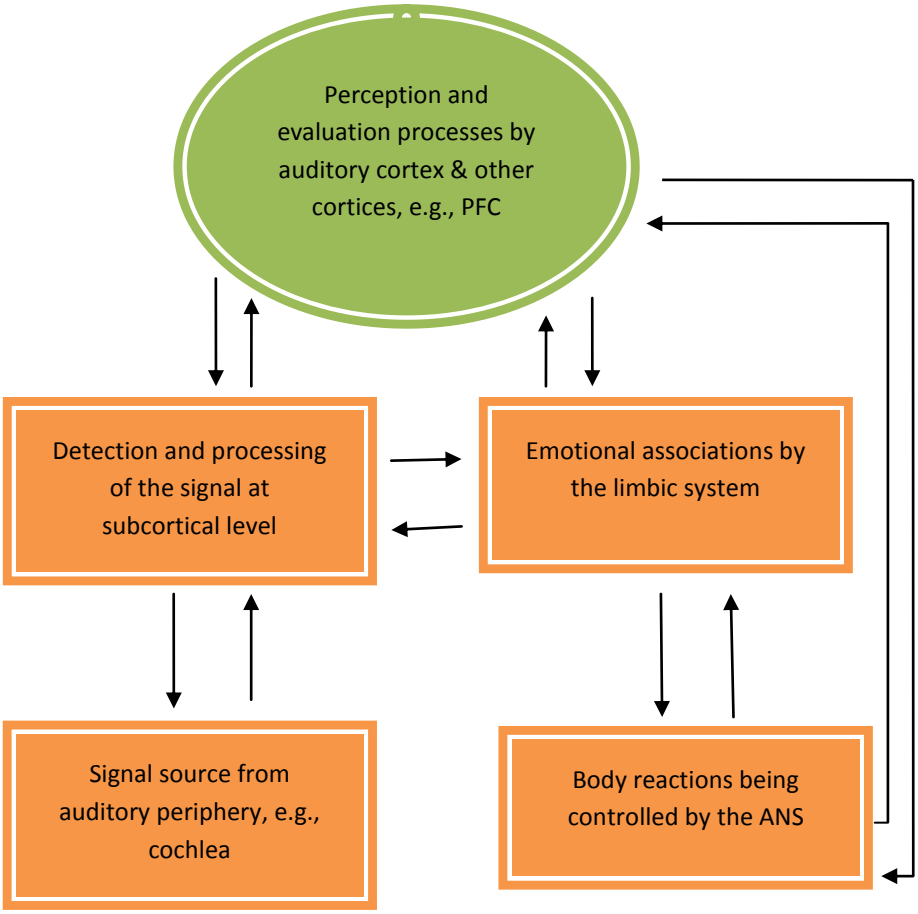


Figure 2.6. Schematic of the neurophysiological model of tinnitus (Jastreboff and Hazell 2004).

2.6 Neural plasticity

Many studies support the hypothesis that the neural plasticity has a major role in tinnitus pathogenesis (Baguley 2002; Cacace 2003; Eggermont 2003; Møller 2003). Evidence from animal studies (Kaltenbach and Afman 2000; Middleton, Kiritani et al. 2011) and human studies (Lockwood, Salvi et al. 1998; Muhinickel,

Elbert et al. 1998) suggest that neural plasticity may contribute to tinnitus. Neural plasticity refers to the structural and functional changes that occur in neurons in response to changing demands (Woolf and Salter 2000). Examples of these demands include postnatal development, pathology and learning (Lledo, Alonso et al. 2006; Møller 2006). Neuroplasticity may be either adaptive or maladaptive with respect to the body's internal environment following the experience of a novel condition. That is, neuroplasticity may be a negative response of the body to a certain stimulus and may result in a pathological condition. Conversely, neuroplasticity may be a positive response, resulting in the learning of new skills, for example (Pascual-Leone, Amedi et al. 2005). Neural plasticity can be induced via different mechanisms. One of these mechanisms involves anatomical changes; for example, a new anatomical connection may be formed, which may involve dendritic growth and sprouting. Another form of neural plasticity involves changes in the synthesis and release of neurotransmitters in neurons or in the metabolic rate of the brain. Functional changes to nerve cells, which may involve changes in the resting membrane potential, may also induce neural plasticity (Nelson 1999; Møller 2006).

The most significant forms of positive neuroplasticity are observed in learning and memory processes. The brain's response to a new experience is characterised by a rearrangement of the connections between neurons. This effect has been observed in learning processes and memory formation in animal studies (Loeser and Melzack 1999; Kaltenbach, Zhang et al. 2005). Environmental enrichment was demonstrated to modify neural plasticity in the hippocampus in older mice. The rate of neurogenesis in the mice that were exposed to environmental enrichment was five times higher than in unexposed mice (Kempermann, Gast et al. 2002).

Another example of the adaptive neural plasticity is the repair processes that occur following brain injury. Such repairs result in the remapping of neural networks to re-establish normal functioning. Unilateral damage to the forelimb representation in the somatosensory cortex of rats was demonstrated to result in altered dendritic morphology in the contralateral homologous cortex, which was characterised by an increase in the arborisation of this cortex. These morphological changes in the opposite hemisphere were believed to compensate for the loss in the damaged hemisphere.(Jones and Schallert 1994).

Structural and functional imaging studies of humans have demonstrated that skill acquisition and learning are associated with a brain reorganisation. For example, an increase in grey matter was observed in individuals who practised juggling compared to a those who did not (Draganski, Gaser et al. 2004). Training-induced structural changes in the brain were also demonstrated in London taxi drivers, who exhibit increased grey matter volume in the posterior hippocampus (Maguire, Gadian et al. 2000). Functional reorganisation in the primary motor cortex following motor skill training has also been reported (Ungerleider 1995). Although structural and functional studies may reveal that neural reorganisation underlies features such as skill acquisition, none of these studies have clarified the underlying microscopic changes.

Maladaptive neuroplasticity can be observed in many pathological conditions, such as focal hand dystonia, limb amputation and tinnitus. Musician's cramp, or focal hand dystonia, is a condition in which professional musicians become unable to make skilled hand movements due to excessive training involving repetitive hand motions. Magnetoencephalography studies have demonstrated reduced distance between digit representations in the somatosensory cortex in musicians

with focal hand dystonia compared to controls (Elbert, Candia et al. 1998; Melzack, Coderre et al. 2001; Flor, Nikolajsen et al. 2006). In limb amputation, a large area of the somatosensory cortex would not be expected to respond to any stimulus due to deprivation of neural inputs from the amputated limb. However, neighbouring neural inputs may be able to stimulate this brain region. Consequently, the neural networks will be reorganised in the affected somatosensory cortex (Ursano 2002).

With regard to the role of maladaptive neuroplasticity in the auditory system, neuroplasticity changes may result in an imbalance between excitation and inhibition as a result of an auditory system pathology, such as hearing loss (Saunders 2007). Hearing loss is a form of injury that induces neural plasticity and changes in neural mapping. Similar to the mechanism of phantom limb pain, a certain area in the auditory cortex will be unresponsive due to the absence of inputs. Following hearing loss, an area of missing frequencies appears in the reorganised tonotopic map (Saunders 2007). Tinnitus signals may arise because of this re-organisation of the neural network (Kaltenbach, Zhang et al. 2005).

According to Kaltenbach (2005), neuroplasticity that is associated with tinnitus perception can be classified into four types. The first type is referred to as injury-induced plasticity, which describes the changes that occur as a result of normal input loss. The loss of normal inputs due to trauma was demonstrated to cause a reduction or even a loss of the functional or anatomical inputs from the inner ear to the cochlear nucleus. The second type of neuroplasticity that is associated with tinnitus perception refers to changes in the perception of tinnitus over time in the absence of any external cause. This is referred to as temporal plasticity and is primarily observed in rapid and slow changes in the intensity of the tinnitus and its

perceived location. Stimulus-dependant plasticity is the third type of plasticity-induced tinnitus. In this proposed type of neural plasticity, the neural changes that occur following acoustic stimulation are below the threshold of cochlear injury. The affect of stimuli on neural plasticity persists even following the removal of that stimulus. The fourth way in which the stimulation of non-auditory pathways is involved in tinnitus is referred to as modulatory plasticity. This type of plasticity resembles stimulus-dependant plasticity in that it involves a stimulus but differs in its involvement of interactions between one or more sensory modalities. It is worth noting that these types of plasticity that were defined by Kaltenbach et al were each based on scientific explanations.

Changes in cortical organisation and tonotopic mapping in the auditory cortex as a consequence of (1) hearing loss and (2) ototoxic drug-induced tinnitus have been previously reported. These changes were represented by an increase in both the strength and the degree of the shift in tinnitus frequency in the auditory cortex (Muhinickel, Elbert et al.1998). According to this evidence, it was hypothesised that neurons in deafferentated cortical regions alter their tuning to represent frequencies of the lesion, including the threshold frequency. Eggermont and Roberts (2004) proposed that the tonotopic map of the auditory cortex is re-organised following noise exposure. This type of re-organisation is represented by unresponsiveness of the cortical neurons that respond to frequencies in which there is hearing loss. In addition, these neurons have been shown to exhibit increased spontaneous activity and neural synchrony.

2.6.1 Tinnitus and phantom pain

Melzack and Wall (1967) first demonstrated the actual mechanism of pain in the gate control theory of pain. These authors suggested that sensory inputs from a

physical injury are modulated by a gate control system and that this modulation results in pain perception. Pain perception was, therefore, hypothesised to result not only from a direct activation of pain receptors in the injured area but also in response to alterations in neural interactions. An injury will create a non-homeostatic environment in the body. In response, the body attempts to return to its normal status and enacts neural, hormonal and behavioural changes that may aid the body in adapting to the new situation (Henry, Loovis et al. 2007). In certain chronic pain conditions in which the body is incapable of healing, e.g., the amputation of an extremity, the body will not be able to restore itself completely. In such cases, changes that may involve neural activity re-arrangement occur (Henry, Loovis et al. 2007). In a study performed by Flor et al. (2006), a strong positive relationship between the degree of cortical reorganisation and the extent of phantom limb pain (classified as chronic pain) following arm amputation was observed. The involvement of the sympathetic nervous system in certain forms of pain, such as neuropathic pain (Woolf and Mannion 1999) and complex regional pain syndrome (Vogel, Gradl et al. 2010), has been demonstrated. During chronic pain, the sensation of pain is intensified and pain receptors in the skin are stimulated, resulting in a condition known as reflex sympathetic dystrophy. Melzack and others reported that neural plasticity occurs during chronic pain. These authors used phantom limb pain as an example. The evidence for neural plasticity was revealed by the presence of the somatotopic map of the amputated hand on the map of the forearm and was associated with functional changes in the targeted cells in the brain. It is worth noting that the neurons that previously responded to the amputated hand subsequently responded to a stimulus in the forearm, indicating that the response of the neurons in this region of the brain was

modulated and that no new neurons were generated (Melzack and Wall 1967; Melzack and Loeser 1977; Melzack, Coderre et al. 2001).

The relationship between tinnitus and chronic pain has been an area of interest for many researchers attempting to better understand tinnitus pathophysiology and treatment. In a comparison study between chronic pain and tinnitus sufferers that was performed by Møller (2007; Møller, Langguth et al. 2010), it was demonstrated that both chronic pain and severe tinnitus are associated with emotional reactions, such as anxiety and feeling ill. Similarly, in patients with tinnitus, there was evidence from MEG recordings that a reorganisation of the auditory cortex occurs, the magnitude of which was correlated with the strength of the tinnitus (Muhinickel, Elbert et al. 1998). The hair cells of the cochlea have sympathetic innervations; therefore, activation of sympathetic system increases the sensitivity of cochlear hair cells (i.e., a stimulation that resembles the stimulation of pain receptors in a region of skin where chronic pain is experienced).

Tonndorf (1987) clearly demonstrated several similarities between chronic pain and tinnitus. Similar to pain, tinnitus is controlled by the central nervous system in the vast majority of cases. In addition, the peripheral origin of both pain and tinnitus allow both sensations to be more easily suppressed than were they to have a central origin. It was revealed that chronic pain and tinnitus could be alleviated by electrical stimulation in selected cases. In somatic tinnitus, in which the perception of tinnitus is associated with disorders of the head and neck, transcutaneous electrical nerve stimulation of the upper cervical nerve was observed to suppress tinnitus (Vanneste, Plazier et al. 2010). In complex regional pain syndrome, electrical stimulation of the motor cortex has been revealed to significantly reduce pain (Velasco, Carrillo-Ruiz et al. 2009). Both chronic pain

patients and those with tinnitus report severe insomnia, anxiety and depression, adding to the similarities between these two conditions (Møller 2007). Neural plasticity, which may be present in several forms, such as hyperactivity, hypersensitivity and the spread of activity, has been observed in chronic pain. In tinnitus, injuries to the auditory system components, such as those that are caused by noise exposure, lead to deafferentation of the central auditory system. This deafferentation may involve a reduced number of auditory nerve fibres, altering the input to the brain and inducing the adoption of a new adaptation strategy. Similar to the situation in hand amputation that was posited by Melzack et al, alterations in the somatosensory inputs result in neural plasticity, which may be represented as a cortical reorganisation (Tonndorf 1987; Kaltenbach, Zhang et al. 2005).

2.6.2 Tinnitus and post-traumatic stress Disorder (PTSD)

Post-traumatic stress disorder (PTSD) is a psychiatric disorder that occurs when an individual develops a variety of symptoms, such as anxiety and deep depression. These symptoms develop after being exposed to traumatic events, such as wars, natural disasters and sexual abuse. The symptoms are characterised by sleep disturbances, chronic depression, and social avoidance (Duckworth 1987; Creamer and O'Donnell 2002). Exploiting advances in neuroimaging techniques, investigators have worked to establish a neurophysiological model of PTSD. Liberzon et al. (1999) observed an increase in the regional cerebral blood flow in the limbic system and the left amygdala in Vietnam veterans compared to the control group. consistent with these findings, Rauch et al. (2000) reported exaggerated amygdalar activity in veterans with PTSD compared to combat-exposed veterans without PTSD.

Analogies between PTSD and tinnitus have been reported in the literature, as the two pathologies are characterised by similar symptoms, such as anxiety and depression. However, very few neuroimaging studies have indicated similarities between PTSD and tinnitus. There is strong evidence suggesting the involvement of the limbic system in tinnitus induction, and this brain region also plays a major role in PTSD. In addition, tinnitus becomes worse when its presence or loudness evokes PTSD-like annoyance and anxiety (Fagelson 2007). It is believed that these conditions share similar neuro-functional mechanisms, a hypothesis that has been supported in certain functional and structural brain studies. Grey matter reductions in the hippocampus have been reported in several MRI volumetric studies that involved subjects with PTSD (Bremner, Narayan et al. 2000; Kitayama, Vaccarino et al. 2005). This effect was also observed in tinnitus sufferers (Muhlau, Rauschecker et al. 2006). The role of the amygdala in PTSD has been emphasised in many neuroimaging studies, which have reported abnormal amygdalar function in PTSD sufferers (Armony, Corbo et al. 2005; Shin, Rauch et al. 2006). With respect to the neurophysiological model of tinnitus, the involvement of the amygdala is strongly suspected (Jastreboff and Hazell 2004). The contribution of the frontal cortex in both tinnitus and PTSD has been observed. Patients with tinnitus were observed to exhibit hyperactivation in several regions of the prefrontal cortex in a PET study (Mirz, Gjedde et al. 2000), indicating that these regions may underlie tinnitus perception. Diminished volume of the prefrontal cortex is associated with hyperactivity in PTSD subjects and has been observed in many neuroimaging studies (Zubieta, Chinitz et al. 1999; Carrion, Weems et al. 2001). In summary, many similarities have been observed between the functional and structural brain differences that characterise tinnitus

and PTSD, suggesting the requirement for further investigations to better understand tinnitus-related negative emotions.

2.7 Neuro-functional imaging and tinnitus

In certain tinnitus cases, audiometric investigations, such as tympanometry and speech discrimination tests, are sufficient to determine the underlying cause of the tinnitus. The conditions that can be identified in this way include otosclerosis, Meniere's disease and noise-induced hearing loss (Crummer and Hassan 2004). However, in the vast majority of patients with tinnitus, radiological evaluation is required to investigate whether the experienced tinnitus is a symptom of an underlying disease. Recent advances in imaging modalities, such as computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI) and functional MRI (fMRI), have provided clinicians with images that contain more information regarding the anatomy and physiology of both the ear and the brain. Certain imaging modalities, such as CT and MRI, provide clinicians with anatomical information. Other techniques, such as PET, fMRI and single photon emission tomography (SPECT), can identify brain regions that exhibit abnormal dynamic processes (Mirz, Brahe Pedersen et al. 1999). In CT, the use of contrast media enhances anatomical details, such as the base of the skull and the soft tissue of both the brain and auditory system. The use of strong magnets and image reconstruction techniques in MRI aid in this enhanced resolution of soft tissues, allowing for the identification of the underlying pathology in many patients with tinnitus.

In addition, PET and fMRI provide investigators with crucial data regarding hearing processes and tinnitus-related brain regions in the presence or the absence of a stimulus (Melcher, Sigalovsky et al. 2000; Lockwood, Burkard et al. 2003).

Functional imaging techniques are considered the gold standard with respect to increasing our understanding of neural responses to different sensory modalities, particularity sound. Exploiting such advances in neuro-functional imaging modalities will improve our understanding of tinnitus pathogenesis.

2.7.1 Positron Emission Tomography (PET)

Positron emission tomography (PET) is a functional imaging modality that primarily involves the use of radionuclides (tracers) that emit positrons. The radionuclides that are used in PET include oxygen-15 (^{15}O), nitrogen-13 (^{13}N), carbon-11 (^{11}C) and fluorine-18 (^{18}F). ^{18}F is commonly used in clinical applications to label substrates such as deoxyglucose (DG) to produce a radio-pharmaceutical known as fluorodeoxyglucose (FDG). FDG is then injected into the patient, enters the bloodstream and is transported to the organ of interest, where it undergoes metabolism and emits positrons. When a positron strikes an electron, the two particles are annihilated, giving rise to two photons with paths that lead in opposite directions. PET scanners consist of a ring of gamma ray detectors that can detect these two photons simultaneously. In clinical investigations, PET detectors record a very large number of these photons (approximately 10^6 to 10^9 events). PET detectors can also locate the decay line of the radiopharmaceutical by determining the two interaction vertices of the photons with the gamma ray detectors. By measuring all of the lines along which the decay occurs, cross-sectional images are produced using computational algorithms. These images reflect the concentration of the radionuclide in the organ of interest (Valk, Delbeke et al. 2003; Phelps 2006).

PET is one of the radiological modalities that has been used to map the central auditory pathways in normal adults. These studies were performed using water-

labelled radionuclides to measure cerebral blood flow. Changes in cerebral blood flow indirectly indicate the neural activity within a specific area. The scan is divided into two stages: during the response to a stimulus and during the absence of a stimulus. The blood flow in the brain during the two stages is compared, enabling the identification of the brain regions that respond to a stimulus. Using MRI combined with PET, it is possible to superimpose the resultant images. In this way, anatomical sites that respond to the stimulus can be clearly visualised (Lockwood, Burkard et al. 2003). However, PET is very limited because of its invasiveness, as it requires an injection of a radionuclide into the bloodstream. Additionally, the temporal resolution of PET is primarily dependant on the half life of the tracer, i.e., 2 minutes for ^{15}O (Phelps 2006). This time scale may not be sufficient to obtain information regarding brain responses. Another drawback of PET as a neuroimaging modality is its poor spatial resolution (Valk, Delbeke et al. 2006). However, PET is superior to functional MRI due its low acoustic noise level. Therefore, there is no scanner noise interference with the brain responses, and this is a highly important factor when performing experiments that involve acoustic stimuli.

Lockwood et al. (1998) observed changes in cerebral blood flow in patients with tinnitus who performed oro-facial movements to control the loudness of their tinnitus. These changes were observed in portions of the auditory and limbic regions, such as the hippocampus. However, Lockwood's study is statistically limited given its small population size (6 patients). Furthermore, these findings cannot be generalised because not all patients are able to control the loudness of their tinnitus. Thus, the tinnitus of other patients may involve different neural substrates. The involvement of the auditory cortex, particularly the primary

auditory cortex (PAC), in tinnitus perception has been demonstrated in several PET studies. These studies concluded that the left PAC is more likely to be involved in tinnitus induction regardless of tinnitus laterality or even-handedness (Lockwood, Salvi et al. 2002; Henry, Loovis et al. 2007; Henry, Loovis et al. 2007). The observed asymmetry in the metabolic rate may be correlated with the anatomical asymmetry in the PAC of normal adults and to a possible development of asymmetries in response to a new condition, such as tinnitus.

Farhadi et al. (2010) demonstrated the involvement of the temporal, parieto-temporal and frontal regions in tinnitus pathophysiology. These observations were based on the increased uptake of FDG in SPECT study. The increased uptake of FDG reflects an increase in the metabolic rate in these areas due to an abnormality or hyperactivity. However, no abnormality was identified in the auditory areas, which is quite inconsistent with other findings and with the neurophysiological mechanisms of tinnitus.

2.7.2 Functional magnetic resonance imaging (fMRI)

The success of tinnitus treatment is primarily dependent on specifying the cause and the underlying mechanism by which it developed. The radiological evaluation of tinnitus in both humans and experimental animals has resulted in a better understanding of tinnitus pathophysiology and has increased treatment success rates. Early imaging strategies of subjects with tinnitus were based on comparing their brain anatomy to that of subjects who do not suffer from tinnitus. However, as a basis for tinnitus imaging, it is important to know what type of tinnitus the patient has, i.e., subjective or objective and pulsatile or continuous. It is also important to differentiate between the effects of tinnitus and the functioning of the normal auditory system (Lockwood, Burkard et al. 2003).

FMRI of the auditory system has been widely implemented to examine cortical organisation in subjects with normal and abnormal hearing. It is well established that certain pathological conditions, such as cochlear damage and cochlear nerve resection, may result in the functional reorganisation of the auditory system. Compared to electroencephalography (EEG) and PET, which do not have the required spatial resolution to localise tinnitus-related anatomical regions, fMRI has been successful in this respect in tinnitus sufferers. One of the primary limitations of fMRI is scanner noise. Notably, the majority of auditory studies require sound stimulation to be delivered to the subjects during the scanning. Interactions are possible between the experimental stimulus and the noise of the scanner; however, numerous paradigms have been applied to reduce the effect of scanner noise in functional studies of the auditory cortex of patients with tinnitus. The ability of fMRI to measure the relative change in oxygen consumption between a stimulus response and the resting state has been exploited to obtain in vivo, real-time brain maps of blood oxygenation. Particularly, it is well established that increased oxygen consumption is related to an increased metabolic rate, which is believed to be correlated with increased neural activity (Arthurs and Boniface 2002; Attwell and Iadecola 2002).

Smits et al. (2007) introduced an fMRI paradigm through which the activation of cortical and subcortical auditory pathway structures is visualised in patients with lateralised tinnitus. These authors reported that there is a lateralisation of the affected areas (Heschel's gyrus and inferior colliculus) in patients with lateralised tinnitus, with lower fMRI activity on the side contralateral to tinnitus perception. That is, a different pattern of activation was observed in the maximum signal intensity change on the side of the perceived tinnitus. Smits and colleagues

calculated the maximum change in fMRI signal intensity bilaterally in the primary and secondary auditory cortices and the inferior colliculus by dividing the number of significantly active voxels in each of these regions in one hemisphere by the total number of active voxels in both hemispheres. Using this method, these authors separately calculated the activation ratio for each region of interest, concluding that the activation ratio for unilateral tinnitus is significantly higher on the side of tinnitus perception. These results support the hypothesis that tinnitus may be considered a phantom auditory sensation with abnormal neural activity. Lanting et al. (2008) applied a sparse sampling fMRI paradigm to reduce the effect of background noise on patients with unilateral tinnitus to examine neural activity in the auditory system. Their analyses indicated enhanced neural activity in the inferior colliculus of patients with tinnitus compared to controls. In contrast to Smits et al, Lanting et al observed an elevated neural activity in the IC that was contralateral to the perceived tinnitus. Melcher et al. (2000) performed a similar study of patients with unilateral tinnitus using a binaural and monaural fMRI stimulation paradigm. In agreement with Smits et al, Melcher et al conclusively demonstrated the elevation of neural activity in the IC that was ipsilateral to the tinnitus perception and decreased neural activity contralaterally.

Increased or decreased neural activity corresponds to excitatory and inhibitory synaptic processes and the associated neural discharges. These processes may result in increased metabolism and consequent increased blood flow and oxygenation. Accordingly, the fMRI signal strength of a given voxel is correlated with the performance of a task (Ogawa, Menon et al. 1993; Arthurs and Boniface 2002). The difference between the findings of Lanting et al and others concerns the side on which increased IC neural activity occurs relative to the perceived

tinnitus. Melcher et al. (2000) explained their findings as follows: tinnitus perception is associated with an increased neural activity in the contralateral IC during silent conditions until saturation is reached. When a sound stimulus is introduced, a decrease in neural activity (as it has reached its maximum) can be observed in the contralateral IC compared to the ipsilateral IC. However, differences in the experimental paradigms, protocols and tinnitus characteristics may result in different findings. In particular, Lanting et al used sparse imaging, whereas Smits et al and Melcher et al did not sufficiently apply the sparse imaging protocol. It has been reported that scanner noise and stimulus interactions may occur (Hall, Haggard et al. 1999), with a potential effect on signal strength.

2.7.3 Magnetoencephalography (MEG)

Magnetoencephalography (MEG) is a non-invasive neuroimaging modality that detects the magnetic field generated by neural activity, with no involvement of any external magnetic field. Neural activity in the brain is the primary electrophysiological basis of MEG signals. There are two primary forms of neural activity: (1) rapid depolarisation of the neural membrane, which results in action potentials, and (2) prolonged changes in the membrane potential, which result in a postsynaptic potential. Action potentials consist of rapid changes in membrane potential that occur over 1 or 2 milliseconds, after which the intracellular potential returns to its resting state. There are there are two types of postsynaptic potentials (PSPs): excitatory (EPSPs) and inhibitory (IPSPs). In EPSPs, a positive electric current due to the inflow of positive ions is directed into the cytoplasm, resulting in nerve cell depolarisation. During IPSPs, a positive electric current is directed extracellularly due to the inflow of negative ions or the outflow of positive ions. This latter type of PSP results in nerve cell hyperpolarisation. The electric current

that is due to cell EPSP-induced depolarisation generates a magnetic field that can be measured using MEG. There are two types of cortical neurons, namely, stellate and pyramidal neurons. Only pyramidal neurons give rise to MEG signals, as they generate a coherent magnetic field when activated. This magnetic field is extremely weak (approximately 50 femto-Tesla). However, this signal can be detected by very sensitive superconducting quantum interference devices (SQUIDs), which are present in MEG instruments (Hämäläinen, Hari et al. 1993; Lu and Kaufman 2003; Hansen, Kringelbach et al. 2010).

The ability of MEG to provide functional data regarding actual neural activity has assisted researchers in localising functional regions within the brain cortex, such as the auditory area. The images that are obtained from MEG are generally superimposed on anatomical images that are obtained from either CT or MRI to obtain more information regarding the location of the neural activity and its duration (Neil Cuffin and Cohen 1979; Masahiro, Hiroaki et al. 2000).

MEG has been exploited to investigate the neural correlates of tinnitus. Weisz et al. (2005) attempted to measure the abnormal spontaneous brain activity by MEG in patients with tinnitus and compared the results to those obtained from a control group with normal hearing. These authors demonstrated a noticeable reduction in alpha power and an associated enhancement in delta power in patients with tinnitus over temporal areas. These activity patterns extended into the posterior regions of the right hemisphere. Enhanced delta (i.e., slow wave) activity is associated with tinnitus perception given that it is characterised by the presence of magnetic slow wave activity (≈4 Hz). However, neither alpha attenuation nor delta enhancement was clearly related to increased spontaneous brain activity, which can be perceived as tinnitus. Additionally, this previous study raised the possibility

of right temporal and left frontal cortical involvement in tinnitus perception. This conclusion was drawn given that the temporal lobe is associated with certain perceptions, such as volume, and the left frontal cortex is associated with affective distress, such as tinnitus (Chayer and Freedman 2001). In previous studies that involved individuals who were clinically diagnosed with depression, a significant enhancement of slow-wave activity was observed (Fernández, Rodriguez-Palancas et al. 2005). This result may suggest a correlation in the brain activity between the neural substrates of tinnitus and depression. Unfortunately, no sufficient MEG group studies of patients with tinnitus have been performed. An altered tonotopic map of the auditory cortices of tinnitus sufferers was reported in two MEG studies, and this reorganisation was determined to be correlated with tinnitus severity (Muhinickel, Elbert et al. 1998; Wienbruch, Paul et al. 2006).

With regard to the importance of MEG in locating cortical sources of tinnitus that may be therapeutically modified, Bowyer et al. (2007) performed a clinical MEG study of two patients with debilitating tinnitus who had been treated with conventional therapies). Tonotopic maps of the auditory cortices of the two patients were obtained and uploaded to a surgical neuronavigational system to guide the placement of electrodes. These electrodes were used to electrically stimulate the source of tinnitus. Interestingly, the MEG-based localisation of the cortical sources of tinnitus was fairly accurate. The surgery was successful in reducing tinnitus, which was nearly eliminated in one patient; the other patient reported the elimination of the tinnitus in one ear. MEG studies of patients with tinnitus also demonstrated the benefits of electrical cortical stimulation of the auditory cortex by reducing the abnormal spontaneous brain activity in these

regions, which is believed to be one of the underlying causes of tinnitus (Ramirez, Kopell et al. 2009).

There is widespread agreement that tinnitus is associated with structural and functional changes in either the ear or brain. One of these changes may be a hearing threshold shift, which would suggest cochlear alterations. Shulaman and Goldstein conclusively demonstrated hearing threshold shifts in a study involving 21 patients with tinnitus who were examined with quantitative electroencephalography (QEEG). These authors observed an abnormal incidence of significant central nervous system electrical dysfunction in each patient. The abnormality could be characterised by an increase or decrease in spontaneous neural activity. These findings certainly indicate that neural changes are associated with tinnitus (Shulman and Goldstein 2002).

2.7.4 Structural studies

Given that it is broadly accepted that tinnitus is associated with maladaptive neuroplasticity, the characteristics of which are changes in neuro-functional mechanisms, it is unsurprising that structural alterations have also been identified. High-resolution MRI has made it possible to non-invasively quantify the volume of specific brain structures. In many studies, the quantification of brain structure volumes, such as those of the grey and white matter, is performed using high-resolution MR and voxel-based morphometry (VBM), which relies on an unbiased comprehensive approach to detect brain structural differences between two groups (Ashburner and Friston 2000).

Although numerous studies have demonstrated functional changes in patients with tinnitus compared to normal controls, few studies have similarly demonstrated structural brain changes. Mahlau et al. (2006) observed a reduction in GM in the

subcallosal region and an increase in GM in the posterior thalamus. However, these findings are not in agreement with those of Landgrebe et al. (2009), who did not observe any changes in either the thalamus or the subcallosal areas with respect to GM volumes. This disagreement occurred despite the fact that both studies had equal sample sizes (i.e., 28 patients with tinnitus). Landgrebe et al observed a significant reduction in the GM in the right inferior colliculus and the left hippocampus. The discrepancy between the two studies may be related to tinnitus characteristics, such as severity and lateralisation. Although both of the studies included patients suffering from unilateral and bilateral tinnitus, there were differences in the size of each group. Other possible explanations for this discrepancy include differences in the methodological approaches, such as the scan acquisition parameters and analytical issues, which have a significant impact on the results. Schneider et al. (2009) demonstrated a reduction in the volume of Heschel's gyrus in tinnitus sufferers compared to controls. This reduction was observed ipsilateral to the affected ear, representing a type of neurodegenerative disease or a form of neuroplasticity. The PFC is not far from the areas where structural alterations that are believed to be related with tinnitus are observed. Leaver et al. (2011) reported GM reductions in the ventro-medial PFC of tinnitus sufferers compared to healthy subjects.

A possible tinnitus-associated alteration in white matter has recently come to the attention of many scientists. Diffusion tensor imaging (DTI) is the only non-invasive MR imaging tool that can assess the microstructure of WM tracts in the brain (Le Bihan, Mangin et al. 2001). An extensive explanation of this technique and its application is discussed in chapter 5. Because DTI is considered a relatively new technique, very few studies have investigated WM integrity in

patients with tinnitus. Lee et al. (2007) reported a reduction in fractional anisotropy (FA) in the left frontal actuate fasciculus and the right parietal arcuate fasciculus in patients with tinnitus compared to controls. However, these findings may be limited by the unmatched mean age between the patients with tinnitus (mean age 48.3 years) and the participants in the control group (mean age 26.5 years) that were included in this study. A positive correlation between age and FA reduction has been reported (Voineskos, Rajji et al. 2012). Therefore, homogeneous age groups should be recruited to avoid such complications. Crippa et al. (2010) reported an FA reduction in the WM tracts that connect the auditory cortex with the amygdala, the inferior colliculus with the amygdala, and the auditory cortex with the inferior colliculus. Nonetheless, more studies are required to better understand WM integrity in patients with tinnitus.

2.8 Hypotheses

Tinnitus is an auditory phantom in which a sufferer senses sounds when no external stimuli are present. It is an irritating phenomenon that causes the sufferer to experience negative emotions and symptoms, such as anxiety, depression, sleep deprivation and attention deficits (Jastreboff and Hazell 2004). I hypothesise that tinnitus and its negative associations are associated with increased brain activation in the auditory region, the PFC and the limbic system. I employed fMRI to investigate the brain functional changes that may be associated with tinnitus. During these experiments, I introduced emotionally evocative standardised visual and acoustic stimuli to two groups of participants: those with tinnitus and healthy subjects. I also hypothesise that tinnitus perception is associated with brain structural deficits in the auditory region, the PFC and the limbic system. The structural deficits that may be associated with tinnitus include (1) grey matter

reduction and (2) changes in the WM integrity, which can consist of either an increase or a decrease in the fractional anisotropy or in the mean diffusivity of the fibres. Because the amygdala receives inputs from the auditory cortex, I hypothesise that the integrity of the fibres that connect these two regions is disrupted in the tinnitus sufferers group compared to controls. A similar hypothesis is also made with respect to the fibres that connect the hippocampus and the auditory cortex. This latter hypothesis is based on the role of the hippocampus in long-term auditory memory formation (Squire, Schmolck et al. 2001). To investigate brain structural changes, I employed high-resolution MRI and diffusion tensor imaging sequences, by which I quantitatively assessed measures of grey and white matter characteristics in the two groups.

3. Chapter 3. Methods

3.1 Inclusion and Exclusion Criteria

The exclusion criteria for the tinnitus and the control subjects included the following: conductive hearing loss; hearing loss worse than 40 dBHL at 2 kHz and 60 dBHL at 4 kHz; head injuries; any neurological disorder, such as acoustic neuroma or microvascular ischemia; migraines; physiological disorders, including motor or sensory disability; and psychological disorders, such as schizophrenia or panic disorders. The patients were asked if they had a lower tolerance to noise than other individuals to assess the presence of hyperacusis. "The Khalfa score" (Khalfa, Dubal et al. 2002) was used to assess the severity of hyperacusis. Only those who had suffered from tinnitus for more than 6 months were included in this study. Controls with chronic tinnitus were not included in this study and none of the controls experienced tinnitus on the scan day. The inclusion and exclusion criteria for both the healthy participants and those with tinnitus are summarised in Table 3.1.

Table 3.1 The inclusion and exclusion criteria

Inclusion criteria	Exclusion Criteria
hearing loss no worse than 40 dBHL at 2 kHz and 60 dBHL at 4 kHz (for both the controls and the subjects with tinnitus)	severe health problems, head injuries, neurological disorders (acoustic neuroma), for both controls and subjects with tinnitus
no conductive hearing loss (for both controls and subjects with tinnitus)	metallic implant or pacemakers (for both controls and subjects with tinnitus)
Newman Tinnitus Handicap Inventory (Newman THI) ≥38 for newly diagnosed patients and existing patients with tinnitus (for subjects with tinnitus only) minimum tinnitus duration >6 months (for subjects with tinnitus only)	physiological disorders (sensory and motor) and psychological disorders (any mental disorders that affect behaviour, such as schizophrenia and panic disorder) (for both controls and subjects with tinnitus)
symmetric hearing loss with < 15 dB asymmetry up to 4 kHz (for both tinnitus and controls subjects)	
age between 30-60 years (for both controls and subjects with tinnitus)	

3.2 Recruitment

After obtaining ethical approval from the Sefton Research Ethics Committee to conduct these studies, announcements for healthy controls and participants with tinnitus were released. The announcement that was made to recruit patients with tinnitus was placed in audiology clinics in Aintree University NHS Foundation Trust Hospitals. The volunteer announcement was advertised through the University of Liverpool web facilities. A full participation information sheet was provided to all of the interested individuals. The participation information sheet

contained the following items: the aim of the study; an explanation of the full procedure, including images explaining the procedure; the duration of the investigations; and the contact information of the researcher in case further information was required. The referrals for the participants with tinnitus were sent to me personally from the audiology department at Aintree University NHS Foundation Trust Hospitals. The referrals included information regarding the tinnitus subject, such as the Newman Tinnitus Handicap Inventory (Newman THI) score, the duration of the tinnitus and the side on which the tinnitus was perceived. Prior to any MRI scan, both the healthy volunteers and the participants with tinnitus signed a consent form. The consent form was provided to ensure that participants fully read and understood the information sheet and agreed to take part in the study.

3.3 Audiology Test

To assess the hearing status of both controls and subjects with tinnitus, pure-tone air-conducted audiometry was performed on all of the subjects. The pure-tone signals were presented at six different frequencies: 250, 500, 1000, 2000, 4000 and 8000 Hertz (Hz). The tones were presented at different sound intensities in hearing level decibels (dBHL) that ranged from -10 to 120 dBHL (Audiology 2004). For the tinnitus sufferers, the test was performed in the sound proof room at the audiology department, Aintree University NHS Foundation Trust Hospitals. The control subjects were tested by a consultant audiological physician at the University of Liverpool site.

3.4 Newman Tinnitus Handicap Inventory Questionnaire (Newman THI) (Newman and Jacobson 1996)

The subjects with tinnitus were requested to fill out a Newman THI questionnaire at their visit to the audiology clinics at Aintree University NHS Foundation Trust

Hospitals, where filling out this questionnaire was routine. The Newman THI questionnaire quantifies the impact of tinnitus on three aspects of the sufferer's daily life, namely, the functional, emotional and catastrophic aspects. Questions under the functional scale evaluate the impact of tinnitus using 11 questions that fit into three primary categories: (1) mental, such as, "Because of your tinnitus, is it difficult for you to concentrate?"; (2) social, such as, "Does your tinnitus interfere with your ability to enjoy your social activities?"; and (3) physical, such as, "Because of your tinnitus, do you have trouble falling asleep at night?" Nine questions of the inventory quantify the impact of tinnitus on the sufferer's daily emotional life. Examples of these questions include "Does your tinnitus make you angry?" and "Do you often feel depressed?" The detrimental impact of tinnitus is assessed using five questions. These questions investigate the severity of the sufferer's reaction to tinnitus, such as their inability to cope with tinnitus, a loss of attention and experiences of fear.

For each question in the Newman THI questionnaire, the patient must choose "yes", "sometimes" or "no". Every "yes" is assigned a score of four points, every "sometimes" is assigned a score of two points, and every "no" is assigned a score of zero points. The scores are summed, and the summation ranges from 0 to 100. Based on the total score of the inventory, the severity of the tinnitus is classified into four grades: (1) no handicap is assessed if the total is 16 points or fewer, (2) a mild handicap is assessed for scores between 18 and 36, (3) a moderate handicap is assessed for scores between 38 and 56, and (4) a severe handicap is assessed for scores between 58 and 100 (Newman and Jacobson 1996).

3.5 MRI Scanning

The controls and subjects with tinnitus underwent a safety screening by a research radiographer at the Magnetic Resonance and Image Analysis Research Centre (MARIARC) prior to being taken into MR scan rooms. The subjects were questioned as to whether they had any contraindication for being exposed to high-strength magnetic fields. The contraindications included the following: metallic implementations (e.g., pacemakers and surgical clips), being on medications for certain diseases (e.g., renal failure and heart disease), or claustrophobia. Any subject with any of these contraindications was excluded from the study.

3.6 Clinical MRI scans

The subjects from both of the groups underwent two clinical scans: (1) a T2-weighted scan and (2) a high-resolution scan of the internal auditory meatus (IAM). The results of both of the scans were sent to the Walton Centre NHS Foundation Trust Hospitals and were analysed by neuroradiologists. The purpose of the first scan was to ensure that none of subjects had any neurological disorders, such as tumours or cysts. This scan is routine for all studies that involve brain investigations in the MARIARC. The second scan was particular to this study and was performed to determine whether any of the participants had an acoustic neuroma in the internal auditory meatus, as this condition was one of the exclusion criteria of this study.

4. Chapter 4. Functional MRI Study

4.1 Aims and objectives

This chapter describes the investigation of the neural correlates of tinnitus using an optimised fMRI paradigm. There were five primary objectives of this study. First, I used fMRI to investigate how tinnitus sufferers respond to pleasant and unpleasant emotionally evocative visual and auditory stimuli. This analysis was performed because it has been hypothesised that the tinnitus signal functions as an unpleasant stimulus. Second, I tested Jastreboff's neurophysiological model that postulates that tinnitus is associated with hyperactivation of the: PFC, auditory cortex and limbic system. Third, I used hearing tests, the Newman THI scores and fMRI to examine the relationship between hearing thresholds, tinnitus severity and the brain activity that is associated with tinnitus. Fourth, I investigated whether pleasant and unpleasant emotional stimuli would stimulate similar or distinct brain regions in tinnitus sufferers compared to the control subjects. An additional component of this objective was to determine whether these regions had previously been correlated with positive or negative emotions. The fifth objective of the study described in this chapter was to examine the psychoacoustic characteristics of tinnitus by creating a tinnitus-like condition in healthy volunteers. The same paradigm that was applied in the investigation of tinnitus sufferers was applied for these analyses, with the exception that tinnitus-like sounds were included.

4.2 Introduction

Recent functional neuroimaging techniques have provided valuable information regarding dynamic processes in the brain. Indeed, it has been possible to examine the behaviour of the brain when individuals perform certain tasks. In addition, these techniques have been used to examine pathological and psychological conditions that involve changes in brain functions. fMRI, which is one of these

technologies, has been extensively utilised to investigate the neural correlates of tinnitus. Moreover, fMRI has provided insights into the relationship among (1) clinical findings, such as those that can be obtained in audiography; (2) the severity of the tinnitus that is described by sufferers; and (3) the neural activity of patients with tinnitus (Smits, Kovacs et al. 2007). FMRI has great advantages as a non-invasive neuroimaging tool with high spatial resolution compared to PET, which is an invasive technique that has poor spatial resolution.

Recently, various experimental paradigms have been introduced that aim to better understand the neural substrates of tinnitus and to achieve clinically applicable results. The vast majority of these studies report the identity of brain regions that are involved in tinnitus perception. The auditory system, the PFC and the temporal lobe constitute points of intersection among these studies, although not all of these previous analyses used fMRI (Muhinickel, Elbert et al. 1998; Mirz, Gjedde et al. 2000; Farhadi, Mahmoudian et al. 2010). The limbic system, which is a primary component of Jastreboff's proposed neurophysiological tinnitus model, has been of recent interest to many researchers. Recent functional imaging studies of tinnitus sufferers have reported the involvement of the limbic system in tinnitus perception (Lockwood, Salvi et al. 1998; Mirz, Gjedde et al. 2000; Leaver, Renier et al. 2011). Working in parallel to these studies, I aimed to investigate the neural correlates of tinnitus from an emotional prescriptive. Hence, I employed pure emotional visual and acoustic stimuli to investigate the emotional responses of patients with tinnitus by comparing their neural activity with the activity that was observed in the control group. For the first time, I employed internationally standardised emotionally evocative pleasant and unpleasant visual stimuli and unpleasant acoustic stimuli in an fMRI study of patients with tinnitus. I aimed to

investigate whether tinnitus perception involves functional reorganisation in the auditory region, the PFC and the limbic system. I hypothesised that tinnitus sufferers would exhibit increased activity in these three regions when they listened to unpleasant sounds, particularly the auditory cortex and the amygdala. Because the amygdala and the auditory cortex are connected (Romanski and LeDoux 1993), the unpleasant tinnitus signal may induce functional changes in these regions. Hence, I compared the BOLD activation map that was obtained from tinnitus sufferers with a map that was obtained from healthy controls. I also introduced unpleasant and pleasant visual stimuli to investigate how tinnitus sufferers respond to different emotional stimuli that involve different sensory modalities. I expected to observe increased activation in the auditory, PFC and limbic regions when tinnitus sufferers viewed unpleasant or even pleasant images. This prediction was based on my hypothesis that tinnitus perception involves neural plasticity, which may manifest as functional reorganisation in these regions. Thus, I investigated the primary effect of tinnitus when subjects viewed pleasant and unpleasant images. Even under the baseline conditions, during which no stimulus was present, I assumed that there would be an increased activation in the auditory, PFC and limbic regions. I compared the BOLD activation map between the subjects with tinnitus and healthy controls during baseline conditions in the two experimental sessions. In the first session, all of the subjects listened to unpleasant sounds. These sounds were followed by baseline conditions, after which pleasant images were viewed. In the second experimental session, all of the subjects listened to unpleasant sounds. These sounds were followed by baseline conditions, after which unpleasant images were viewed.

I investigated the psychoacoustic features of tinnitus by performing an experiment on healthy volunteers in which they experienced tinnitus-like sounds. This experiment aimed to investigate whether tinnitus of a known origin (i.e., the auditory cortex) would exhibit similar underlying neural substrates as shown by tinnitus of unknown origins (i.e., the tinnitus in the study patients). I examined the neural activity of the healthy volunteers when they viewed (1) pleasant and unpleasant images that were combined with tinnitus-like sounds and (2) pleasant and unpleasant images in the absence of tinnitus-like sounds; these results were compared with those of tinnitus sufferers when viewing pleasant and unpleasant images, assuming that they continuously experience tinnitus sounds. I hypothesised that the psychoacoustic features of the tinnitus signal would evoke hyperactivation in the limbic system in the healthy volunteers when they experienced tinnitus-like sounds. Thus, I performed within-subject analyses of variance on data from healthy volunteers that were obtained when they viewed pleasant and unpleasant images that were combined with tinnitus-like sounds or in the absence of these sounds. I also investigated the brain regions that exhibited hyperactivation in the patients with tinnitus when they viewed pleasant and unpleasant images. Next, I investigated whether tinnitus-like sounds specifically activated similar brain regions in the control patients to the regions that were observed to be activated in tinnitus patients.

4.3 Materials and Methods

Prior to conducting the fMRI study, a pilot study was performed on 5 healthy volunteers. The aim of the pilot study was to develop an efficient experimental paradigm in terms of defining significant activation and determining the optimum scanning duration. I tested three block designs and one silent event-related design.

In the three block designs, I applied different stimuli durations, which were separated by baseline conditions. In the first design, the stimulus time was set at 7 seconds and the baseline was set at 12.5 seconds. In the second block design, I applied a stimulus time of 10 seconds and a baseline time of 10 seconds. In the third design, the stimulus time was set at 21 seconds and the baseline time was 21 seconds. During the 21 seconds of stimulation, 7 different auditory or visual stimuli were presented, each lasting for 3 seconds. In the silent-event-related design, each stimulus was presented for 5 seconds and was followed by 5 seconds of a baseline condition. The stimulus was triggered by the MR scanner in the non-acquisition periods, thereby avoiding the noise contribution of the scanner. However, this design did not result in any significant activation of any of the brain regions that were under consideration. This design also involved a longer presentation time for all of the stimuli given that each stimulus was triggered by the scanner during non-acquisition intervals. These aspects of this design made the scanning uncomfortable for the participants; the design was therefore excluded. Compared with the first and second block designs, the block design with the 21 s of stimuli followed by 21 s of baseline resulted in an optimised BOLD signal and a reasonable scanning time.

4.3.1 Subjects

This study obtained ethical approval from the Sefton Research Ethics Committee in Liverpool. Between November 2009 and March 2011, 18 patients who suffered from tinnitus were recruited from the audiology clinics at Aintree University NHS Foundation Trust Hospitals, Liverpool. For purposes of comparison, 15 healthy volunteers were recruited through the University of Liverpool Web facilities. The inclusion and exclusion criteria for both the patients and the healthy volunteers are

summarised in Table 3.1. All of the volunteers were assessed by an audiologist to ensure that they exhibited normal hearing thresholds. The patient and volunteer characteristics are listed in Table 4.1. Seven patients with tinnitus had a history of noise exposure, and none of the controls had a history of noise exposure. One tinnitus subject had tinnitus because of ototoxicity. None of the controls had tinnitus or experienced tinnitus on the day of study. Seventeen patients with tinnitus reported sleep disturbance because of their tinnitus. No Anxiety and Depression scale collected. Only one subject with tinnitus reported hyperacusis with a Khalfa score of 26 (Khalfa, Dubal et al. 2002). To exclude the possibility of acoustic neuroma, all of the subjects were scanned using a high-resolution MRI sequence to obtain detailed images of the internal auditory meatus (IAM). To exclude any neurological disorder in the brain, all subjects were scanned using T2 MRI sequence. Following a detailed explanation of the experimental procedure and a reading of the provided information sheet, all of the subjects gave written informed consent. The scan results were sent to the Walton Centre NHS Foundation Trust and were reviewed by neuroradiologists. None of subjects from either group exhibited any neurological disorders such as acoustic neuroma or microvascular ischemia.

Table 4.1 Patient and control characteristics.

Characteristics	Controls (n=15)	Unilateral tinnitus (n=3)	Bilateral tinnitus (n=15)
age (years)			
range	31-60	42-58	33-60
Mean±SD	48.5±8.76	48.66±8.32	51.62±8.28
gender			
male	9	2	9
female	6	1	6
tinnitus duration			
range		9 months-2 years	1-25 years
Mean±SD (years)	-	1.3±0.66	6.08±8.47
average Newman score	-		
range		46-74	34-86
Mean±SD		59.33±14.04	58.13±13.05

4.3.2 Stimuli

The visual stimuli consisted of pleasant and unpleasant photographs that were taken from the International Affective Picture System (IAPS) (Lang, Bradley et al. 2008). The baseline images consisted of a cross-hair that was presented in the middle of the screen. I employed 50 pleasant images and 50 unpleasant images. The description, slide number and the mean and the standard deviation (SD) of the valance and their associated arousal levels of the images are listed in Tables 4.2 and 4.3. The auditory stimuli consisted of a set of unpleasant sound examples in which an infant is crying and a woman is screaming, which were obtained from

the International Affective Digitised Sound system (IADS) (Bradley and Lang 2007). A list of these sounds, including the mean and the SD of their valence and associated arousal levels, is presented in Table 4.4. The selection of the images and the sounds was based on a previous study (Lang, Bradley et al. 2008), and no ratings were collected from the individuals who participated in the present study. These stimuli were internationally standardised, and their ratings were published by Bradley el al. (Lang, Bradley et al. 2008). Furthermore, several studies of emotions have employed these stimuli without prior collection of ratings. Rather, these studies used ratings based on the study by Bradley et al (Hariri, Tessitore et al. 2002; Hariri, Mattay et al. 2003; Britton, Taylor et al. 2006).

Table 4.2. Means and standard deviations (SD) of the valance and arousal levels of the pleasant IAPS images that were employed in this study. The ratings are taken from the technical manual of the IAPS (Lang, Bradley et al. 2008).

Description	Slide number	Valence Mean(SD)	Arousal Mean(SD)
kittens	1463	7.45(1.76)	4.79(2.19)
puppies	1710	8.34(1.12)	5.41(2.34)
monkeys	1811	7.62(1.59)	5.12(2.25)
Mickey Mouse	1999	7.43(1.47)	4.77(2.40)
father	2057	7.81(1.28)	4.54(2.41)
infant	2058	7.91(1.26)	5.09(2.48)
infants	2080	8.09(1.47)	4.70(2.59)
children	2158	7.31(1.48)	5.00(2.20)
father	2165	7.63(1.48)	4.55(2.55)
children	2345	7.41(1.72)	5.42(2.47)
boat	2398	7.48(1.32)	4.74(2.11)
couple	2550	7.77(1.43)	4.68(2.43)
child	2655	6.88(2.09)	4.57(2.19)
garden	5199	6.93(1.91)	4.70(2.52)
harbour	5215	6.83(1.70)	5.40(2.15)
waterfall	5260	7.34(1.74)	5.71(2.53)
nature	5270	7.26(1.57)	5.49(2.54)
fireworks	5480	7.53(1.63)	5.48(2.35)
skydiver	5621	7.57(1.42)	6.99(1.95)
hiker	5629	7.03(1.55)	6.55(2.11)
mountain	5814	7.15(1.54)	4.82(2.40)
sea	5825	8.03(1.18)	5.46(2.72)
beach	5833	8.22(1.08)	5.71(2.66)
brownie	7200	7.63(1.74)	4.87(2.59)
Ice cream	7330	7.69(1.84)	5.14(2.58)
cup cake	7405	7.38(1.73)	6.28(2.16)
pasta	7480	7.08(1.62)	4.55(2.42)
ferry	7492	7.41(1.68)	4.91(2.46)
castle	7502	7.75(1.40)	5.91(2.31)
ferries wheel	7508	7.02(1.46)	5.09(2.11)
skyline	7570	6.97(1.69)	5.54(2.34)
desert	7580	7.51(1.60)	4.59(2.72)
skier	8030	7.33(1.76)	7.35(2.02)
sailing	8080	7.73(1.34)	6.65(2.20)
athlete	8120	7.09(1.36)	4.85(2.13)
hang glider	8161	6.71(1.64)	6.09(2.24)
parachute	8163	7.14(1.61)	6.53(2.21)
sky surfer	8168	7.01(1.57)	6.84(2.01)
cliff diver	8178	6.50(2.00)	6.82(2.33)
skydivers	8185	7.57(1.52)	7.27(2.08)
skier	8190	8.10(1.39)	6.28(2.57)
water skier	8200	7.54(1.37)	6.35(1.98)
surfers	8206	6.43(1.75)	6.41(2.19)
pilot	8300	7.02(1.60)	6.14(2.21)
tennis player	8350	7.18(1.56)	5.18(2.28)
rafting	8370	7.77(1.29)	6.73(2.24)
tubing	8420	7.76(1.55)	5.56(2.38)
Happy teens	8461	7.22(1.53)	4.69(2.20)
waterslide	8496	7.58(1.63)	5.79(2.26)
roller coaster	8499	7.63(1.41)	6.07(2.31)

Table 4.3. The means and standard deviations (SD) of the valance and arousal levels of the unpleasant IAPS images that were employed in this study. The ratings are taken from the technical manual of the IAPS (Lang, Bradley et al. 2008).

description	Slide number	Valance Mean(SD)	Arousal Mean(SD)
toddler	2095	1.79(1.18)	5.25(2.34)
hospital	2205	1.95(1.58)	4.53(2.23)
black eye	2345.1	2.26(1.46)	5.50(2.34)
woman	2375.1	2.20(1.31)	4.88(2.21)
sad children	2703	1.91(1.26)	5.78(2.25)
funeral	2799	2.42(1.41)	5.02(1.99)
sad child	2800	1.78(1.14)	5.49(2.11)
crying boy	2900.1	2.56(1.41)	4.61(2.07)
mutation	3016	1.90(1.31)	5.82(2.44)
mutation	3017	2.45(1.35)	5.34(2.39)
burnt face	3101	1.91(1.19)	5.60(2.46)
battered female	3181	2.30(1.43)	5.06(2.11)
stitches	3195	2.06(1.23)	6.36(2.25)
hospital	3220	2.49(1.29)	5.52(1.86)
dying man	3230	2.02(1.30)	5.41(2.21)
injured child	3301	1.80(1.28)	5.21(2.26)
infant	3350	1.88(1.67)	5.72(2.23)
assault	6022	2.14(1.55)	6.09(2.47)
soldier	6212	2.19(1.49)	6.01(2.44)
aimed gun	6243	2.33(1.49)	5.99(2.23)
police	6838	2.45(1.44)	5.80(2.09)
starving child	9040	1.67(1.07)	5.82(2.15)
plane crash	9050	2.43(1.61)	6.36(1.97)
starving child	9075	1.66(1.10)	6.04(2.40)
cow	9140	2.19(1.37)	5.38(2.19)
soldiers	9163	2.10(1.36)	6.53(2.21)
dead cows	9181	2.26(1.85)	5.39(2.41)
injured dog	9184	2.47(1.52)	5.75(2.43)
dead dog	9185	1.97(1.16)	5.65(2.35)
injured dog	9187	1.81(1.36)	6.45(2.30)
cemetery	9220	2.06(1.54)	4.00(2.09)
assault	9254	2.03(1.35)	6.04(2.35)
garbage	9295	2.39(1.30)	5.11(2.05)
crying woman	9332	2.25(1.33)	5.34(2.00)
soldier	9420	2.31(1.59)	5.69(2.28)
soldier	9421	2.21(1.45)	5.04(2.15)
assault	9428	2.31(1.31)	5.66(2.41)
accident	9435	2.27(1.47)	5.00(2.03)
kids	9520	2.46(1.61)	5.41(2.27)
duck in oil	9560	2.12(1.93)	5.50(2.52)
dog	9570	1.68(1.23)	6.14(2.31)
cat	9571	1.96(1.50)	5.64(2.50)
ship	9600	2.48(1.62)	6.46(2.31)
KKK rally	9810	2.09(1.78)	6.62(2.26)
car accident	9900	2.46(1.39)	5.58(2.13)
car accident	9901	2.27(1.25)	5.70(2.22)
car accident	9902	2.33(1.38)	6.00(2.15)
car accident	9903	2.36(1.35)	5.71(2.28)
execution	9914	2.06(1.48)	6.49(2.26)
fire	9921	2.04(1.47)	6.52(1.94)

Table 4.4. The means and standard deviations (SD) of the valance and arousal levels of the unpleasant IADS sounds that were employed in this study. The ratings were taken from the technical manual of the IADS (Bradley and Lang 2007).

description	Sound number	Pleasure Mean(SD)	Arousal Mean(SD)
bees	115	2.16 (1.33)	7.03 (1.33)
buzzing	116	3.02 (1.65)	6.51 (1.65)
male cough	241	2.46 (1.53)	5.87 (1.53)
female cough	242	2.80 (1.86)	5.39 (1.86)
man wheeze	244	2.44 (1.34)	6.31 (1.34)
infants crying	260	2.04 (1.39)	6.87 (1.39)
baby crying	261	2.75 (1.68)	6.51 (1.68)
scream	275	2.05(1.62)	8.16 (1.62)
female scream2	276	1.93(1.63)	7.77 (1.63)
female scream3	277	1.63 (1.13)	7.79 (1.13)
child abuse	278	1.57 (1.43)	7.27 (1.43)
fight 2	282	2.92 (2.34)	7.20 (2.34)
attack 2	285	1.80 (1.56)	7.79 (1.56)
victim	286	1.68 (1.18)	7.88 (1.18)
fight 1	290	1.65 (1.27)	7.61 (1.27)
male scream	292	1.99 (1.41)	7.28 (1.41)
women crying	296	2.06 (1.22)	6.07 (1.22)
car horns	420	2.34 (1.51)	7.08 (1.51)
tire skids	422	2.22 (1.47)	7.52 (1.47)
car wreck	424	2.04 (1.52)	7.99 (1.52)
plane crash	501	2.74 (1.76)	6.93 (1.76)
bike wreck	600	2.13 (1.55)	7.28 (1.55)
air raid	624	2.82 (1.75)	7.10 (1.75)
busy signal	703	2.65 (1.59)	5.68 (1.59)
alarm clock	709	2.78 (1.93)	7.54 (1.93)
siren 1	711	2.61 (1.59)	7.39 (1.59)
buzzer	712	2.42 (1.62)	7.98 (1.62)
sirens	713	2.95 (1.71)	6.98 (1.71)
dentist drill	719	2.89 (1.67)	6.91 (1.67)
crash	732	2.89 (1.68)	6.98 (1.68)

The IAPS and IADS were developed to provide investigators in the fields of emotions and attention with a set of standardised, emotionally evocative stimuli. In the development of the IAPS images and IADS sounds, 100 subjects (50 female) rated each image and each sound. The rating instrument that was used in the present study is referred to as the Self-Assessment Manikin (SAM). The SAM is a graphic figure that contains a range of happy, smiling faces, neutral faces and unhappy faces. The valance of each IAPS image and IADS sound is rated using this scale. The SAM instrument also contains five figures with expressions that

range from excited (wide eyes) to relaxed (sleepy eyes), representing the arousal dimension. Every participant rated each image or sound using a score range from 1 to 9. A score of 9 indicates that the image or sound is highly pleasant and arousing, whereas a score of 1 indicates extreme unhappiness and low arousal. The participants were instructed to assign the score of 5 in the SAM instrument if they felt completely neutral, i.e., neither happy nor unhappy, or if they felt neither excited nor calm.

The selection of the IAPS and IADS stimuli was aimed at targeting the emotions of happiness (e.g., a baby, a couple and sailing) and unhappiness (e.g., a starving child and an injured child) in the two groups of participants. I selected images with a mean valance > 6 (on the 9-point SAM scale) to represent pleasant emotional stimuli and a mean valance < 2.5 to represent unpleasant emotional stimuli. The mean arousal for the pleasant images was 5.6±0.8 and for the unpleasant images was 5.65 ±0.6. For the auditory stimuli, I selected sounds with a mean valance of < 3.5 to represent unpleasant auditory emotional stimuli. The mean arousal for the selected unpleasant IADS sounds was 7.08±0.71. Both the IAPS images and the IADS sounds were selected based the mean valance and arousal levels of all of the participants (50 males and 50 females) (Bradley and Lang 2007; Lang, Bradley et al. 2008). I obtained tinnitus-like sounds from the British Tinnitus Association (BTA), and these sounds were presented to the controls only.

4.3.3 Paradigm

The experimental paradigm consisted of a block design of 20 epochs of 21 s in which either visual or auditory stimuli were presented. The following sequence was used: 21 s of auditory stimuli delivered binaurally followed by 21 s of the

baseline condition (i.e., a cross-hair on the screen). The visual stimuli were then presented for 21 s through MR compatible headphones (MR Confon; MR Confon GmbH, Magdeburg, Germany). This sequence was repeated 10 times. The experiment was performed over two sessions. During experimental session 1, the participants listened to unpleasant auditory stimuli, which was followed by the baseline condition and the pleasant visual stimuli. In experimental session 2, the participants listened to unpleasant auditory stimuli, which was followed by the baseline condition and the unpleasant visual stimuli. The visual and auditory stimuli were programmed using E-Prime software (Psychology Software Tools). The participants lay in the MRI scanner and viewed, via a mirror, large stimuli on the same type of computer monitor.

I added two other experimental sessions to the control group, during which the participants, in addition to being presented with pleasant and unpleasant IAPS images, experienced tinnitus-like sounds that were obtained from the BTA. The participants underwent precisely the same paradigm except unpleasant IADS acoustic stimuli, i.e., tinnitus-like sounds, were introduced.

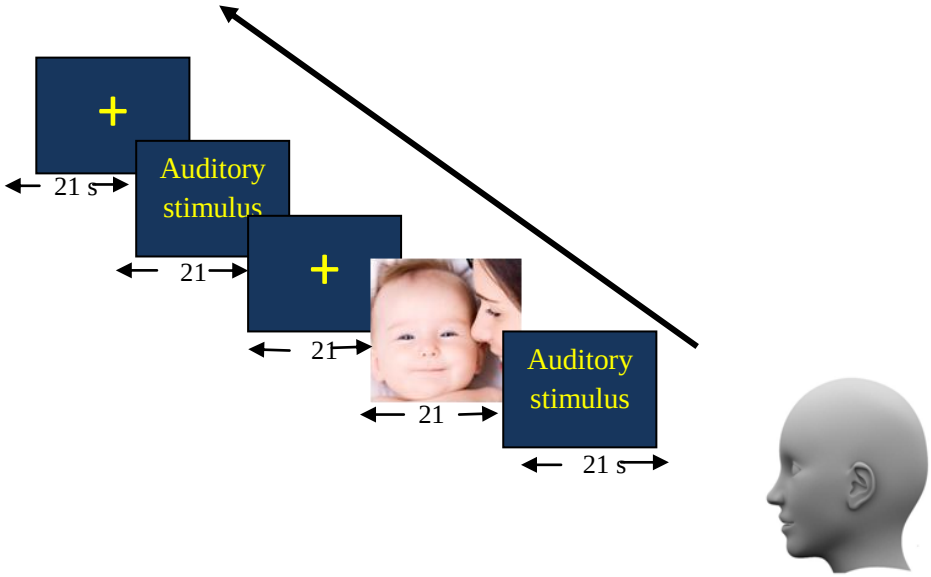


Figure 4.1 Schematic diagram of the experimental paradigm. Each block consisted of 7 stimuli, each lasting for 3 s, followed by a 21 s baseline condition in which a cross-hair was presented.

4.3.4 fMRI Acquisition

For spatial normalisation and localisation, T1-weighted anatomical images were acquired using the following acquisition parameters: a field of view (FOV) of 256 mm, a 1 mm slice thickness, a 2040 ms TR, a 5.57 TE, and a flip angle of 8°. The fMRI scan was performed using a Siemens 3T Trio MRI Scanner (Siemens, Germany). I applied an echo planar imaging (EPI) sequence using a repetition time (TR) and an echo time (TE) of 3000 and 30 ms, respectively. Each acquired volume consisted of 44 slices, with a slice thickness of 2.5 mm and a 0.5 mm gap between the slices. A flip angle of 90° was used. The FOV was 192 mm, and the matrix size was 64×64 mm, giving a voxel size of 3×3×3 mm. As noted above, I added a high-resolution T2-weighted sequence to rule out the presence of acoustic neuroma. The acquisition parameters were the following: a 0.5 mm slice thickness

without gapping between slices and a 750 ms TR. The Functional coverage is shown in Figure 4.3.

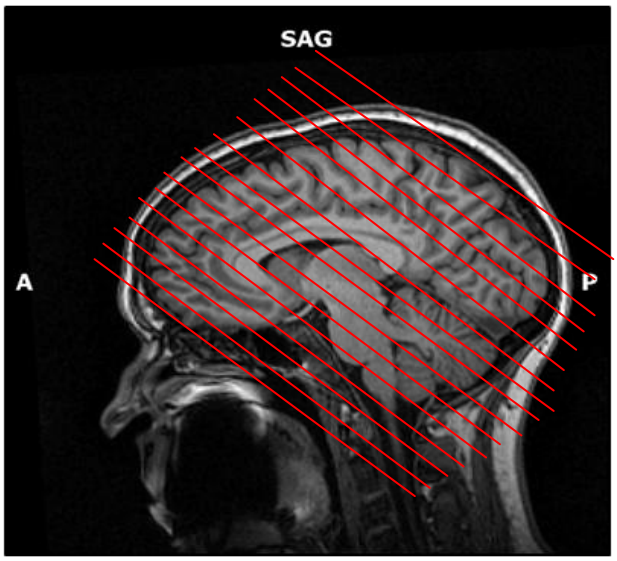


Figure 4.2 Functional coverage on a T1-weighted image. Each volume consisted of 44 slices with a slice thickness of 2.5 mm.

4.3.5 Data Analysis

The images that were obtained from fMRI were processed using the Brain Voyager QX software package (Brain Innovation, the Netherlands). All of the image data from the functional sessions for each subject underwent the following pre-processing steps: head motion correction was performed using trilinear/sinc interpolation by spatially aligning all of the acquired volumes to the first volume using rigid body transformations. All of the subjects exhibited acceptable degrees of head motion (within 1 mm). A slice-time correction was performed using cubic spline interpolation with an ascending slice scanning order. All of the functional images for each subject were spatially smoothed using a Gaussian filter of 6 mm FWHM. High-pass temporal filtering of 2 or fewer cycles was applied. The

anatomical images were transformed into 1×1×1 mm^3 isovoxels using sinc interpolation and then transformed to Talairach space as a preparatory step for the group analysis. For each of the functional sessions, a protocol file was created using BV to define each stimulus timing of the block design. The functional images were co-registered with the 3-D isovoxeled anatomical data and the volume time course (VTC) file. This file contained the complete 4-D functional data set. For the first level of analysis, the GLM was computed for each experiment, applying separate predictors for each subject. The statistical analysis on the group level was performed using random effect analysis (RFX) and a three-way analysis of variance (ANOVA) approach. A three-factor ANOVA was performed using repeated measures on factor A (auditory stimulus), factor B (visual stimulus) and factor C (between groups).

For the control group, who underwent the tinnitus-like perception experiment, a single-factor ANOVA within-subjects analysis was performed. The results of this analysis were compared with the results of the previous group analyses.

4.3.6 Correlation analysis

I examined the correlations between the mean fMRI BOLD signal and the hearing thresholds in both of the groups. The correlation between tinnitus severity as measured using the Newman THI score and the mean fMRI BOLD signal was also investigated. The correlation maps were produced using Brain Voyager software to identify the whole-brain regions that exhibited significant correlations between changes in the observed fMRI BOLD signals and the other measures. The maps were corrected for multiple comparisons using False Discovery rate (FDR) approach (Genovese, Lazar et al. 2002), with $p<0.05$. Following this step, I extracted the beta values and ran the correlation analysis externally.

4.4 Results

4.4.1 Demographics

Independent sample t-tests revealed no significant difference between the tinnitus sufferers and controls with respect to hearing threshold (HT) (right ear, t=0.2 p= 0.7; left ear, t=0.16, p= 0.87, degrees of freedom (df) = 31). The paired-sample t-test revealed no significant difference within the subjects with tinnitus between the HTs of the right and left ears (t=0.3, p= 0.7, df= 16). The statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois).

4.4.2 fMRI

4.4.2.1 Tinnitus versus controls

Unpleasant auditory stimuli: Two-sample t-tests revealed significantly (p_{FDR} <0.05) increased activation in the subjects with tinnitus compared to the controls in the bilateral amygdalae, primary auditory cortices (BA41) and superior temporal gyri. A significantly increased activation was observed in the subjects with tinnitus versus the controls in the right middle frontal gyrus (BA 46) and in the left inferior frontal gyrus. The activation map of the subjects with tinnitus contrasted with that of the control group following corrections for multiple comparisons p_{FDR}<0.05 (Genovese, Lazar et al. 2002) (see Figure 4.4). The detailed statistics regarding the active brain regions are given in Table 4.5.

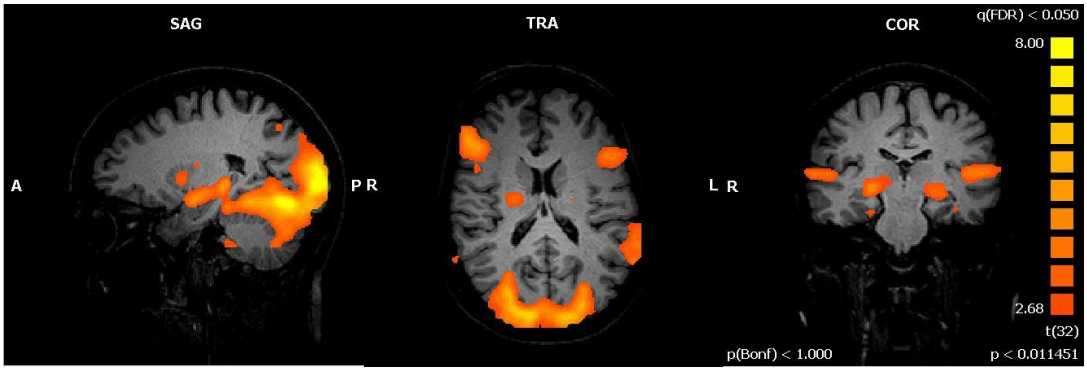


Figure 4.3. The subjects with tinnitus versus the control group ANOVA (t-test) for the unpleasant auditory stimuli. The activation map has been corrected for multiple comparisons, with $p_{FDR} < 0.05$.

Table 4.5. Significantly active brain regions based on an ANOVA (t-test) of the unpleasant auditory stimuli in patients with tinnitus in comparison with the control group. Whole-brain analyses were performed with $p_{FDR} < 0.05$. The volumes of interest were created from the significantly active clusters, considering only clusters that were larger than 200 contiguous voxels. The regions are presented in Talairach coordinates with their peak t and p values and cluster size in voxels.

brain region	peak voxels in Talairach coordinates			cluster size (voxels)	peak *t* value	p value	z score
	x	y	z				
right hemisphere							
amygdala	22	-6	-11	332	4.2	0.0002	4.3
primary auditory cortex (BA41)	55	-21	8	236	3.9	0.0004	3.7
superior temporal gyrus (BA22)	58	-22	9	467	3.6	0.0001	3.6
middle frontal gyrus (BA46)	52	29	20	1200	4.9	0.00002	4.9
left hemisphere							
amygdala	-26	-4	-11	289	5.5	0.000005	5.2
primary auditory cortex (BA41)	-46	-21	10	570	4.1	0.0002	4
superior temporal gyrus (BA22)	-44	-22	6	869	4.2	0.0001	4.3
inferior frontal gyrus	-50	19	20	707	3.6	0.001	3.7

Between-group within-stimulus interactions in experimental session 1: A repeated-measures ANOVA revealed a significant ($p_{FDR} < 0.05$) main effect of tinnitus in the left middle frontal gyrus ($F(2, 60)= 13.8$, $p<0.0008$), the left anterior cingulate ($F(2, 60)= 12.6$, $p<0.001$), the left precuneus ($F(2, 60)= 11.1$, $p < 0.002$), the right superior frontal gyrus ($F(2, 60)= 11.7$, $p < 0.003$), the right middle frontal gyrus ($F(2, 60)= 14.5$, $p < 0.0006$), the right cingulate gyrus ($F(2, 60)= 9.4$, $p< 0.004$) and the right precuneus ($F(2, 60)= 13.1$, $p <0.001$). The significantly active clusters were converted to volumes of interests, and detailed statistics regarding these regions were obtained. The patients with tinnitus exhibited significantly increased activation in all of these regions compared to the controls, except for the right and left precuneus, in which the controls exhibited significantly increased activation compared to the subjects with tinnitus. The detailed statistics regarding the significantly active clusters are listed in Table 4.6. The group interaction map was corrected for multiple comparisons using $p_{FDR}<0.05$ (Genovese, Lazar et al. 2002) and is shown in Figure 4.5.

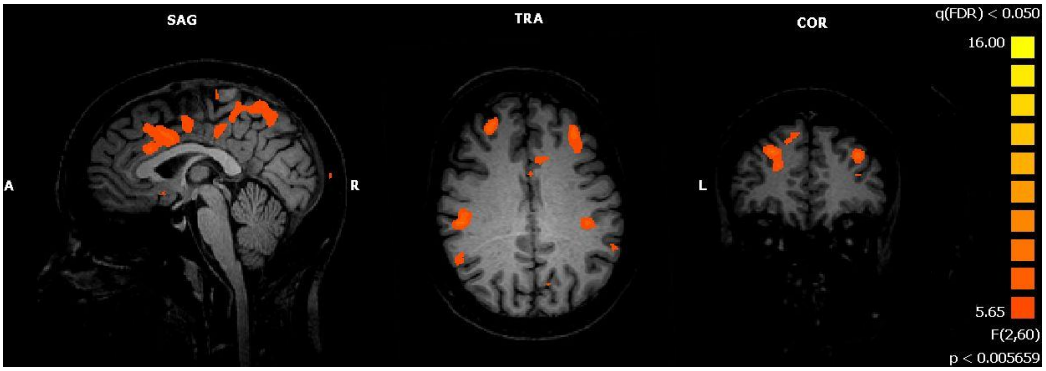


Figure 4.4. F-test map indicating significant within-stimuli group interactions in experimental session 1. The interaction map was corrected for multiple comparisons, applying P_{FDR} <0.05.

Table 4.6. Brain regions that exhibited significant within-stimuli group interactions in experimental session 1 in patients with tinnitus versus the healthy controls. The interaction map was corrected for multiple comparisons, with p_{FDR} <0.05. The volumes of interests were created from significant interaction clusters, considering only the clusters that were larger than 200 contiguous voxels. The regions are presented in Talairach coordinates with their peak t and p values and cluster size in voxels.

Region	Talairach coordinates			cluster size (voxels)	peak *t* value	p value	z score
	x	y	z				
patients>controls left middle frontal gyrus (BA8)	-24	19	47	435	3.5	0.001	3.6
left anterior cingulate (BA32)	-9	21	-6	346	4.04	0.0003	4
right superior frontal gyrus	20	19	49	278	3.9	0.0005	3.7
right middle frontal gyrus (BA10)	24	54	19	355	3.8	0.0006	3.8
right cingulate gyrus (BA23)	2	-8	40	309	3.9	0.0005	4
controls>patients left precuneus	-13	-66	35	466	3.5	0.001	3.6
left postcentral gyrus	-42	-26	30	809	4.3	0.0001	4.5
right precuneus	7	-60	35	556	4.3	0.00001	4.4
right precentral gyrus	45	-25	30	372	3.6	0.0002	3.2

Between-group within-stimuli interactions in experimental session 2: A repeated-measures ANOVA revealed a significant main effect of tinnitus in the left amygdala ($F(2, 60) = 13$, $p < 0.001$) and the right parahippocampal gyrus (BA34) ($F(2, 60) = 11.9$, $p < 0.001$). The patients with tinnitus exhibited increased

activation in both the left amygdala (t= 3.8, p < 0.0008, z= 3.9) and the right parahippocampal gyrus (t= 3.4, p < 0.002, z= 3.1). The interaction map is shown in Figure 4.6.

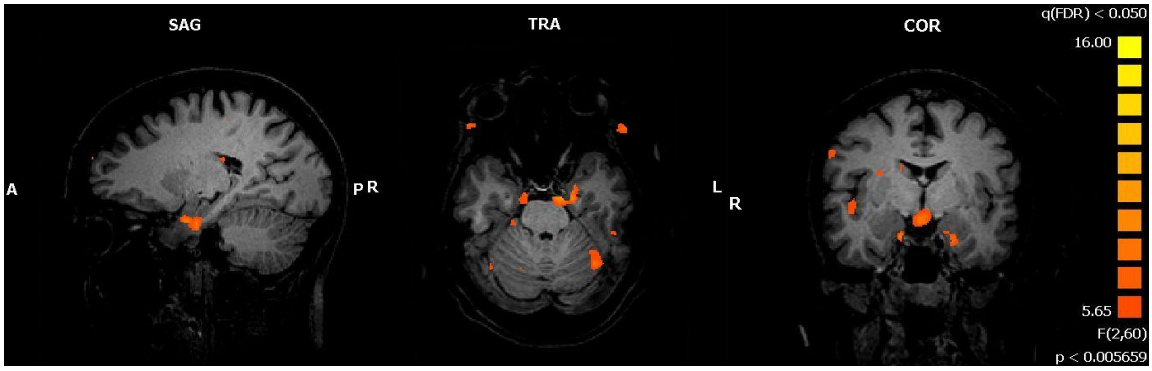


Figure 4.5. F-test map illustrating significant within-stimuli group interactions in experimental session 2. The interaction map was corrected for multiple comparisons, with P_{FDR} <0.05.

Baseline condition in experimental session 1: A repeated-measures ANOVA and t-tests of the baseline conditions revealed significantly (p_{FDR}<0.05) increased activation in the left middle frontal gyrus (t= 2.7, p <0.009, z= 2.5) in the subjects with tinnitus versus the controls.

Baseline condition in experimental session 2: A repeated-measures ANOVA and t-tests of the baseline conditions did not reveal any significant difference (p_{FDR}<0.05) between the subjects with tinnitus and the controls in experimental session 2.

Between groups analyses with the pleasant visual stimuli: A repeated-measures ANOVA revealed a significant difference (p_{FDR} <0.05) between the tinnitus and controls in the left-middle frontal gyrus (F (1, 31) = 12.6, p < 0.001), the left

cingulate gyrus (F (1, 31) = 13.8, p < 0.0008), the left parahippocampal gyrus (F(1, 31)= 10.8, p < 0.004), the left supramarginal gyrus (F(1, 31)= 11.8, p < 0.001), the right superior frontal gyrus (F (1, 31) = 16.6, p <0.0003), the right medial frontal gyrus (F(1, 31)= 11.1, p < 0.002) and the right postcentral gyrus (F(1,31)= 11.2, p <0.002). The between-groups analysis with respect to the pleasant visual stimuli activation map was corrected for multiple comparisons, with $p_{FDR} < 0.05$, and is shown in Figure 4.6. The detailed statistics regarding these regions are given in Table 4.7.

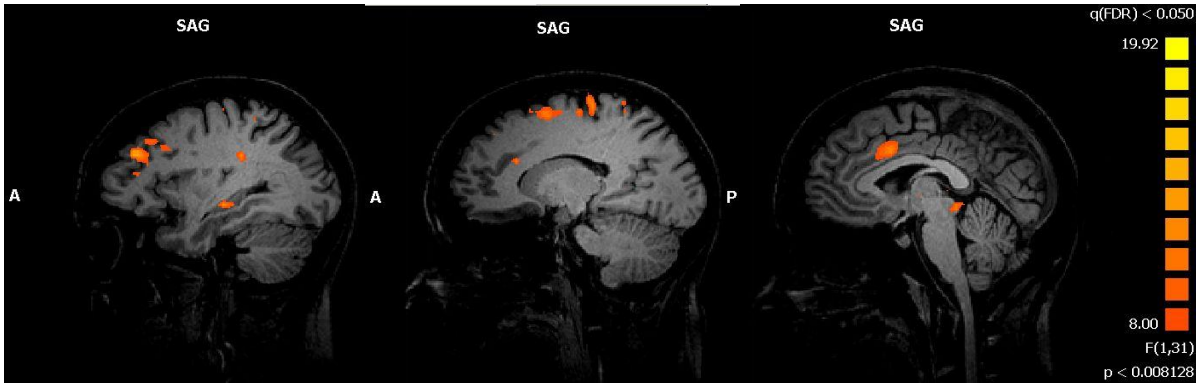


Figure 4.6. F-test map illustrating a significant difference between the tinnitus patients and the controls for the pleasant visual stimuli. The map was corrected for multiple comparisons, with P_{FDR} <0.05.

Table 4.7 The brain regions that exhibited significant difference between groups during the pleasant visual stimuli in patients with tinnitus versus the controls. The activation map was corrected for multiple comparisons, with p_{FDR} <0.05. The volumes of interest were created from significant active clusters, considering only clusters that were larger than 200 contiguous voxels. The regions are presented in Talairach coordinates with their peak t and p values and cluster size in voxels.

brain region	peak voxels in Talairach coordinates			cluster size (voxels)	peak *t* value	p value	z score
	x	y	z				
patients > controls left middle frontal gyrus	-29	38	28	480	3.7	0.0008	3.8
left cingulate gyrus (BA24)	-3	13	33	608	3.5	0.001	3.4
left parahippocampal gyrus	-21	-28	-10	280	4.02	0.0003	4
right superior frontal gyrus (BA9)	22	44	30	560	4.5	0.00008	4.5
right medial frontal gyrus	11	6	58	403	3.7	0.0009	3.6
controls > patients left supramarginal gyrus	-56	-48	29	603	3.4	0.003	3.4
right postcentral gyrus	39	-23	32	590	4.6	0.00005	4.3

Between groups analyses with the unpleasant visual stimuli: A repeated-measures ANOVA revealed a significant ($p_{FDR} < 0.05$) difference between the tinnitus patients and the controls in the left parahippocampal gyrus ($F(1, 31) = 9.7$, $p < 0.003$), the left middle frontal gyrus ($F(1, 31) = 11.7$, $p < 0.002$) and the right middle frontal gyrus ($F(1, 31) = 10.4$, $p < 0.002$). The between-groups activation map for the unpleasant visual stimuli is shown in Figure 4.7. The detailed statistics regarding these regions are given in Table 4.8.

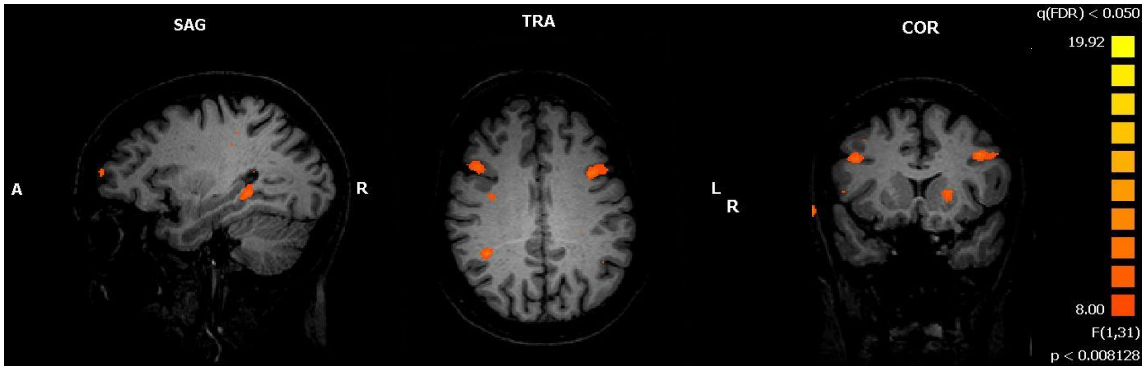


Figure 4.7. F-test maps indicating the significant difference between the tinnitus patients and the controls for the unpleasant visual stimuli. The activation map was corrected for multiple comparisons, with P_{FDR} <0.05.

Table 4.8. The brain regions that exhibited significant difference between groups during the unpleasant visual stimuli in patients with tinnitus versus the controls. The activation map was corrected for multiple comparisons, with p_{FDR} <0.05. The volumes of interest were created from significant active clusters, considering only clusters that were larger than 200 contiguous voxels. The regions are presented in Talairach coordinates with their peak t and p values and cluster size in voxels.

region	peak voxels in Talairach coordinates			cluster size (voxels)	peak *t* value	p value	z score
	x	y	z				
patients > controls left parahippocampal gyrus	-29	-36	-6	269	3.4	0.002	3.3
left middle frontal gyrus (BA9)	-39	15	30	349	4.5	0.0001	4.7
right middle frontal gyrus	42	14	30	312	5.6	0.00004	5.5

4.4.2.2 Tinnitus-like perception

As described above, I performed two experiments on healthy volunteers by having them listen to tinnitus-like sounds and simultaneously view pleasant or unpleasant images. I performed within-subject ANOVAs and t-tests on the brain activation data that were obtained during viewing of the pleasant and unpleasant IAPS images relative to the baseline condition in the following three groups: 1- healthy volunteers when they viewed pleasant and unpleasant images and listened to tinnitus-like sounds, 2- healthy volunteers when they viewed pleasant and unpleasant images with no auditory stimuli is present, and 3- patients with tinnitus when they viewed pleasant and unpleasant images. Pleasant images combined with tinnitus-like sounds yielded significant neural activity ($p_{FDR}<0.05$), primarily in the PFC, the limbic system, the auditory cortex and the temporal lobe. The details of the regions that exhibited significant activation are listed in Table 4.9.

Table 4.9. Tinnitus-like sounds combined with pleasant visual stimuli. The results of the within-subject ANOVA (stimuli > baseline condition) are shown, corrected for multiple comparisons with $p_{(FDR)}$ <0.05 and clusters ≥ contiguous 200 voxels.

Region	Talairach coordinates x	y	z	peak t value	cluster size (voxels)	p value	z score
left hemisphere hippocampus	-29	-20	-12	5.5	683	0.00004	5.5
amygdala	-23	-5	-13	7.5	890	0.000001	7.4
inferior frontal gyrus (BA47)	-33	30	-6	5.1	850	0.00002	5.2
superior temporal gyrus	-30	8	-20	4.8	614	0.00006	4.4
right hemisphere anterior cingulate (BA32)	5	34	-10	4.3	335	0.00005	4.3
cingulate gyrus	9	10	41	5.4	491	0.00003	5.5
amygdala	20	-5	-12	5.5	467	0.00004	5.5
parahippocampal gyrus	20	-2	-12	6.2	652	0.000001	6
inferior frontal gyrus	39	-1	30	6.1	1000	0.000002	6
temporal lobe	32	4	-29	6.5	680	0.0000008	6.6

When the healthy volunteers viewed the pleasant IAPS images without tinnitus-like sounds, a significantly increased activation ($p_{FDR} < 0.05$) was observed in the right superior and medial frontal gyri, the PFC, the anterior and posterior cingulate cortices and the temporal lobe. In the left hemisphere, significantly increased activation ($p_{FDR} < 0.05$) was observed in the superior and middle frontal gyri and the temporal lobe. The detailed statistics regarding these regions are given in Table 4.10.

Table 4.10. The results of the within-subject ANOVA for the controls (pleasant images > neutral condition), corrected for multiple comparisons, with $p_{(FDR)} < 0.05$ and clusters ≥ 200 voxels.

brain region	Talairach coordinates			cluster size (voxels)	peak *t* value	p	z score
	x	y	z				
left hemisphere							
temporal lobe (BA20)	-52	-4	-23	208	3.4	0.003	3.3
superior frontal gyrus	-28	39	27	388	3.6	0.0006	3.9
middle frontal gyrus	-20	39	27	281	4.7	0.0003	3.7
right hemisphere							
superior frontal gyrus	17	13	52	590	6.1	0.0008	4
medial frontal gyrus (BA10)	5	47	11	468	4.6	0.0005	3.9
PFC (BA8)	26	23	39	586	4.3	0.0003	3.9
anterior cingulate (BA24)	5	29	20	280	3.8	0.001	4.01
posterior cingulate gyrus (BA31)	5	-30	31	680	4.7	0.00004	5.5
temporal lobe	54	-18	-13	205	3.7	0.0002	4.8

The within-subjects ANOVA for tinnitus sufferers viewing the pleasant images revealed significantly ($p_{FDR} < 0.05$) increased activation in the left inferior frontal and medial gyri, the parahippocampal gyrus, the amygdala and the cingulate gyrus. In the right hemisphere, there was a significant increase in the BOLD signal in the posterior cingulate cortex, the middle and medial frontal gyri, the parahippocampal gyrus, the amygdala and the temporal lobe. Detailed statistics regarding these regions are given in Table 4.11. The activation maps for the following conditions are shown in Figure 4.9: (1) the tinnitus-like condition combined with the pleasant images in the control group, (2) patients with tinnitus when they viewed pleasant images, and (3) controls when they viewed pleasant images. All of the maps were corrected for multiple comparisons, with $p_{FDR} < 0.05$.

Table 4.11. The results of the within-subjects ANOVA for tinnitus sufferers (pleasant images > neutral condition), corrected for multiple comparisons, with $p_{(FDR)}$ <0.05 and clusters ≥ 200 voxels.

brain region	Talairach coordinates			cluster size (voxels)	peak *t* value	p	z score
	x	y	z				
left hemisphere							
inferior frontal gyrus	-42	9	27	708	5.2	0.00001	4.6
medial frontal gyrus	-3	-2	54	336	3	0.004	2.8
parahippocampal gyrus	-21	-5	-12	448	5.4	0.00005	5.9
amygdala	-25	-2	-14	499	5.6	0.00003	5.6
cingulate gyrus (BA24)	-5	3	34	249	3.2	0.003	2.8
right hemisphere							
posterior cingulate cortex	4	-48	11	675	4.3	0.0001	3.5
medial frontal gyrus (BA6)	4	2	55	508	3.5	0.001	3.5
middle frontal gyrus	48	4	38	690	6.9	0.000001	6.95
parahippocampal gyrus	24	-4	-11	550	7.55	0.000009	7.3
amygdala	25	-2	-13	344	5.3	0.00007	5.02
temporal lobe	31	-3	-24	467	4.7	0.00008	4.9

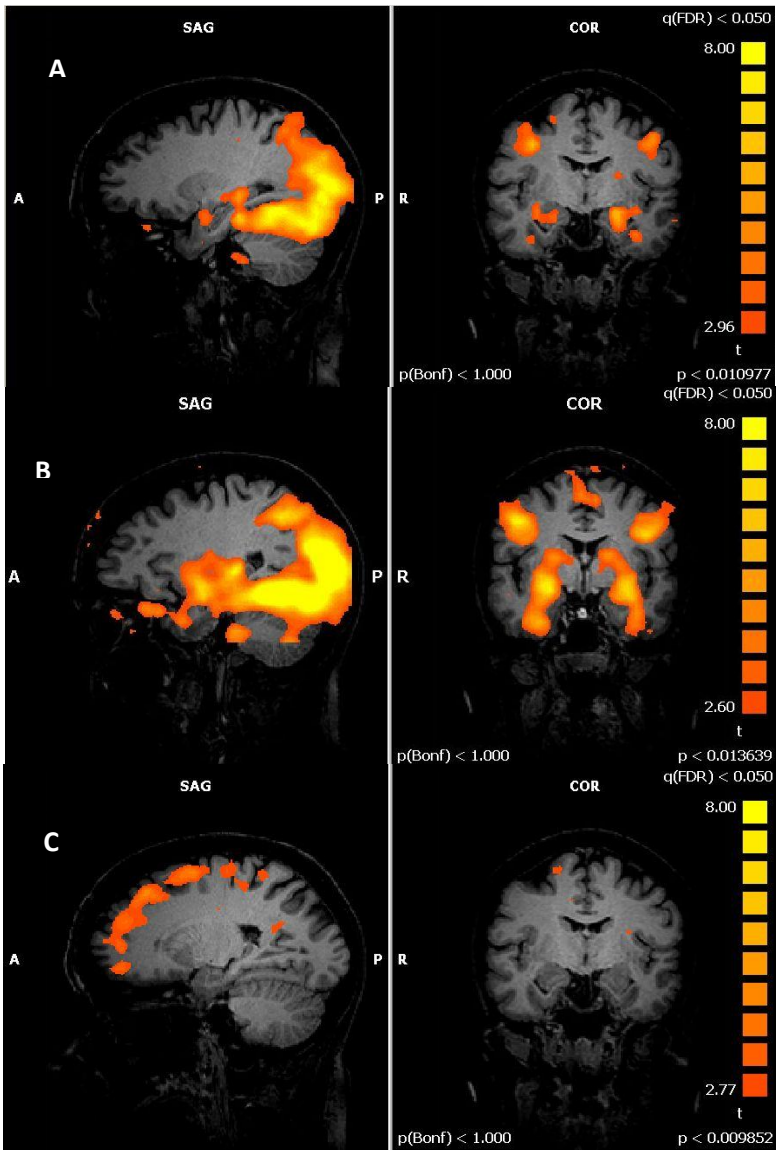


Figure 4.8. Within-subjects ANOVA, applying a contrast t-test, of pleasant images > neutral condition. (A) Activation map for the tinnitus-like condition in the healthy controls when combined with the pleasant images, (B) activation map for the patients with tinnitus while viewing the pleasant images and (C) activation map for the healthy controls while viewing the pleasant images only. All of the activation maps were corrected for multiple comparisons, with $p_{FDR} < 0.05$.

Processing both the unpleasant images and the tinnitus-like sounds involved significant activation in the limbic system, such as the hippocampus and the amygdala, in addition to the temporal lobe. Detailed statistics regarding these regions are presented in Table 4.12.

Table 4.12. Tinnitus-like sounds combined with unpleasant visual stimuli. The results of the within-group ANOVA for the control subjects (stimuli > neutral condition) are shown, corrected for multiple comparisons, with $p_{(FDR)} < 0.05$ and clusters ≥ 200 voxels.

brain region	Talairach coordinates			peak t value	cluster size in voxels	p value	z score
	x	y	z				
left hemisphere hippocampus	-28	-24	-9	5.4	498	0.00003	5.4
amygdala	-27	-5	-13	4.7	524	0.00006	4.4
parahippocampal gyrus (BA36)	-37	-26	-15	5.6	500	0.00001	5.6
right hemisphere hippocampus	31	-18	-12	5.2	320	0.00002	5
amygdala	20	-5	-12	5.5	467	0.00001	5.5
parahippocampal gyrus	20	-2	-12	6.2	787	0.000003	6.3
temporal lobe	32	4	-29	6.5	680	0.000001	6.2

When the controls viewed the unpleasant images with no acoustic stimuli, significantly ($p_{FDR} < 0.05$) increased bilateral activation was observed in the amygdala, the hippocampus, the parahippocampal gyrus, the fusiform gyrus and the inferior frontal gyrus. There was also increased activation in the left anterior

cingulate gyrus and the right temporal lobe. The detailed statistics regarding these regions are presented in Table 4.13.

Table 4.13. The results of the within-group ANOVA for the control subjects (unpleasant images > baseline condition), corrected for multiple comparisons, with $p_{(FDR)} < 0.05$ and clusters ≥ 200 voxels.

brain region	peak voxels in Talairach coordinates			cluster size in voxels	peak t value	p value	z score
	x	y	z				
left hemisphere							
amygdala	-25	-7	-11	464	6.9	0.000001	6.4
hippocampus	-27	-8	-15	388	4.2	0.00002	4.8
parahippocampal gyrus	-23	-38	-10	558	6.38	0.000001	7.1
fusiform gyrus	-41	-63	-13	1076	10.1	0.0000001	9.3
anterior cingulate gyrus (BA24)	-4	14	-10	466	4.2	0.00003	3.5
inferior frontal gyrus	-44	7	24	552	4	0.0003	4
right hemisphere							
amygdala	24	-6	-12	598	6.6	0.000003	6.2
hippocampus	27	-15	-11	639	5.1	0.000005	5
parahippocampal gyrus	26	-4	-11	778	8.8	0.0000004	7.7
fusiform gyrus	26	-64	-10	1055	9.5	0.0000002	9.47
temporal lobe	32	-4	-26	923	6.5	0.000006	5.8
inferior frontal gyrus	35	6	26	686	5	0.00007	5.1

A *t*-test analysis of the response to the unpleasant images relative to the baseline condition revealed significantly increased bilateral activation ($p_{FDR} < 0.05$) in subjects with tinnitus in the amygdala, hippocampus, parahippocampal gyrus, medial and inferior frontal gyri, and fusiform gyrus. I also observed increased activation in the left anterior cingulate gyrus and the right middle frontal gyrus.

The detailed statistics regarding these regions are given in Table 4.14. Figure 4.10 shows the activation maps for (1) the tinnitus-like condition combined with the unpleasant images in the control group, (2) patients with tinnitus when they viewed unpleasant images and (3) the controls when they viewed unpleasant images. All of the maps were corrected for multiple comparisons, with $p_{FDR} < 0.05$.

Table 4.14. The results of the within-group ANOVA for subjects with tinnitus (unpleasant images > baseline condition), corrected for multiple comparisons, with $p_{(FDR)} < 0.05$ and clusters ≥ 200 voxels.

brain region	peak voxels in Talairach coordinates			cluster size in voxels	peak t value	p value	z score
	x	y	z				
left hemisphere							
amygdala	-23	-6	-12	520	6.4	0.000004	6.4
hippocampus	-27	-12	-15	722	4.4	0.0001	4.4
parahippocampal gyrus	-22	-7	-15	336	6.5	0.000003	6.6
fusiform gyrus	-34	-33	-18	1050	12	$1x10^{-7}$	12.3
anterior cingulate gyrus (BA24)	-1	12	-9	358	4.7	0.00004	4.2
inferior frontal gyrus	-42	31	-4	636	4.1	0.0002	4.1
medial frontal gyrus	-1	35	-12	298	3.4	0.001	3.3
right hemisphere							
amygdala	23	-5	-12	466	6.8	0.0000007	6.86
hippocampus	28	-16	-11	523	5.5	0.000004	5.3
parahippocampal gyrus	31	-4	-19	722	4.5	0.00007	4.48
middle frontal gyrus (BA9)	42	19	30	336	8.5	0.00000008	8.8
inferior frontal gyrus	40	5	30	1162	8.1	0.0000005	8.2
medial frontal gyrus (BA6)	5	1	52	577	3.5	0.001	3.48
fusiform gyrus	39	-16	-23	609	4.3	0.0001	4.3

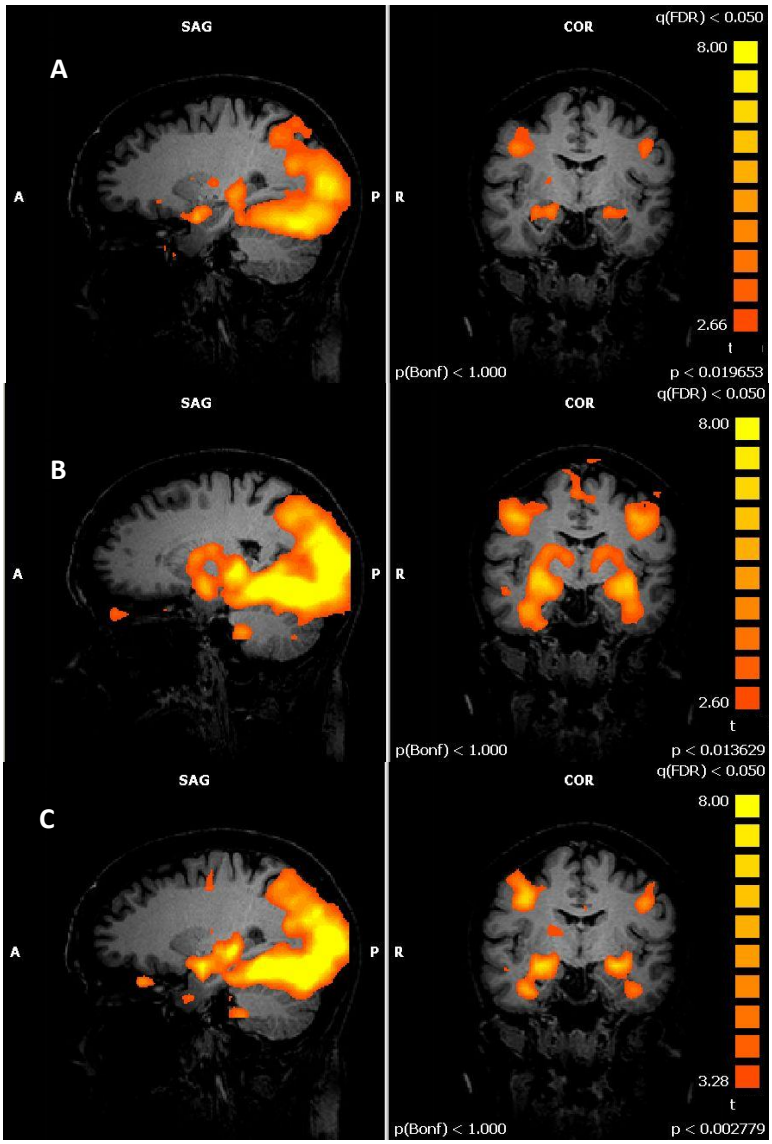


Figure 4.9. Within-group ANOVA, applying t contrast, of unpleasant images > baseline condition. (A) The activation map for the tinnitus-like condition in the healthy controls combined with unpleasant images, (B) the activation map for the patients with tinnitus while viewing the unpleasant images and (C) the activation map for the healthy controls while viewing the unpleasant images without acoustic stimuli. All of the activation maps were corrected for multiple comparisons, with $p_{FDR} < 0.05$.

4.4.3 Correlation analysis

I examined the correlations between (1) the changes in the fMRI BOLD signal in regions that exhibited significantly elevated activation in subjects with tinnitus, (2) the hearing thresholds of both ears, and (3) the severity of the tinnitus as measured using the Newman THI. Whole-brain maps were generated using Brain Voyager software to identify brain regions where these correlations were significant. The maps were corrected for multiple comparisons using an FDR approach at $p<0.05$. Following this step, I extracted the beta values and ran the correlation analysis externally. No correlations were observed between the observed changes in fMRI BOLD signal and hearing thresholds when the subjects viewed pleasant images and heard unpleasant sounds. However, when subjects viewed unpleasant images and heard unpleasant sounds, the following positive correlations were observed between changes in the fMRI signal and the hearing thresholds. A strong positive correlation ($r=0.7$, $p< 0.0002$ and $r= 0.63$, $p < 0.0003$ $df = 16$) was observed between the activation of the right cingulate gyrus and the right superior temporal gyrus ($r= 7$, $p < 0.0002$ and $r= 0.65$, $p < 0.00028$ $df= 16$) and the subjects' hearing threshold in the right and the left ear, respectively. Furthermore, a positive correlation was observed between changes in the fMRI BOLD signal in the right medial frontal gyrus ($r=0.6$, $p < 0.001$ $df= 16$) and the left superior temporal gyrus (BA22) ($r= 0.6$, $p <0.00035$ $df= 16$) and the mean hearing thresholds. I also observed that changes in the fMRI BOLD signal in the left inferior frontal gyrus correlated positively with subjects' hearing thresholds in the right and the left ears ($r=0.57$, $p < 0.0004$ and $r= 0.6$, $p < 0.0003$, respectively, $df = 16$). The correlations between the hearing thresholds and fMRI BOLD signal are illustrated in Figures 4.11, 4.12 and 4.13.

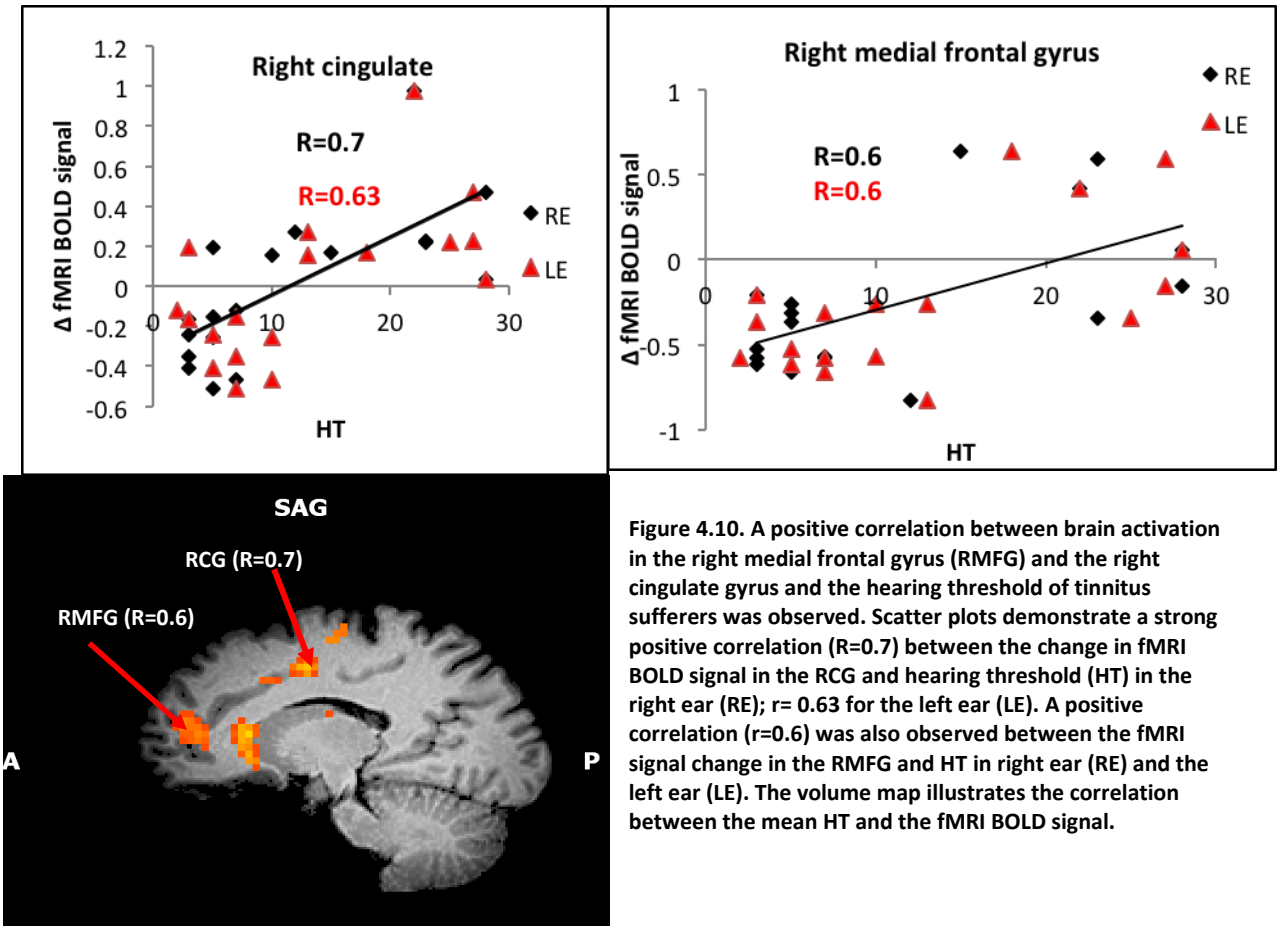


Figure 4.10. A positive correlation between brain activation in the right medial frontal gyrus (RMFG) and the right cingulate gyrus and the hearing threshold of tinnitus sufferers was observed. Scatter plots demonstrate a strong positive correlation (R=0.7) between the change in fMRI BOLD signal in the RCG and hearing threshold (HT) in the right ear (RE); r= 0.63 for the left ear (LE). A positive correlation (r=0.6) was also observed between the fMRI signal change in the RMFG and HT in right ear (RE) and the left ear (LE). The volume map illustrates the correlation between the mean HT and the fMRI BOLD signal.

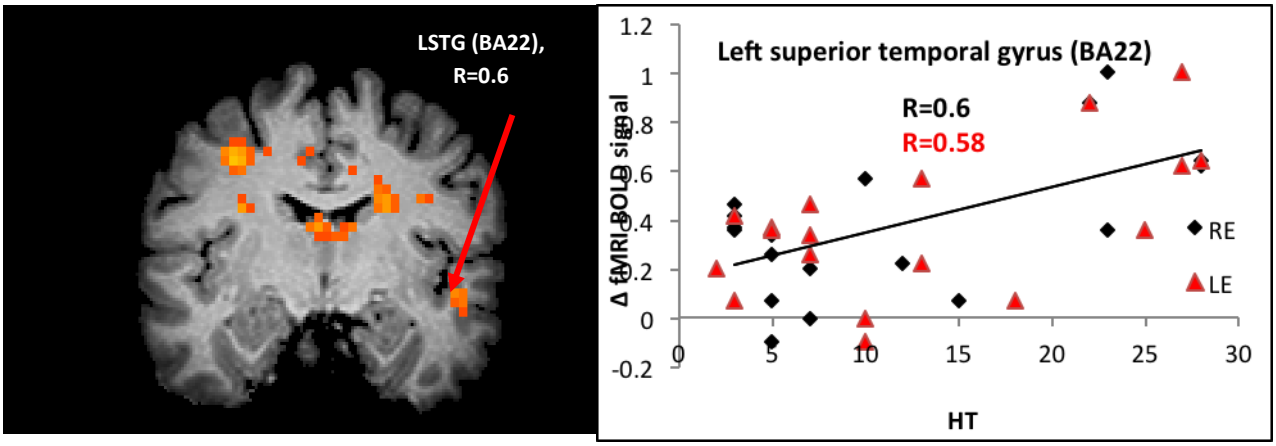
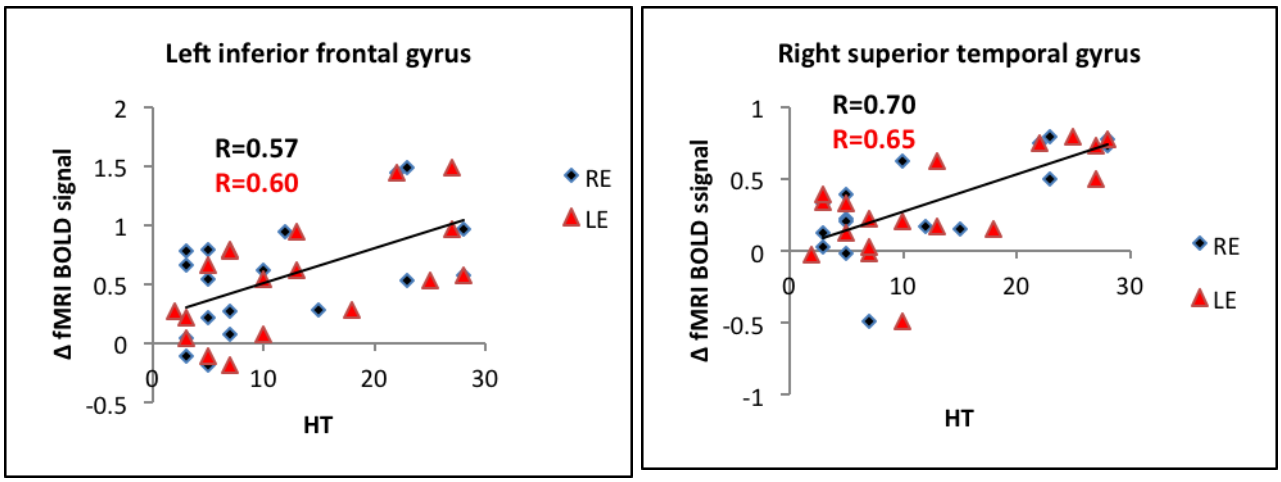


Figure 4.11. Correlation map overlaid with a T1 image showing a positive correlation between the fMRI BOLD signal in the left superior temporal gyrus (LSTG) and the hearing threshold (HT) in both ears. The scatter plot indicates the relationship between the extracted beta values from the LSTG and HT in subjects with tinnitus, with r=0.6 and 0.58 for the HT in the right ear (RE) and left ear (LE), respectively.



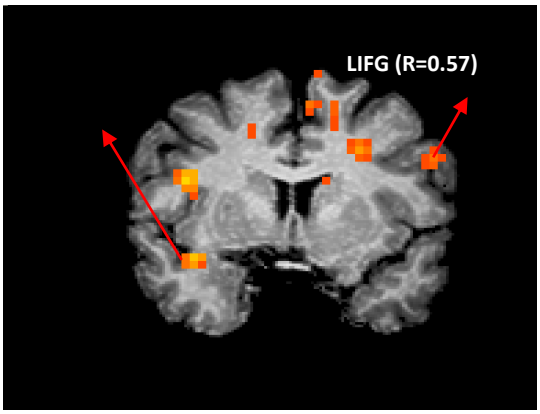
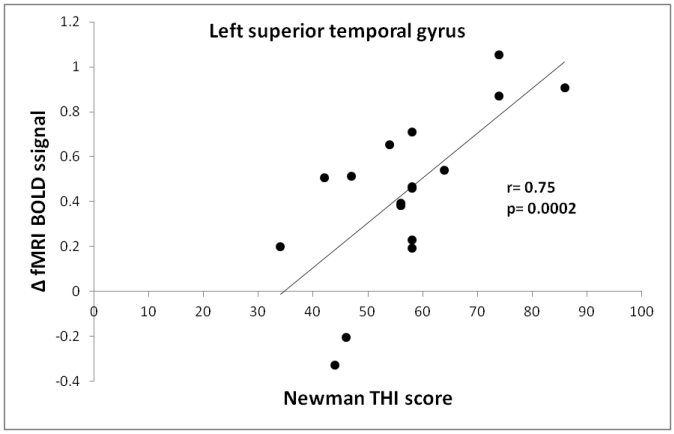
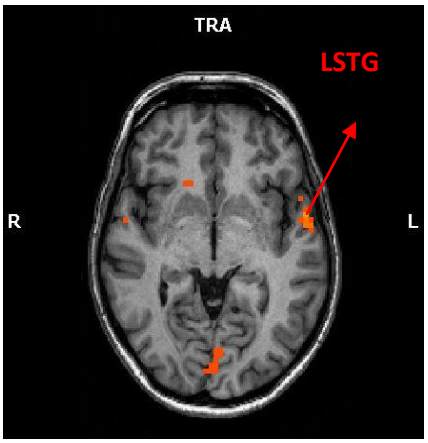
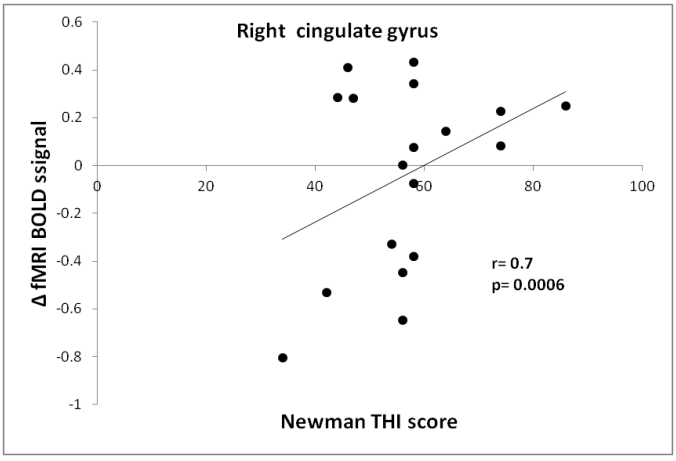


Figure 4.12. Correlation between brain activation in the right superior temporal gyrus (RSTG) and the left inferior frontal gyrus (LIFG) and the mean hearing threshold (HT) in subjects with tinnitus. A correlation map overlain on a T1 image reveals a positive correlation between the RSTG and LIFG and HT, with r=0.7, and 0.57, respectively. The scatter plots demonstrate the relationship between the extracted beta values and the HT in these regions.

A correlation analysis between the mean fMRI BOLD signal and the Newman THI score revealed a significant positive correlation ($p_{FDR} < 0.05$) between the severity of the tinnitus and the fMRI BOLD signal in the right cingulate gyrus (r= 0.7, p= 0.0006, df= 16) and the left superior temporal gyrus (r= 0.75, p= 0.0002, df= 16). The correlation between the mean fMRI BOLD signal and the Newman THI score is shown in Figure 4.14.







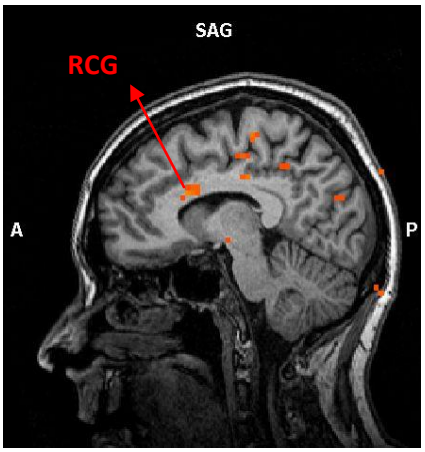


Figure 4.13 Significant ($p_{FDR} < 0.05$) positive correlation between the fMRI BOLD signal and the Newman THI score in the left superior temporal gyrus (LSTG) (top row) and the right cingulate gyrus (RCG) (bottom row).

4.6 Discussion

In the present study, emotionally evocative auditory and visual stimuli were employed for the first time in an fMRI study targeting patients with tinnitus. I hypothesised that tinnitus sufferers would exhibit increased activation in the auditory region, the PFC and the limbic system compared to controls. I utilised both pleasant and unpleasant emotionally evocative visual stimuli, whereas only unpleasant acoustic stimuli were applied. This protocol was chosen because tinnitus sufferers experience tinnitus sounds, which are considered to be "unpleasant", and employing pleasant IADS sounds would mask the tinnitus signal, making it impossible to specifically examine the effect of pleasant acoustic stimuli on tinnitus sufferers. In this chapter, it was revealed that patients with tinnitus exhibit hyperactivation near the PFC and in the limbic and auditory regions.

Elevated PFC activation in response to unpleasant auditory stimuli, as well as pleasant and unpleasant visual stimuli in patients with tinnitus compared to controls, supports Jastreboff's neurophysiological model of tinnitus (Jastreboff 1990). In this model, it is postulated that increased PFC activity enhances the responses of the autonomic nervous system to tinnitus perception and vice versa (Jastreboff 1990). The fundamental contribution of the PFC to behaviour, cognition and working memory is widely accepted (Miller and Cohen 2001). In addition to theorists' hypothesises concerning this crucial role of the PFC, functional neuroimaging studies (Wagner, Maril et al. 2001; Luna, Padmanabhan et al. 2010) have directly confirmed this role. The observed increased PFC activity in tinnitus sufferers in the present work is consistent with the findings of Mirz et al (2000) and Farhidi et al (2010), who both reported elevated PFC activity using

PET and SPECT imaging. The patients with tinnitus exhibited elevated activity in the PFC, reflecting an abnormality in this region that was not observed in controls. That is, hyperactivation in the PFC may increase the sufferer's awareness of their tinnitus given the presence of a positive feedback between the PFC and the autonomic nervous system (Jastreboff 1990; Jastreboff and Hazell 2004). The observations from tinnitus clinics show that the majority of tinnitus sufferers exhibit cognitive, attentional and memory-related deficits (Andersson 2002; Jastreboff and Hazell 2004). Hence, cognitive behavioural therapy (CBT) has been widely introduced as a treatment programme in tinnitus clinics and has achieved a certain degree of success (Jakes, Hallam et al. 1992; Andersson and Kaldo 2004). Tinnitus sufferers tend to concentrate on tinnitus sounds rather than on other aspects of their environment (Jastreboff and Hazell 2004). I believe that increased PFC activation may be associated with neural plasticity induced by over-stimulation of this region. Furthermore, I propose and that this over-stimulation is characterised by continuous attention of the patient to the tinnitus.

The amygdala, which is the most important structure of the limbic system, has been demonstrated to be involved in tinnitus. I observed significantly increased activation in the amygdala bilaterally in tinnitus sufferers when the participants listened to unpleasant sounds. When the participants viewed unpleasant images and listened to unpleasant sounds, increased activation was observed in the left amygdala in subjects with tinnitus compared to controls. Ridder et al (2006) reported the involvement of the amygdalohippocampal region in tinnitus perception via injections into the anterior choroidal artery, which is known to supply the amygdala and hippocampus. Following the injection, these authors reported 30% tinnitus suppression ipsilaterally and 70% contralaterally. The major

contribution of the amygdala in emotional processing and the acquisition of conditioned fear is well established. The amygdala does not function in isolation but, rather, receives sensory inputs from other cortical and neo-cortical regions, including the auditory system (LeDoux 1992; LeDoux 2000). With respect to the neurophysiological model of tinnitus, the auditory system is considered to be the source of the tinnitus signal (i.e., the stimulus). The rapid onset of the tinnitus signal that occurs in many patients is capable of creating a functional connectivity between the sender of the signal (i.e., the auditory cortex) and the receiver (i.e., the amygdala), despite this connection being maladaptive (Jastreboff and Hazell 2004). The excessive and pathological stimulus thereby causes increased activation in the amygdala, which, in turn, forms negative associations, including emotional stress, with the signal. In the current study, I highlighted this behaviour of the amygdala from a functional perspective. The lateralisation of the amygdalar response in experimental session 2 may not be of significance given that the statistical thresholding of the active voxels itself was not symmetric (Davidson and Irwin 1999); thus, I subjected whole-brain data to statistical analysis.

The hippocampus and the cingulate cortex, as components of the limbic system, were also demonstrated to be involved in tinnitus perception. These results are consistent with those of Mirz et al (2000). Lockwood et al (1998) also reported changes in cerebral blood flow in the hippocampal region when tinnitus sufferers performed oral-facial movements. Nevertheless, such findings are associated with sufferers who are capable of controlling the loudness of their tinnitus by performing oral facial movements. The generalisation of this observation is therefore limited to a subset of the tinnitus population. Findings from functional neuroimaging studies have reported the involvement of the hippocampus in PTSD

sufferers (Bremner, Staib et al. 1999; Brohawn, Offringa et al. 2010). Similarities in the neuro-functional mechanisms that underlie tinnitus and PTSD are widely accepted. In addition, sufferers of these conditions share common symptoms, such as anxiety and sleep disturbance (Jastreboff and Hazell 2004; Fagelson 2007; Møller, Langguth et al. 2010).

Predictably, tinnitus sufferers exhibited hyperactivation of the auditory cortex compared to the control group while hearing unpleasant sounds. It is believed that tinnitus pathogenesis primarily involves a dysfunction of the auditory system (Jastreboff 1990; Eggermont and Roberts 2004; Jastreboff and Hazell 2004). Muhinickel et al. (1998) reported tonotopic reorganisation in the auditory cortex in tinnitus, and this reorganisation was correlated with the severity of the condition. Numerous human neuroimaging studies have reported the involvement of the auditory system and its associative regions in tinnitus perception (Giraud, Chery-Croze et al. 1999; Mirz, Gjedde et al. 2000; Smits, Kovacs et al. 2007). Nevertheless, the exact nature of this contribution, the underlying mechanism and its position in the hierarchy of the factors that underlie tinnitus pathogenesis are largely uncharacterised. Although the current study focused on investigating the neural correlates of the emotional characteristics of tinnitus, the observed hyperactivation in the auditory cortex of tinnitus sufferers was unsurprising. In addition to the role of the auditory cortex in tinnitus perception and as a provider of the tinnitus signal (acoustic role) (Jastreboff and Hazell 2004), this brain region significantly contributes to the brain's emotional circuitry. This function is evidenced by the observation that sensory inputs from the auditory cortex terminate in the amygdala (LeDoux 2000). Furthermore, the auditory cortex is considered to be a source of emotional signals (Adolphs 2002). It has been

extensively argued that chronic tinnitus is conditioned by reorganisation of neural pathways in the auditory cortex (Rauschecker, Leaver et al. 2010); however, pre- and post-tinnitus perception investigations are required to determine whether this reorganisation causes tinnitus or vice versa. I believe that the hyperactivation of auditory-associative regions that was observed in the present analysis may indicate neural plasticity in the auditory cortex, which would be in agreement with Jastreboff's model.

Correlation analyses revealed a strong correlation between the fMRI BOLD signal and HT in patients with tinnitus. Significant positive correlations between changes in the fMRI BOLD signal and the HTs were observed in the right cingulate gyrus, the right and the left superior temporal gyrus, the right medial frontal gyrus and the left inferior frontal gyrus. Previous studies have demonstrated that there is a strong correlation between hearing loss and tinnitus perception (Axelsson and Ringdahl 1989). Although I did not include patients with tinnitus who also exhibited significant hearing loss, a subset of the subjects had hearing deficits. The subjects with higher HTs exhibited stronger changes in the fMRI BOLD signal, i.e., stronger activation compared to subjects who exhibited no or minor hearing deficits. It is accepted that fMRI, in its measurement of the haemodynamic response to a certain task, is an indirect measure of neuronal activity (Bandettini, Jesmanowicz et al. 1998; Attwell and Iadecola 2002). Therefore, I suggest that the hearing deficits that are associated with tinnitus may participate in the maladaptive neuroplasticity that underlies tinnitus pathogenesis.

Tinnitus severity, as measured using Newman THI scores, was also demonstrated to be positively correlated with the mean fMRI BOLD signal in the left superior temporal gyrus and the right cingulate gyrus. The patients with higher Newman

THI scores (i.e., higher tinnitus severity) exhibited higher functional reorganisation in these regions. The positive correlation between the severity of the tinnitus and functional brain reorganisation has been reported in several studies. Muhinickel et al. (1998) demonstrated a positive correlation between subjective tinnitus strength as assessed using the Multidimensional Tinnitus Inventory score and the degree of shift of the tinnitus frequency in the auditory cortex. Schecklmann et al. (2011) reported a positive correlation between tinnitus distress, as measured using the tinnitus questionnaire score, and bilateral brain activation in the posterior inferior temporal gyrus and the parahippocampal-hippocampal interface. In agreement with the current findings, Golm et al. (2012) reported a positive correlation between tinnitus distress, as measured using the Hospital Anxiety and Depression Scale, and the fMRI BOLD signal in the left temporal gyrus and the right anterior and mid-cingulate cortices. The positive correlations that were revealed in the present study confirm the role of the limbic system and the auditory region in tinnitus-related distress. It is possible that neural activity in these regions reflects the negative associations that characterise tinnitus perception. These findings support the role of the auditory and limbic regions in neuro-modulatory treatment approaches for tinnitus.

The perception of the combined tinnitus-like sounds and pleasant IAPS images in healthy volunteers revealed significant activation in regions that were similar to those observed to be activated in tinnitus sufferers, i.e., the amygdala and the hippocampal region. The amygdala and the hippocampal region exhibited increased activation when the healthy volunteers listened to tinnitus-like sounds and viewed pleasant images, and a similar activation pattern was observed when the patients with tinnitus viewed pleasant images. However, when the healthy

volunteers viewed the pleasant images in the absence of tinnitus-like sounds, the amygdala and the hippocampal region did not exhibit a significant hyperactivation. Tinnitus sounds may evoke activation in the limbic structures, suggesting that tinnitus with known origins (i.e., the induced condition) may have similar neural substrates as do tinnitus sounds of unknown origins (i.e., pathological tinnitus). The current results are in agreement with Mirz et al (2000), who induced a tinnitus-like condition in healthy volunteers using tinnitus-like sounds, which were based on sufferers' descriptions, and other aversive acoustic stimuli. Indeed, the results of the Mirz et al study are consistent with numerous neuroimaging studies (Lockwood, Salvi et al. 1998; Mirz, Gjedde et al. 2000) of tinnitus sufferers, including the current analysis.

Considering all of these findings, it is worth emphasising that tinnitus sufferers exhibited exaggerated brain activity, particularly in the limbic system, in response to emotional stimuli. Within-subject analyses of the control group revealed activation in the limbic system, thereby validating the fMRI paradigm and stimuli. However, comparison analyses revealed an overactivation of these regions in tinnitus sufferers relative to the control group, highlighting the role of neural plasticity in tinnitus pathogenesis. The clinical implication of the present study is the validation of fMRI as an objective diagnostic tool for tinnitus; I observed that this technique was able to differentiate between tinnitus sufferers and healthy controls. The follow-up of tinnitus sufferers using fMRI will make it possible to evaluate the efficacy of treatments by analysing pre- and post-treatment brain activation patterns.

5. Chapter 5. Diffusion Tensor Imaging

5.1 Aims and Objectives

The aim of study described in this chapter was to investigate the role of WM integrity in tinnitus pathophysiology using MR diffusion tensor imaging. The first objective was to investigate whether tinnitus sufferers exhibit differences compared to controls with respect to (1) white matter integrity, as measured using fractional anisotropy, and (2) mean diffusivity. The second objective was to investigate cortical connectivity in tinnitus sufferers by analysing the primary WM tracts. The third objective was to investigate the WM connectivity between the auditory regions, the amygdala and the hippocampus.

5.2 Introduction

Diffusion tensor imaging (DTI) is a non-invasive magnetic resonance imaging technique that is used to measure water molecule diffusivity in vivo. DTI measures the thermal, or Brownian, motion of water molecules in WM tracts and is expressed in units of mm^2/s. It is well established that water is the major component of WM and of the DTI signal (Agartz, Andersson et al. 2001; Johansen-Berg and Behrens 2009). One of the most informative measures that can be obtained by DTI is fractional anisotropy (FA). FA is based on the concept that water diffusion in WM is not identical in all directions, which describes a type of diffusion that is referred to as anisotropic. In contrast, the diffusion of water in the CSF, in which the water molecules move freely, is identical in all directions and is therefore referred to as isotropic (see Figure 5.1). In WM, diffusion is greatly affected by the orientation and the architectural features of the WM fibres (Pierpaoli, Jezzard et al. 1996; Le Bihan, Mangin et al. 2001). The FA of the WM is a representation of the incoherent directional distribution of molecular diffusivity of water molecules. FA values range from 0 to one; the closer the value is to one, the more anisotropic, i.e., the more unidirectional, the diffusion is

(Johansen-Berg and Behrens 2009). Anisotropy in WM has been observed in cats (Moseley, Cohen et al. 1989cited in; Chenevert, Brunberg et al. 1990) and in humans (Chenevert, Brunberg et al. 1990). Another metric that can be obtained with DTI is the apparent diffusion coefficient (ADC), or the mean diffusivity (MD), which quantifies the magnitude of water diffusion in a given tissue (Basser 1995).

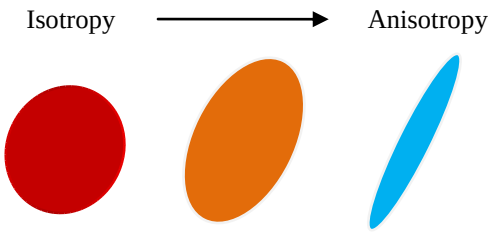


Figure 5.1 Diffusion ellipsoids with different degrees of isotropy. Red indicates isotropic diffusion, orange indicates a higher degree of anisotropy and blue indicates anisotropy (Johansen-Berg and Behrens 2009).

DTI has been widely used in clinical medicine. The clinical applications of DTI include the diagnosis ischemic stroke, brain trauma imaging, MS, psychiatric conditions and other neurodegenerative diseases (Schaefer, Grant et al. 2000). DTI has been implemented as a routine protocol in many clinics because of its advantages over other techniques in terms of disease diagnosis. Compared to conventional MRI, DTI can better differentiate dysmyelinating diseases, in which FA is observed, from demyelinating pathologies, in which no FA is observed (Sundgren, Dong et al. 2004). This sensitivity impacts the diagnosis of these pathologies and, therefore, the choice of treatment. However, DTI requires further improvements as well as the standardising of the post-processing analysis to

become a robust platform from which to address clinical and research-related questions.

DTI has been used to investigate the WM alterations that may be associated with aging or pathologies of the auditory system. Lutz et al. (2007) reported increased FA in the inferior colliculus and decreased FA in both the auditory radiation and the transverse temporal gyrus in elderly subjects relative to younger subjects. Reduced FA in the anterior thalamic radiation, the superior and inferior longitudinal fascicule, the corticospinal tract, the inferior fronto-occipital tract and the superior occipital fasciculus has been reported in patients who suffer from hearing loss (Husain, Medina et al. 2011). Furthermore, reduced FA in the inferior colliculus, the auditory radiation, the trapezoid body and the lateral lemniscus has been reported in sensorineural hearing loss (Chang, Lee et al. 2004). DTI was recently used to investigate the pathological mechanism of tinnitus perception, although this use has not yet been adopted for clinical applications. The first DTI study of patients with tinnitus was conducted by Don-Sik et al. (2006). In this study, the authors quantified the total WM volume in a group of tinnitus sufferers using DTI images. However, this study was limited because it lacked a comparable control group, making the findings very limited in their generalisability. Lee et al. (2007) observed reduced integrity of the WM that connects the auditory cortex with the frontal and parietal lobes in patients with tinnitus compared to a control group. The significant findings of these authors may be correlated with the negative associations of tinnitus, such as distress and cognitive deficits, which are regulated by the frontal cortex (Davidson and Irwin 1999). Crippa et al. (2010) reported increased FA in the WM pathway that connects the auditory cortex with the amygdala in tinnitus sufferers compared to

controls. Blast-induced tinnitus in rats was demonstrated to cause an increase in the FA and axial diffusivity in the inferior colliculus and an increase in the mean diffusivity of the medial geniculate body. These changes are believed to represent an underlying mechanism of blast-induced tinnitus and hearing loss (Mao, Pace et al. 2012). Additional studies of tinnitus perception, either involving animals or humans, must be performed using DTI, which is a relatively new technique, to better understand the relationship between cortical connectivity and tinnitus. The aim of the present study was to investigate whether WM is involved in tinnitus pathogenesis. I hypothesised that tinnitus sufferers would exhibit reduced integrity of the WM that connects the brain regions that are involved in attention, emotions, and auditory processing. In this chapter, I also used DTI to track WM connectivity between the auditory cortex (as a seed region) and the amygdala and the hippocampus (as target regions). Based on a tractography study by Crippa et al.(2010), I expected disrupted FA and MD of the pathway connecting the auditory cortex with the amygdala. Based on several preceding neuroimaging studies (Lockwood, Salvi et al. 1998; De Ridder, Fransen et al. 2006; Landgrebe, Langguth et al. 2009), I expected reduced integrity of the pathway connecting the auditory region with the hippocampus in tinnitus sufferers compared to the controls.

5.3 Materials and Methods

5.3.1 Subjects

For the subjects' characteristics and for the inclusion and exclusion criteria, see chapters 3 and 4. I included 17 patients with tinnitus (one tinnitus participant did not complete the DTI session) and 15 normal healthy controls in this study.

5.3.2 Image acquisition

A single-shot echo-planar imaging (EPI) sequence (TR=8000 ms, TE= 111 ms) was used. Diffusion gradients were applied in 60 directions (b=1000 s/mm^2), together with 5 non-diffusion acquisitions (b=0 s/mm^2). For each DTI scan, 50 axial slices were acquired using a slice thickness of 2.5 mm and no slice gapping.

5.3.3 Image analysis

The DTI data set was corrected for eddy current distortion and motion using FDT (FMRIB's toolbox) as part of the FMRIB Software Library, Oxford, UK (available free online at http://www.fmrib.ox.ac.uk/fsl/, release 4.1.3.). The brain extraction tool (BET) was used to remove extra cerebral tissues, such as the skull and the eyes. For the fractional anisotropy (FA) and mean diffusivity (MD) calculations, the DTI images were imported to Brain Voyager QX Software package version 2.2 (Brain Innovation, Maastricht, the Netherlands). First, the DT images were co-registered on a high-resolution 3-D volume that was acquired during the same session. This acquisition was performed by applying an affine alignment of 12 parameters. Next, a volume-diffusion-weighted (VDW) data set was created for each subject using sinc interpolation. The FA and MD maps were then constructed in native space after applying a brain mask to remove the noise from outside of the brain. As a preparatory step for the group analyses, the FA and MD maps were transformed into ACPC space and then into TAL space. The statistical analyses were performed using the following two approaches: (1) whole-brain analysis with $p_{FDR} < 0.05$ and (2) a threshold p value of <0.005 with a minimum cluster size of 10 voxels. For the latter approach, no correction for multiple comparisons was performed. Significant clusters based on the random effects analysis of variance (RFX ANOVA) calculations were labelled with

reference to the JHU-ICBM-DTI WM labels, which are part of FSL atlas tools (see Figure 5.2).

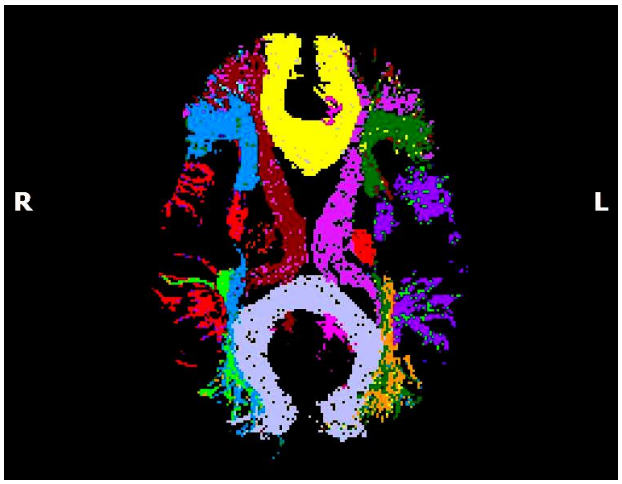


Figure 5.2 Primary white matter tracts, with significant clusters from the group comparisons labelled.

5.*3.4 Regions of interest analysis*

I investigated the WM connectivity between the auditory cortex and the amygdala and between the auditory cortex and the hippocampus. The auditory cortex ROI for each subject was manually selected using the functional MR data in sound minus baseline (sound-baseline) BOLD activation maps. The auditory cortex was used as a seeding point, and both the amygdala and the hippocampus were used as target points in each hemisphere. The mean FA and MD for the connectivity between the auditory cortex and the amygdala and between the auditory cortex and the hippocampus were calculated for each single path and for each subject. Region of interest tractography is demonstrated in Figure 5.3. The mean FA and MD for

each subject were exported to SPSS version 18.0 (SPSS Inc., Chicago, Illinois) for statistical analysis.

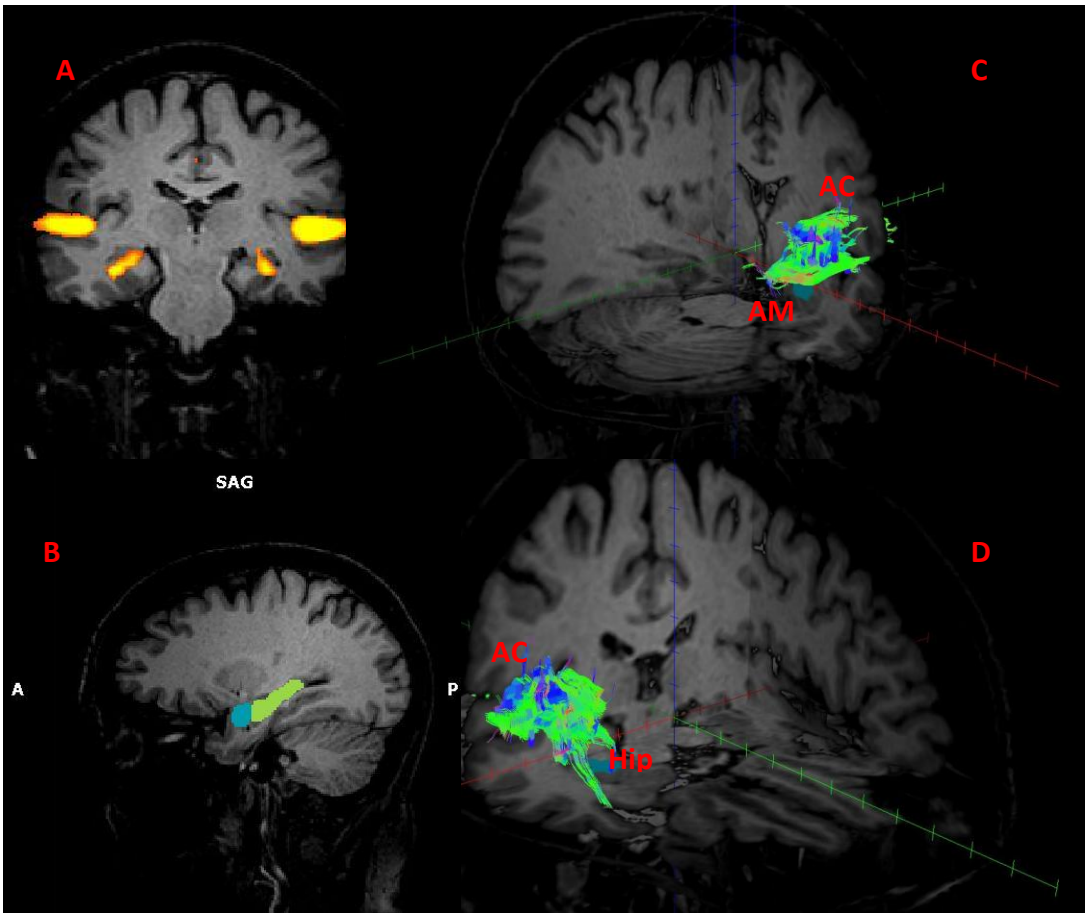


Figure 5.3. region of interest tractography. (A) the right and left auditory cortices were identified for each subject using the fMRI data and were employed as seed points. (B) The hippocampus (green) and the amygdala (blue) were employed as target points for each subject. (C) DTI tractography showing the fibres connecting the auditory cortex (AC) and the amygdala, (D) DTI tractography demonstrating the white matter connectivity between the auditory cortex (AC) and the hippocampus (Hip).

5.4 Results

5.4.1 Demographics

See chapter 4.

5.4.2 FA and MD

The whole-brain analyses, after being corrected for multiple comparisons and applying a false discovery rate statistical (FDR) approach, did not reveal any significant differences between the tinnitus sufferers and the controls.

With respect to the controls versus patients with tinnitus contrast *t*-test (controls > patients), which had a threshold of $p<0.005$ (uncorrected for multiple comparisons) with a minimum cluster size of 10 voxels, significant reductions in FA were observed in the following regions: the right PFC (inferior fronto-occipital fasciculus), the left inferior and superior longitudinal fasciculi, the corpus callosum, and the left anterior thalamic radiation (see Table 5.1 and Figure 5.3). We also observed an increase in the MD in the right anterior thalamic radiation and the left PFC (see Table 5.2). I ran a second contrast *t*-test, namely, patients > controls, applying the same statistical threshold. This analysis revealed a significant increase in the FA in the right inferior longitudinal fasciculus in the tinnitus patients (see Figure 5.4); however, no significant differences were observed in the MD in this contrast t-test.

Table 5.1 ANOVA RFX of the FA, controls>patients. The statistical threshold was set at p<0.005, uncorrected for multiple comparisons, with a minimum cluster size of 10 voxels.

Region	**Peak voxels in Talairach coordinates**				
	x	y	z	t	p
Right hemisphere					
PFC					
Inferior fronto-occipital fasciculus (medial frontal gyrus, BA9)	**4**	**55**	**14**	**3.9**	**0.0005**
Corpus callosum					
Body	**3**	**-15**	**25**	**3.7**	**0.0008**
Splenium	**3**	**-38**	**14**	**3.6**	**0.001**
Left hemisphere					
superior longitudinal fasciculus	**-44**	**-37**	**-13**	**4.8**	**0.00004**
inferior longitudinal fasciculus	**-37**	**-52**	**-11**	**4.3**	**0.0002**
Anterior thalamic radiation	**-26**	**-31**	**-1**	**4.1**	**0.0003**

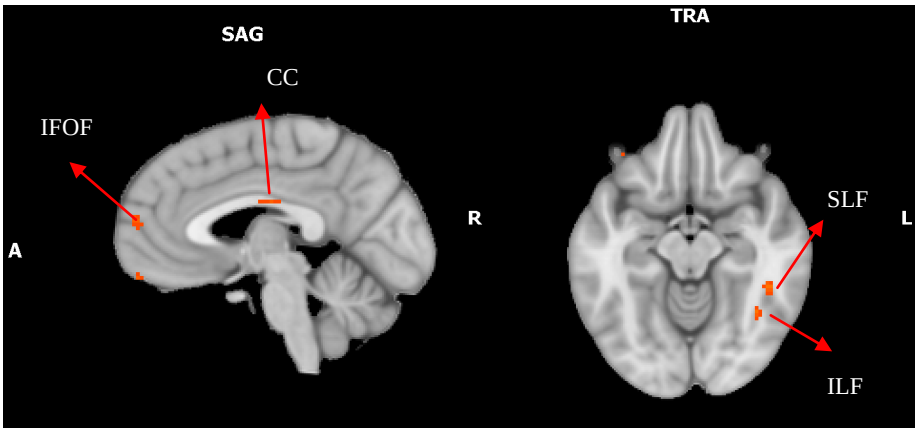


Figure 5.4 ANOVA RFX FA controls>patients. The statistical threshold was set at p<0.005, uncorrected for multiple comparisons, with minimum clusters of 10 mm^3. IFOF=inferior fronto-occipital fasciculus, cc= corpus callosum, SLF= superior longitudinal fasciculus, ILF= inferior longitudinal fasciculus.

Table 5.2 ANOVA RFX MD controls>patients. The statistical threshold was set at p<0.005, uncorrected for multiple comparisons, with a minimum cluster size of 10 voxels.

Region	**Peak Voxels in Talairach coordinates**				
	x	y	z	t	p
Right hemisphere Anterior Thalamic radiation	**3**	**-18**	**6**	**-3.4**	**0.002**
Left hemisphere Inferior fronto-occipital fasciculus	**-31**	**-32**	**1**	**-3.5**	**0.001**

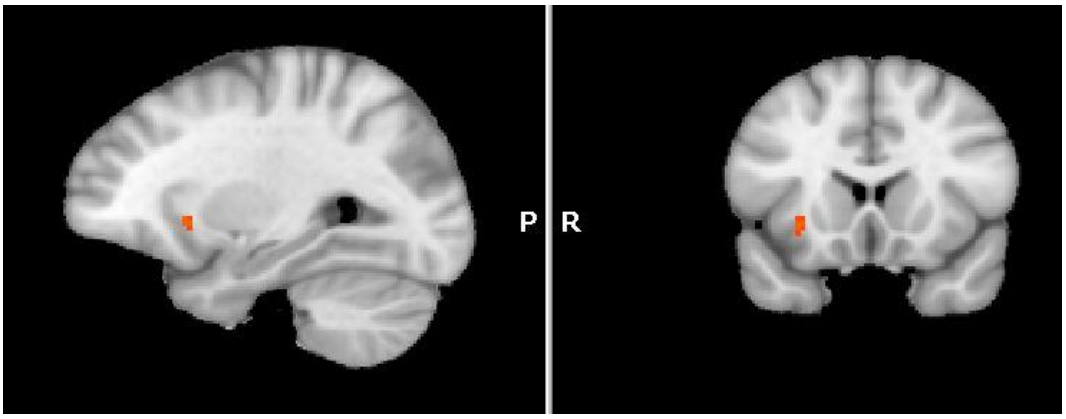


Figure 5.5 ANOVA RFX MD patients >controls. The statistical threshold set at p<0.005, uncorrected for multiple comparisons, with minimum cluster sizes of 10 contiguous voxels. Increased FA in subjects with tinnitus compared to the controls was observed in the right inferior longitudinal fasciculus.

The FDR statistical approach is effective in avoiding Type I errors, in which false-positive results are observed due to noise contribution (in neuroimaging studies). Alternatively, due the loss of sub-threshold voxels, this approach may be prohibitively conservative with respect to identifying a true effect (i.e., a Type II error) (Lieberman and Cunningham 2009). An activation likelihood estimation meta-analysis of four groups of functional neuroimaging studies reported closely matching results between statistical maps that were set at a threshold of $p < 0.0001$ and were uncorrected for multiple comparisons and maps made using the FDR

approach (Laird, Fox et al. 2005). Lieberman and Cunningham (2009) demonstrated that the use of an uncorrected p <0.005 with a cluster size of 10 voxels provides a significance level that is comparable to the FDR approach. The results of the uncorrected multiple-comparisons statistical approach have been analysed in a large body of literature (Muhlau, Rauschecker et al. 2006; Teipel, Stahl et al. 2007; Whitwell, Avula et al. 2010). The uncorrected statistical map, although it is statistically not significant, may provide qualitative information regarding certain neurodegenerative diseases at early stages (Ashburner, Csernansk et al. 2003). Hence, I applied the uncorrected statistical approach, with p <0.005 and with a cluster size > 10 voxels, to maximise the possibility of detecting subtle differences between the WM in patients with tinnitus and controls.

5.4.3 Region of interest analysis

Independent-samples *t*-tests did not reveal any significant differences ($p_{Bonferroni} < 0.05$) between the tinnitus and control group with respect to the FA of the WM between the right auditory cortex and either the right amygdala (t= -1.7, p= 0.4, df= 28) or the right hippocampus (t= -0.5, p= 2.5, df= 28). Similar findings were observed in the left hemisphere; i.e., no significant differences were observed in the FA of the WM between the auditory cortex and either the amygdala (t= -0.37, p= 2.85, df= 28) or the hippocampus (t= -1.6, p= 0.5, df= 28).

No significant difference was observed ($p_{Bonferroni} < 0.05$) between patients with tinnitus and controls with respect to the MD of the pathways that connect the right auditory cortex with either the right amygdala (t= 0.35, p= 2.9, df= 28) or the right hippocampus (t= 0.5, p= 2.4, df= 28). No significant differences were observed between patients with tinnitus and controls with respect to the MD of the pathways

that connect the left auditory cortex with either the left amygdala (t= 1.2, p= 0.9, df= 28) or the hippocampus (t= -0.882, p= 1.56, df= 28).

5.4.4 Correlations

No significant correlation was identified in the tinnitus patient group between the mean FA of the entire brain and any of the following metrics: age (r= 0.03, p= 2.8, df= 15), hearing threshold (r= 0.2, p= 2.6, df= 15), tinnitus duration (r=0.002, p=3.6, df= 15) or the severity of the tinnitus as measured using the Newman THI (r=0.01, p=3.2, df= 15). The whole-brain MD was not significantly correlated with any of the following metrics: age (r= 0.25, p= 0.4, df= 15), hearing threshold (r= 0.03, p= 1.2, df= 15), severity of the tinnitus (r= 0.035, p= 3.6, df= 15), or tinnitus duration (r= 0.05, p= 2.8, df= 15). All of the correlations were corrected for multiple comparisons by applying a Bonferroni correction with $p<0.05$. However, none of these correlations reached significance, even prior to correcting for multiple comparisons.

In the control group, no significant correlations (applying a Bonferroni correction) were observed between the FA and age (r= 0.2, p= 1, df= 13) or the mean hearing threshold (r= 0.07, p= 1.2, df= 13). No significant correlations were observed between the MD and age (r= 0.04, p=1.3, df= 13) or between MD and the hearing threshold (r= 0.1, p= 0.4, df= 13). None of these correlations reached significance when performed without corrections for multiple comparisons. The correlation analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois).

5.5 Discussion

In this DTI study, patients with tinnitus exhibited significant reductions in FA compared to the healthy controls in multiple brain regions, including the right anterior PFC (i.e., the inferior fronto-occipital fasciculus), the corpus callosum, the

left temporal lobe (i.e., the superior and inferior longitudinal fascicule), and the left anterior thalamic radiation. Compared to the healthy controls, the patients with tinnitus exhibited increased mean diffusivity in the right anterior thalamic radiation and the left inferior fronto-occipital fasciculus. Increased FA in the inferior longitudinal fasciculus was observed in patients with tinnitus compared to the healthy controls.

The underlying causes of reduced FA in WM tracts vary and may reflect any of the following processes: loss of connectivity between cortical regions, demyelination or dysmyelination of neuronal axons, changes in WM organisation, or changes in WM fibre architecture (Basser 1995). The underlying causes of increased FA are unknown; however, it is believed that there are certain factors that contribute to increases in FA, including increased myelination, deficits in axonal structure or decreased axonal diameter (Beaulieu 2002).

A deficit in the PFC WM integrity has been reported in major depressive disorder (MDD) (Li, Ma et al. 2007; Cullen, Klimes-Dougan et al. 2010) and attention deficit/hyperactivity disorder (ADHD) (Konrad, Dielentheis et al. 2010), which share some symptoms with tinnitus. These WM deficits in MDD and ADHD were characterised by a reduction in the FA, similar to what was observed in the present study. Jastreboff (1990) suggested the contribution of the PFC to tinnitus perception in his neurophysiological model of tinnitus. Many functional neuroimaging studies have reported abnormal activation in the PFC in tinnitus populations (Mirz, Gjedde et al. 2000; Farhadi, Mahmoudian et al. 2010). With regard to structural alterations that are hypothesised to be associated with tinnitus, Leaver et al. (2011) reported grey matter volume reductions in the PFC of tinnitus sufferers. The inferior fronto-occipital fasciculus (IFOF) consists of long

association fibres that connect the frontal and occipital lobes (Schmahmann and Pandya 2006). Anatomical studies have revealed that the IFOF connects to different cortices in its path between the frontal occipital lobes, including the temporal and parietal lobes (Martino, Brogna et al. 2010). Reduced FA and increased MD in the right and the left IFOF, respectively, were observed in tinnitus sufferers compared to the controls. Similar findings were reported in patients with bipolar disorder during episodes of depression (Zanetti, Jackowski et al. 2009). The observed reduction in white matter FA in the PFC may be an indication of a loss of WM integrity and connectivity with other cortical and neocortical regions.

The superior longitudinal fasciculus (SLF) connects the PFC with the parietal lobe, and reduced integrity of this connectivity may produce abnormal functioning of the connected cortices. My findings, although they differ with respect to the involved WM tract, are in agreement with those of Lee et al. (2007). These authors demonstrated reduced FA in the arcuate fasciculus, which connects the PFC with the temporal lobe (Schmahmann and Pandya 2006). Here, I report on the FA in the PFC-parietal WM tracts. The connectivity of the PFC with other cortices appears to be involved in tinnitus perception. Deficits in the SLF have been demonstrated to correlate with a deficit in working memory in patients with schizophrenia (Karlsgodt, van Erp et al. 2008). Apart from its executive and cognitive functions, the PFC has a major role in memory processing (Goldman-Rakic 1995; Rao, Williams et al. 1999). Defects in working memory have been reported in tinnitus sufferers (Rossiter, Stevens et al. 2006). When examining FA in the grey matter, I observed reduced FA in the approximate location of Brodmann area 9, which is located in the medial PFC. This region has been demonstrated to be connected

with the amygdala and to play a major role in emotions (Milad and Quirk 2002). These findings suggest that the reduced PFC connectivity may be responsible for the affected memory and negative emotions that are associated with tinnitus perception. Alternatively, the disrupted cortical connectivity may be the neural basis of tinnitus pathophysiology. Other effects of this disruption, including memory and emotional deficits, should also be noted.

The inferior longitudinal fasciculus (ILF), which exhibited reduced FA in the left hemisphere and increased FA in the right hemisphere in tinnitus sufferers, connects the occipital lobe with the anterior temporal lobe. The ILF is believed to allow for the rapid transfer of visual signals between the visual areas, the anterior temporal region, the parahippocampal gyrus and the amygdala in projection- or feedback-related processes (Catani, Jones et al. 2003). From this latter finding, it has been suggested that the ILF is responsible for the enhancement of emotionally significant visual stimuli via mediating the transfer of feedback visual signals from the amygdala to the early visual areas. Disrupted integrity of the connectivity between these regions may be responsible for the negative emotional aspects of tinnitus. Based on the negative emotions reported by tinnitus sufferers, Jastreboff (2004) suggested the involvement of the limbic system in tinnitus perception. The results from the current study support Jastreboff's theoretical model by demonstrating reduced integrity of the connective fibres that connect to the limbic system in tinnitus sufferers. This reduced integrity was characterised by both increased and decreased FA. These deficits may also underlie the pathological mechanism that makes tinnitus difficult to cope with emotionally.

Although this result was not predicted, the body and the splenium of the corpus callosum (CC) exhibited reduced FA in patients with tinnitus compared to the

controls. The corpus callosum is the largest white matter fibre and connects the two hemispheres. Reduced WM integrity of the CC has been reported in sufferers of emotional and cognitive deficits, such as bipolar disorder (Wang, Kalmar et al. 2008), ADHD (Cao, Sun et al. 2010) and PTSD (Jackowski, Douglas-Palumberi et al. 2008). The importance of the CC lies in its critical requirement for inter- and intra-hemispheric communication and the consequent integration of cognitive, emotional and motor functions. It has been proposed that there is a mirror negative image of each hemispheric region on the opposite hemisphere and that the CC connects these homologous areas in normal brains. That is, if a certain region in a given hemisphere is activated, the fibres of the CC will act to inhibit functional asymmetry in the opposite homotopic region. Studies of patients with "split brains" have reported that these patients demonstrate conceptual difficulties that are similar to patients with right hemisphere damage. The deficits that were due to right hemisphere damage have been shown to include attention and cognition (Cook 1986; Posner 1989), which are also associated with tinnitus. The reason for these deficits in split-brain patients is the absence of inhibition of the right hemisphere by the left, although the right hemisphere functions normally.

With regard to the pathophysiological mechanisms of tinnitus, the reduced integrity of the CC may cause an imbalance between the two hemispheres in terms of simultaneous excitation and inhibition (Cook 1986; Kitterle 1995). The body of the CC houses projections from motor, somatosensory and auditory regions. The splenium of the CC, which also exhibited reduced FA in subjects with tinnitus, contains fibres from the parieto-temporal and occipital regions (Witelson 1989; Witelson 1995; Bamiou, Sisodiya et al. 2007). The reduction in FA in the body of the CC that is exhibited by tinnitus sufferers may reflect a deficit in the callosal

fibres that connect the auditory regions of the two hemispheres. Therefore, the tinnitus signal may arise as a consequence of such disrupted connectivity.

The observed deficit in the splenium in the subjects with tinnitus may reflect a disrupted integrity of the projections that connect the bilateral temporal regions. The involvement of the temporal region in the context of tinnitus pathophysiology has been revealed in several neuroimaging studies (Mirz, Brahe Pedersen et al. 1999; Mirz, Gjedde et al. 2000; Farhadi, Mahmoudian et al. 2010). The role of interhemispheric connectivity in tinnitus perception has not been identified, and future studies are required to unmask the involvement of inter-hemispheric connectivity in tinnitus.

The anterior thalamic radiation (ATR) connects the PFC with the thalamus (Mori, Kaufmann et al. 2002). The observed reduced FA in the right ATR and increased MD in the left ATR reflect disrupted connectivity between the PFC and the thalamus. Muhlau et al. (2006) suggested the contribution of the posterior thalamus in tinnitus pathogenesis. Dysfunction in PFC-thalamus circuitry was observed to be involved in sufferers of schizophrenia with cognitive dysmetria, in which patients exhibit difficulties in coordinating the receipt, retrieval, processing and expression of information (Andreasen, O'Leary et al. 1996). The bilateral disruption in the integrity of the PFC-thalamus connection may be responsible for the cognitive, attentional and memory deficits observed in tinnitus sufferers who participated in this study. This conclusion can be drawn given that both of these regions have major roles in the integration of these functions (Portas, Rees et al. 1998; Van der Werf, Scheltens et al. 2003).

No significant correlations were observed between FA and MD and the following metrics: age, hearing thresholds, severity of the tinnitus, and tinnitus duration. The lack of a significant correlation between DTI measures and age in both groups may have been due to the relatively narrow age that was used for the tinnitus sufferers, which was between 30 and 60 years, which was matched by the controls. It was also clear that the tinnitus and the control groups did not exhibit hearing loss (see the inclusion and exclusion criteria in chapter 4), although certain deficits in hearing thresholds were present. In sensorineural hearing loss sufferers, the MD was not observed to be correlated with the severity of hearing impairment, although the FA was correlated with this metric (Lin, Wang et al. 2008). Excluding the participants with hearing loss from both groups may impact the FA and hearing thresholds correlation. The severity of tinnitus and its duration are self-determined quantities and are affected by the personal evaluation of the impact of the condition on a person's quality of life. Therefore, this score may vary among individuals (Nobel 2000). Tinnitus duration is based on a self-evaluation of when the tinnitus began to be an irritating phenomenon that caused the sufferer to seek medical assistance. Although there are many functional and structural studies regarding tinnitus perception, only Schecklmann et al. (2011) reported a positive correlation between tinnitus duration and metabolism in the ventromedial PFC and the posterior cingulate cortex.

I observed deficits in different WM tracts, most involving PFC connectivity. Such disrupted integrity in the tinnitus population most likely reflects a role for this condition in the emotional, attentional and cognitive negative associations that are characteristic of tinnitus. Alternatively, the observed deficit in the connectivity between the PFC and other cortices may be an underlying cause of tinnitus, which

would then lead to dysfunctional attention and emotions. Therefore, the tinnitus signal may arise as a consequence of increased or misdirected attention. This hypothesis is supported by the fact that certain individuals identify tinnitus as a problem and others do not (Jastreboff and Hazell 2004). It is difficult to verify what begins first: the tinnitus or the deficits in cortical connectivity. Analysing the effect of reduced brain WM integrity in animals, particularly the connectivity between the PFC and other cortices, would be an ideal approach to addressing this question. The current study, although it provides evidence regarding the involvement of WM integrity in tinnitus, is limited by the small number of recruited patients. This limitation may also explain why no differences were observed when applying the FDR statistical approach (another limitation), which is a more conservative approach but has more statistical power. Further DTI studies of tinnitus sufferers, including studies with larger sample sizes, are required to investigate the possible role of cortical connectivity in tinnitus pathophysiology. The clinical impact of such studies would aid in the better understanding of the neural mechanism of tinnitus induction and would therefore aid in the identification of a cure or the improvement of current treatment strategies.

6. Chapter 6. Cortical Thickness Analysis and Voxel-Based Morphometry

6.1 Aims and Objectives

The aim of the study described in this chapter was to investigate the neural correlates of tinnitus perception in terms of structural alterations using high-resolution MRI and semi-automated computational algorithms. The first objective was to determine whether tinnitus sufferers exhibit cortical thickness alterations compared to the control group. The second objective was to investigate whether tinnitus perception involves structural changes, specifically in terms of grey matter alterations, via cortical thickness analyses and voxel-based morphometry. Third, I investigated whether patients with tinnitus exhibit cortical thickness and grey matter volume changes in the PFC, auditory region and limbic system.

6.2 Introduction

Measuring the thickness of the human cerebral cortex was at one time only possible by post-mortem examination. However, advances in MRI image reconstruction techniques and imaging sequences, which allow for high-resolution images, permit the non-invasive estimation of human cerebral cortical thickness. Cortical thickness analysis (CTA) is a new technique that provides quantitative measures of cortical morphology by measuring the distance between the WM and the grey matter cerebrospinal fluid (CSF) boundaries (Lerch and Evans 2005). Previously, the determination of cortical thickness was based on a skilled radiologist's determination of the three-dimensional thickness based on two-dimensional images. However, this method is considered less accurate as it requires estimating the intersecting imaging plane and the plane that is tangential to the cortex, neither of which are the same across the entire cortex. The cerebral cortex is highly folded such that the folds are not aligned with the core axes by

which the slice data are viewed (Fischl and Dale 2000; Jones, Buchbinder et al. 2000).

Recent advances in computational algorithms and the development of powerful computer-aided high-resolution MR image analysis techniques have made cortical thickness measurements more reliable and valid. Current computational algorithms primarily rely on generating surface representations of grey/white matter and the pial surface when measuring cortical thickness. Each point of the cortical representation is associated with a path-length correspondence that is retrieved from the subject's cortex. The measured cortical thickness reflects the distance between the grey/white matter and the pial surfaces. Computational algorithms have made it possible to measure the cortical thickness of the entire brain at any point with sufficient accuracy (Fischl and Dale 2000; Jones, Buchbinder et al. 2000; Kabani, Le Goualher et al. 2001). Cortical thickness measurements of normal human brains that were made using automatic computational algorithms were compared with thicknesses that were obtained using post-mortem analyses, and significant agreement between these two measures was observed, validating the computational techniques (Fischl and Dale 2000).

CTA is of great interest to the neuroscience community because it allows for the understanding of normal development and of other neurodegenerative diseases. Specifically, it can be determined whether such conditions are related to normal aging or to psychiatric disorders. Variations in cortical thickness may underlie certain pathological or physiological alterations. For example, intellectual abilities have been determined to be positively correlated with cortical thickness in adolescents and healthy adults, particularly in temporal and prefrontal regions

(Shaw, Greenstein et al. 2006; Narr, Woods et al. 2007). In subjects with schizophrenia, cortical thinning was reported in the frontal and temporal regions (Narr, Bilder et al. 2005). Regional cortical thickness changes were also reported in normal aging (Salat, Buckner et al. 2004), Alzheimer's disease (Dickerson, Bakkour et al. 2009) and bipolar disorder (Lyoo, Sung et al. 2006). A clinical value of cortical thickness determination would be achieved if such differences could be linked to neurodegenerative disorders in their early stages (Jones, Buchbinder et al. 2000).

To our knowledge, no study has been performed to investigate cortical thickness in a tinnitus population. However, a small number of studies have used VBM to identify structural alterations of the grey matter of tinnitus suffers. These grey matter alterations were reported in the auditory, PFC and limbic regions (Muhlau, Rauschecker et al. 2006; Leaver, Renier et al. 2011). VBM is an unbiased technique for the quantification of regional cerebral volume and the amount of tissue, such as a grey matter, in a given volume (Good, Johnsrude et al. 2001). For the first time, I used CTA, which is a relatively new technique, in combination with VBM to investigate cortical thickness and grey matter volume in patients with tinnitus compared with age- and sex-matched controls. I hypothesised that tinnitus sufferers would exhibit cortical thickness and grey matter alterations in the frontal, limbic and temporal regions and that these alterations would be associated with hearing deficits.

6.3 Materials and Methods

6.3.1 Subjects

For the subjects' characteristics and for the inclusion and the exclusion criteria, see chapters 3 and 4. One tinnitus subject did not attend the structural scan, and

one tinnitus subject was excluded due to a noisy image. One subject from the control group was also excluded due to a noisy image. Thus, I included 16 of the 18 tinnitus sufferers and 14 of the 15 healthy controls in the CTA study.

6.3.2 Image acquisition

Three-dimensional high-resolution structural MR volumes were acquired using a Symphony 1.5 T whole-body MRI imaging system (Siemens, Magnetom, Germany). The acquisition parameters were the following: 256 mm FOV, an 8° flip angle, a slice thickness of 1 mm, and a matrix size of 256×256 mm. The voxel size was 1×1×1 mm. The TR and the TE were 1660 and 3.04 ms, respectively.

6.3.3 Image analysis

6.3.3.1 CTA

The image analysis was performed using the Brain Voyager QX software package V2.2 (Brain Innovation, Maastricht, the Netherlands). The T1-weighted images were corrected for inhomogeneities to improve brain segmentation. All of the anatomical data were transformed to ACPC and then to Talairach (TAL) standard space as a preparatory step for the pre-processing pipeline of the CTA. The TAL images were transformed to a resolution of 0.5×0.5×0.5 mm using sinc interpolation. Next, the brains were "peeled" from surrounding head tissue using the advanced segmentation tools available in Brain Voyager. The cerebellum and the subcortical structures were removed, and tissue contrast and homogeneity were enhanced using a sigma filter. The white matter-grey matter boundary and grey matter-cerebrospinal fluid (CSF) boundary were segmented and polished by computing the magnitude map, which is based on the calculated gradient maps of the binary segmentation results. The cortical thickness volume maps were calculated for every subject. Because it is not possible to perform group analyses

based on cortical thickness volume maps, reconstructed folded cortical representations for each subject were created. To improve the spatial correspondence between the different subjects and different populations, the reconstructed folded cortical representations (meshes) for each subject and each hemisphere were cortically aligned, matching the macro-anatomical structures, such as the gyri and the sulci. The cortical thickness then was calculated for each cortically aligned mesh for each subject.

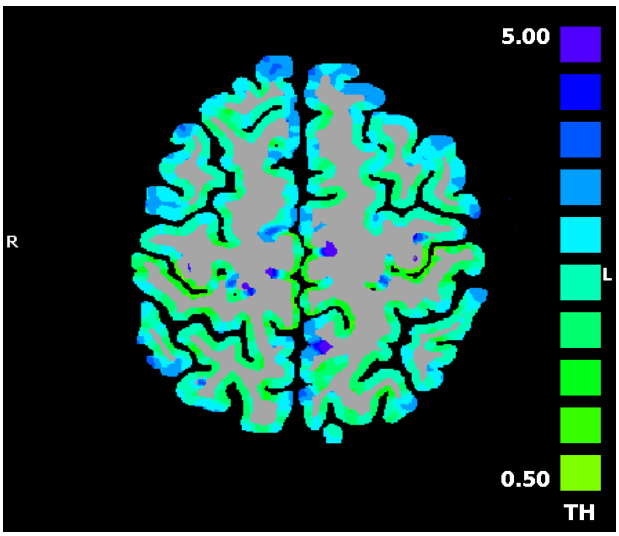


Figure 6.1 Cortical thickness overlain on a volume map. The colour bar indicates thickness in mm.

Whole-brain approaches were applied, which were corrected for multiple comparisons, with a false discovery rate at $p<0.05$ and a priori-defined regions of interest (ROIs). The brain atlas that was provided by Brain Voyager QX (Goebel, Esposito et al. 2006), which contained the four defined lobes (i.e., frontal, temporal, occipital and parietal) was used for these analyses. Of these four lobes, only the bilateral frontal and temporal lobes were investigated (see Figure 6.2). The ROIs included (bilaterally) the superior frontal gyri, middle frontal gyri,

inferior frontal gyri, superior temporal gyri, middle temporal gyri, inferior temporal gyri, anterior cingulate cortex, cingulate gyri, posterior cingulate cortex, and primary auditory cortex (BA41) (see Figure 6.3). The selection of the lobes and ROIs was based on previous fMRI and VBM studies of tinnitus (Mirz, Gjedde et al. 2000; Landgrebe, Langguth et al. 2009; Leaver, Renier et al. 2011).

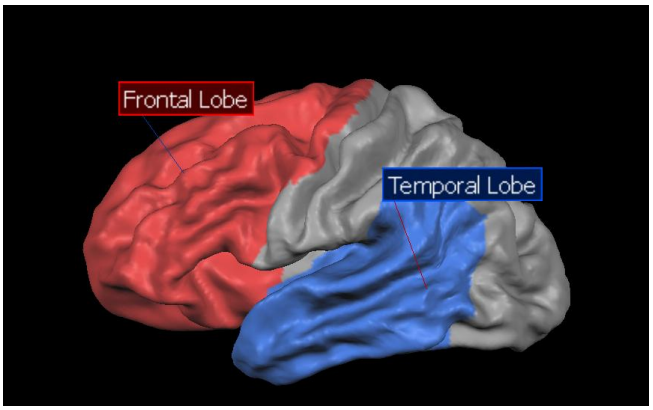


Figure 6.2 Regions of interest. This figure shows the selected lobes in both hemispheres. The frontal lobe is shown in red, and the temporal lobe is shown in blue.

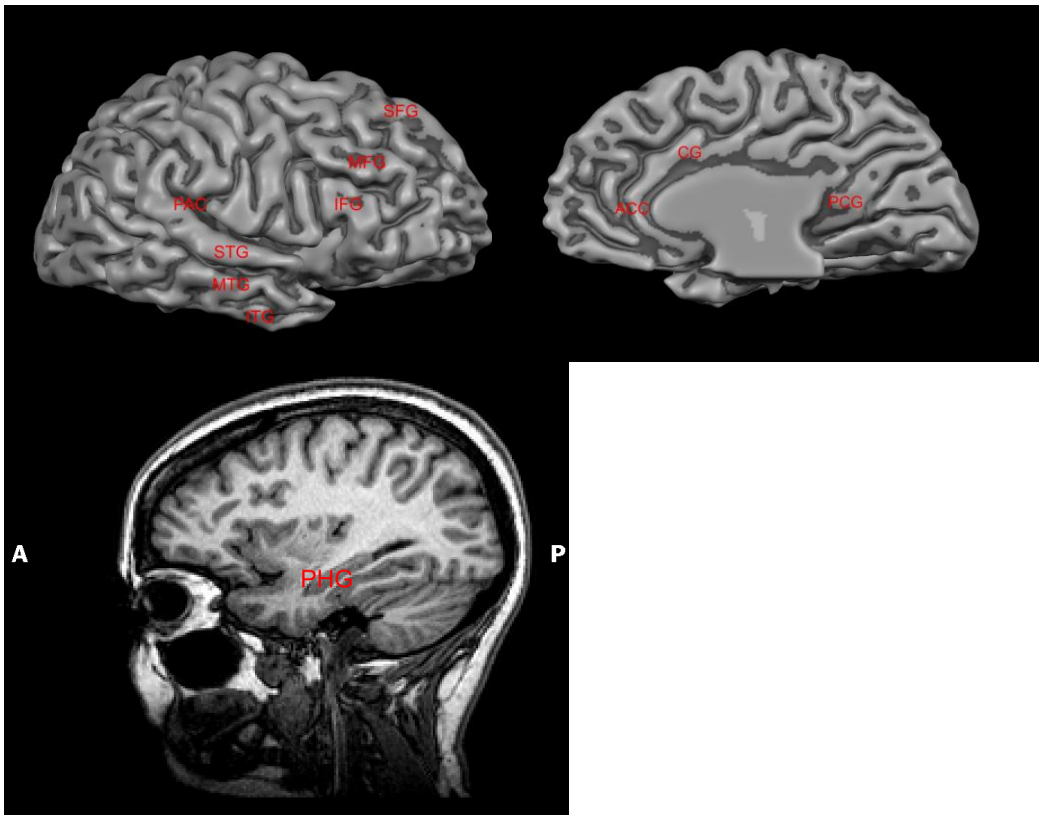


Figure 6.3 Regions of interest. This figure shows the a priori-hypothesised ROIs in both hemispheres and illustrates the right hemisphere only for purposes of visualisation. The included ROIs (bilaterally) are the following: the superior frontal gyrus (SFG), middle frontal gyrus (MFG), inferior frontal gyrus (IFG), superior temporal gyrus (STG), middle temporal gyrus (MTG), inferior temporal gyrus (ITG), primary auditory cortex (PAC), anterior cingulate cortex (ACC), cingulate gyrus (CG), posterior cingulate gyrus (PCG), and parahippocampal gyrus (PHG).

6.3.3.2 VBM

The MRI data were processed using Statistical Parametric Mapping software (SPM8) (Wellcome Department of Cognitive Neurology, London, UK) and its VBM8 toolbox, which are available at http://dbm.neuro.uni-jena.de/vbm. These programs were run on Matlab version 7.9 (The Mathworks, Inc., Natick, MA, USA). The pre-processing steps for the VBM were the following: 1) T1-weighted images were spatially normalised to a standard MNI anatomical space to allow for spatial averaging; 2) the images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF); 3) comparisons of GM volume were performed for the entire brain. The preprocessing was performed as described in

the VMB8 manual. The resulting GM images were smoothed using a Gaussian Kernel of 12 mm FWHM. Voxel-by-voxel *t*-tests using the general linear model (GLM) and considering total brain volume as a covariate were used to detect GM volume differences between patients with tinnitus and the controls (Ashburner and Friston 2000; Ashburner 2007).

6.4 Results

6.4.1 CTA

Whole-brain analysis: The FDR approach, with $p < 0.05$, did not reveal any significant difference in cortical thickness between those with tinnitus and controls.

Pre-defined ROIs: Significant cortical thickness reductions were observed in the right temporal lobe in patients with tinnitus compared with the controls (see Table 6.1). However, when applying Bonferroni correction with $p < 0.05$ none of these comparisons remain significant.

Table 6.1 Patient versus control t-test contrasts for the frontal and temporal lobes.

Lobe	Patients Mean±SDev	Controls Mean ±SDev	*t*	$p_{(uncorrected)}$	$p_{(Bonferroni\ corrected)}$
right frontal lobe	2.8±0.09	3± 0.07	-1.7	0.1	0.4
right temporal lobe	**2.9± 0.088**	**3.2± 0.085**	**-2.6**	**0.022**	0.08
left frontal lobe	2.8± 0.09	2.9± 0.07	-1.1	0.31	1.24
left temporal lobe	2.9± 0.07	3.1± 0.07	-1.8	0.1	0.4

The results of the regional ROI analysis are presented in Table 6.2. In the right hemisphere, cortical thickness reductions were observed in subjects with tinnitus compared to the control group in the middle and inferior frontal gyri; the anterior cingulate cortex; the cingulate gyrus; the superior, middle and inferior temporal gyri; and the right primary auditory cortex (BA41).

In the left hemisphere, a borderline significant cortical thickness reduction was observed in subjects with tinnitus compared to controls in the cingulate gyrus, and a significant cortical thickness reduction was observed in the posterior cingulate gyrus and the superior temporal gyrus. When applying Bonferroni correction with $p < 0.05$, none of these comparisons remain significant. I have reported both corrected and uncorrected p values.

Table 6.2 Patient versus control t-test contrasts of cortical thickness of the a priori-defined ROIs. The thicknesses are displayed in mean (M) ±standard deviation (SDev) in mm.

Region	Patients Mean±SDev	Controls Mean±SDev	*t*	$p_{(uncorrected)}$	$p_{(Bonferroni corrected)}$
right hemisphere superior frontal gyrus	2.7± 0.09	2.9± 0.1	-1.5	0.15	3.3
middle frontal gyrus	**2.9± 0.11**	**3.2± 0.14**	**-2.1**	**0.050**	1.1
inferior frontal gyrus	**3.1± 0.16**	**3.7± 0.23**	**-2.1**	**0.05**	1.1
anterior cingulate cortex	**2.4± 0.08**	**2.7± 0.04**	**-2.9**	**0.011**	0.24
cingulate gyrus*	**2.6± 0.07**	**2.8± 0.06**	**-2.4**	**0.030**	0.66
posterior cingulate gyrus	2.8± 0.08	2.9± 0.07	-1.6	0.23	5.06
parahippocampal gyrus	3.6± 0.24	3.7± 0.36	-0.27	0.8	17.6
superior temporal gyrus*	**2.7± 0.11**	**3± 0.11**	**-2.1**	**0.05**	1.1
middle temporal gyrus	**2.8±0.11**	**3.4± 0.23**	**-2.6**	**0.023**	0.51
inferior temporal gyrus	**3.0± 0.12**	**3.4±0.18**	**-2.8**	**0.015**	0.33
primary auditory cortex (BA41)	**2.4± 0.11**	**2.9± 0.18**	**-2.3**	**0.039**	0.85
left hemisphere superior frontal gyrus	2.8± 0.10	2.8± 0.08	0.69	0.50	11
middle frontal gyrus	2.8± 0.11	2.9± 0.09	-0.65	0.53	11.6
inferior frontal gyrus	2.9± 0.13	3.2± 0.14	-1.8	0.09	1.98
anterior cingulate cortex	2.5± 0.10	2.6± 0.06	-0.9	0.38	8.36
cingulate gyrus	2.6± 0.08	2.9± 0.11	-2	0.062	1.36

posterior cingulate gyrus	**2.6±0.10**	**3.1± 0.13**	**-2.9**	**0.012**	0.26
parahippocampal gyrus	3.6± 0.2	4.1± 0.32	-1.4	0.2	4.4
superior temporal gyrus*	**2.7± 0.10**	**3± 0.11**	**-2.3**	**0.04**	0.88
middle temporal gyrus	2.8± 0.08	3± 0.15	-1.5	0.15	3.3
inferior temporal gyrus	3.1± 0.12	3.4± 0.3	-0.93	0.37	8.14
primary auditory cortex (BA41)	2.5± 0.09	2.6±0.10	-1.3	0.21	4.6

*significant findings in bilateral structures based on the uncorrected p value for multiple comparisons.

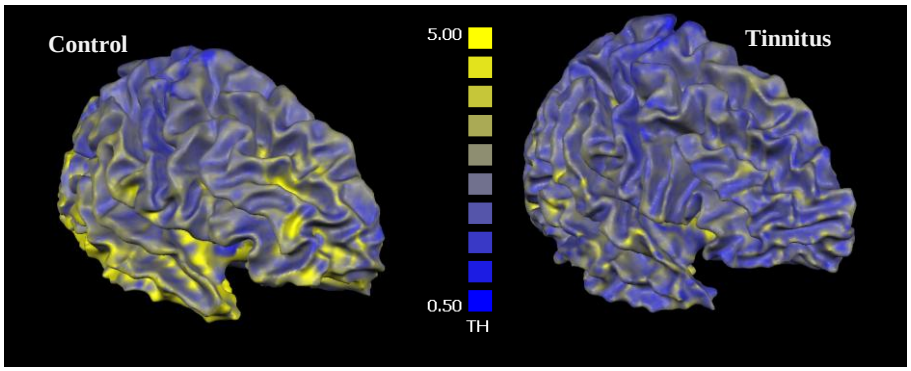


Figure 6.3 Two examples of cortical thickness maps of the right hemisphere. The left map shows the cortical thickness map of a healthy control, and the right map shows the thickness map for an individual with tinnitus.

6.4.2 VBM

Whole-brain analyses, which were corrected for multiple comparisons and performed by applying a Family Wise Error (FWE) <0.05, did not reveal any significant difference between patients with tinnitus and controls. Relaxing the threshold to p uncorrected for multiple comparisons to <0.001, with a minimum

cluster size (k) of 20 voxels, resulted in the detection of a significant GM volume reduction in the left medial prefrontal cortex (BA8) in the subjects with tinnitus compared to controls. For this region, a cluster (k) size of 306 voxels was determined (see Figure 6.4).

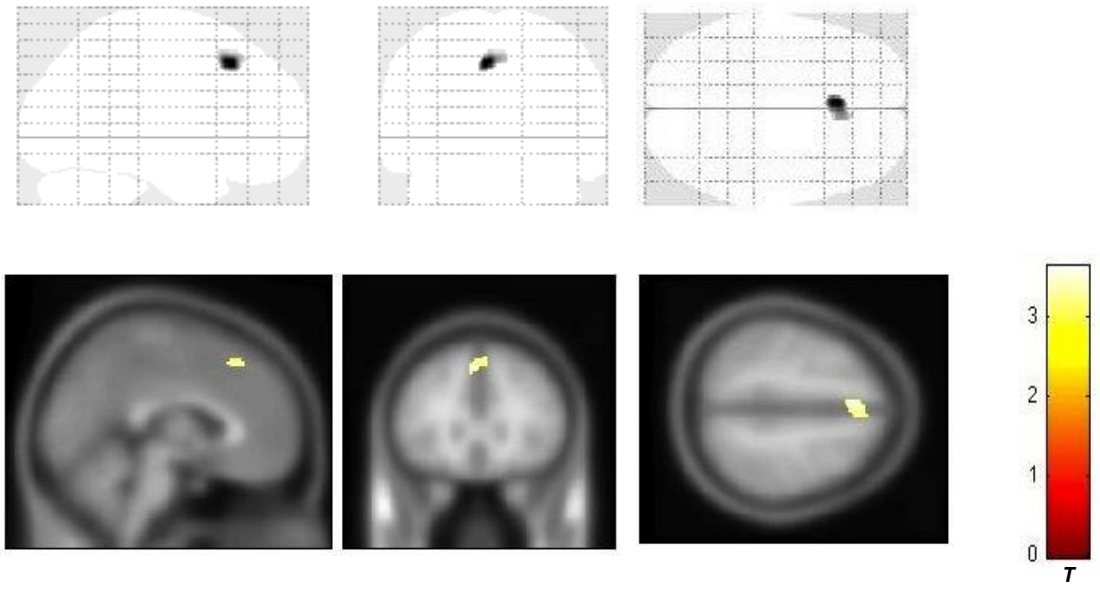


Figure 6.4 Grey matter volume decreases within the left medial prefrontal cortex (PFC) in the subjects with tinnitus compared to controls, with a cluster size of 306 voxels. MNI coordinates of peak voxels: x= -4, y= 26, z= 46, $p_{uncorrected}$< 0.001, with a minimum cluster (*K*) = 20 voxels

6.4.3 Correlations

No correlations were observed between cortical thickness in the frontal and temporal lobes and other measures (i.e., age, hearing threshold, THI) in the subjects with tinnitus. With respect to the controls, no correlations were observed between cortical thickness in the frontal and temporal lobes and age or hearing thresholds.

With respect to the tinnitus sufferers, a significant negative correlation was observed between the hearing threshold and the cortical thickness of the right cingulate gyrus (r = -0.6, p=0.02 for the RE, and r= -0.51, p= 0.04 for the LE, df= 14) in subjects with tinnitus. Cortical thickness in the right anterior cingulate cortex was determined to be correlated with HTs in patients with tinnitus (r = -0.64, p= 0.008 for HT in the right ear and r= -0.54, p= 0.03 for the left ear, df= 14). No correlation was observed between the THI score and cortical thickness measures in any of the ROIs. A borderline significant negative correlation was observed between the HT in the right ear and the thickness of the right primary auditory cortex (r = -0.46, p= 0.06, df= 14) in subjects with tinnitus but not in controls. However, none of these correlations survived the Bonferroni correction at $p<0.05$. A summary of these correlations are presented in Table 6.3. In the left hemisphere, no correlation between the HT of either ear and cortical thickness was observed. The GM volume did not correlate with any of these measures in the subjects with tinnitus.

There was no significant correlation between the behavioural measures, the cortical thickness of any ROI or the GM volume in any of the ROI in the control subjects.

Table 6.3. Summary of the significant correlations between certain ROIs and HT in the right ear and left ear.

brain region	correlations	
	RE	LE
right cingulate gyrus	r= -0.6, $p_{(uncorrected)}$= 0.02, $p_{(Bonferroni)}$= 0.44	r= -0.51, $p_{(uncorrected)}$= 0.04, $p_{(Bonferroni)}$= 0.88
right anterior cingulate cortex	r= -0.64, $p_{(uncorrected)}$= 0.008, $p_{(Bonferroni)}$= 0.17	r= -0.51, $p_{(uncorrected)}$= 0.03 $p_{(Bonferroni)}$= 0.66
right primary auditory cortex	r= -0.46, $p_{(uncorrected)}$= 0.06, $p_{(Bonferroni)}$= 1.32	–

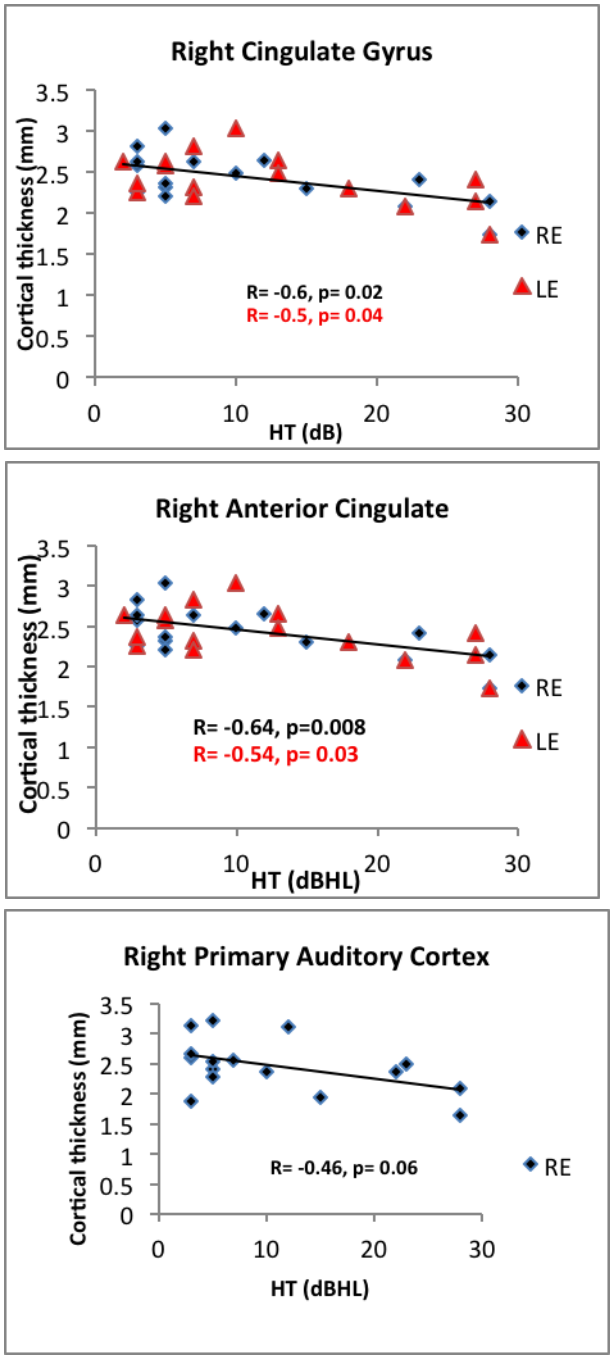


Figure 6.5 Scatter plots showing the correlation between cortical thickness alterations and hearing thresholds (HT) in both the right ear (RE) and the left ear (LE) in the right primary auditory cortex, anterior cingulate cortex and cingulate gyrus in the tinnitus sufferers group.

6.5 Discussion

I employed a semi-automated approach to measure the cortical thickness of the entire brain and in a priori-hypothesised ROIs to investigate structural brain differences in a group of patients with tinnitus versus age- and sex-matched controls. To the best of our knowledge, this is the first study to investigate alterations in the cortical thickness of individuals with tinnitus. Group analyses were applied using pre-defined ROIs, revealing that patients with tinnitus exhibit cortical thickness reductions in the vicinity of the right PFC, including the middle and inferior frontal gyri. In the temporal region, the superior temporal gyri exhibited a significant bilateral cortical thickness reduction in subjects with tinnitus compared to the controls. The cortical thickness reduction was more extensive in the right temporal lobe than in the left temporal lobe and included the middle and inferior temporal gyri. The result of overall cortical thickness reduction in the right temporal lobe was obtained following ROI analysis. The limbic system was also involved in the cortical thickness reductions in subjects with tinnitus compared to the controls. The VBM results revealed a reduction in the GM volume in the left medial PFC (BA8) in subjects with tinnitus compared to the control group.

Cortical thickness reductions in the PFC have been reported in PTSD sufferers (Geuze, Westenberg et al. 2008), adding another similarity between PTSD and tinnitus perception to those discussed by Fagelson (2007) and Møller (2010). Functional alterations in the PFC have been reported in many neuroimaging studies of patients with tinnitus (Mirz, Brahe Pedersen et al. 1999; Mirz, Gjedde et al. 2000; Farhadi, Mahmoudian et al. 2010). The theoretical neurophysiological model of tinnitus that was proposed by Jastreboff (1990) considers the PFC to be

one of the major components of the tinnitus perception model. This model is based on the fact that tinnitus sufferers report deficits in cognition, memory and attention (Andersson 2002; Jastreboff and Hazell 2004), all of which are functions that are regulated by the PFC (Miller and Cohen 2001). Studies that have investigated cortical thickness in attention-related disorders, such as ADHD, revealed cortical thinning in the PFC (Makris, Biederman et al. 2007). The same findings were reported in individuals suffering from bipolar disorder. Most chronic tinnitus sufferers share a common major symptom with bipolar disorder, namely, depression (Jastreboff and Hazell 2004; Lyoo, Sung et al. 2006). Hence, the observed cortical thickness reduction and the GM volume reduction in the PFC may not be due solely to attention-related deficits but may also be associated with the negative emotions experienced by tinnitus sufferers. The PFC, specifically the inferior frontal gyrus, contributes to verbal learning and memory (Hagino, Suzuki et al. 2002). Patients with tinnitus were reported to have impairments in working memory (Rossiter, Stevens et al. 2006). Thus, I believe that the reduction in both cortical thickness and regional grey matter volume in the PFC in the examined subjects with tinnitus compared to the controls may indicate an underlying neurodegenerative mechanism of tinnitus perception and its negative associations. The lack of studies that have investigated cortical thickness alterations in tinnitus populations makes it difficult to compare the results of this study to others. Although certain studies have reported structural changes in tinnitus sufferers by means of voxel-based morphometry (VBM) (Muhlau, Rauschecker et al. 2006; Landgrebe, Langguth et al. 2009), only Leaver et al. (2011) observed structural changes in the PFC, a result that is in agreement with our findings.

The primary auditory cortex, which lies in the temporal lobe, has been strongly linked to tinnitus perception and is considered to be the signal's source (Jastreboff and Hazell 2004). Many believe that tinnitus perception is conditioned by a dysfunction in the auditory cortex (Jastreboff 1990; Eggermont and Roberts 2004). Functional neuroimaging studies of tinnitus population have reported abnormal functioning of the auditory cortex (Giraud, Chery-Croze et al. 1999; Smits, Kovacs et al. 2007). Structural MRI studies of the tinnitus population have revealed GM volume reductions in the auditory cortex (Landgrebe, Langguth et al. 2009) and a reduced volume of Heschel's gyrus (Schneider, Andermann et al. 2009). The findings from the present study, which demonstrate cortical thickness reductions in the right primary auditory cortex (which includes Heschel's gyrus), are in agreement with these previous results. The authors of these previous studies investigated the concentration of grey matter, whereas I estimated cortical thickness, a measure that relies on determining the distance between the grey and white matter surfaces. The observed cortical thickness reduction in the auditory cortex was significantly extended in the right hemisphere to include the middle and inferior temporal gyri. In the left hemisphere, I observed a significant cortical thickness reduction in the superior temporal gyrus in tinnitus sufferers compared to controls. Functional imaging studies have reported abnormal regional blood flow in the temporal lobe in patients with tinnitus (Mirz, Gjedde et al. 2000; Farhadi, Mahmoudian et al. 2010).

A pre-defined ROI statistical approach revealed a cortical thickness reduction in the limbic system in tinnitus sufferers compared to controls. In the right hemisphere, I observed a significant cortical thinning in the anterior cingulate cortex and cingulate gyrus. In contrast, in the left hemisphere, only the posterior

cingulate gyrus exhibited a significant cortical thickness reduction. Negative emotions, which are controlled by the limbic system, are widely believed to be associated with tinnitus perception (Jastreboff and Hazell 2004). Abnormal functioning in the limbic system has been demonstrated in several neuroimaging studies of tinnitus patients (Lockwood, Salvi et al. 1998; Mirz, Brahe Pedersen et al. 1999). However, such activation patterns are believed to indirectly represent the role of neural plasticity in tinnitus. With regard to structural alterations that are believed to be associated with tinnitus, Muhlau et al reported a GM volume reduction in the sub-callosal region, which is part of the limbic system and plays a major role in behavioural responses to environmental stimuli (Muhlau, Rauschecker et al. 2006).

Cortical thickness measurements reflect the status of the grey-white matter boundary and the cortical mantle itself. Thus, cortical thinning may indicate changes in myelination, neuronal density, the grey-white matter boundary and synaptic pruning (Narr, Bilder et al. 2005; Shaw, Lerch et al. 2006). Scientists have observed that cerebral cortical thickness depends on the constituent cell types, cell density and neuronal organisation. Therefore, cortical thinning or thickening may represent deviations from normal cytoarchitecture. It is possible that reduced cortical thickness may reflect a GM reduction and vice versa. Narr et al. (2005) revealed a strong association between GM concentrations and cortical thickness.

Correlation analyses revealed an association between HTs and cortical thickness alterations in subjects with tinnitus compared to the controls. Tinnitus sufferers with hearing deficits (i.e., high hearing thresholds) tend to exhibit thinner cortices compared to subjects with tinnitus with low HT (i.e., no hearing deficits).

However, this was not observed when considering GM volume, as no significant correlation between the VBM results and HT were observed. These results are in agreement with those found by Leaver et al (2011). Based on previous studies, I expected to identify a correlation between age and both cortical thickness alterations and GM volume (Salat, Buckner et al. 2004; Voineskos, Rajji et al. 2012) in both groups. However, several of the correlations failed to reach significance. This result may have occurred because the analysed age range was not sufficiently wide, as we only recruited subjects between 30 and 60 years old. Tinnitus severity, as measured using the Newman THI, was correlated with both cortical thickness and GM volumes, but this correlation did not reach significance. The Newman THI instrument is a subjective measure based on self-reporting of the impact of tinnitus on one's daily life. This score depends on one's personal reaction to the tinnitus sound, the understanding of the term "severity" and other personal factors (Newman, Jacobson et al. 1996; Nobel 2000).

Reduced cortical thickness in patients with tinnitus was more pronounced in the right hemisphere, including the right PFC and the right middle and inferior frontal gyri. These results were not observed for the left hemisphere. I included 2/16 patients with discrete unilateral (left) tinnitus. However, in the subjects with bilateral tinnitus, two patients declared that the tinnitus was stronger in the left ear, and one subject reported that in was stronger in the right ear. Lanting et al. (2008) reported abnormal functionality of the inferior colliculus that was contralateral to the side of tinnitus perception. Muhinickel et el. (1998) also reported a positive correlation between the severity of the tinnitus and the amount of tonotopic map reorganisation in the contralateral hemisphere. With regard to the opposite results that were observed in the unilateral tinnitus patients, my findings are limited by

the small number of unilateral tinnitus subjects, and it was not my intention to examine the neural correlates of unilateral tinnitus specifically. Thus, I cannot generalise that the observed cortical thickness alterations associated with left-sided tinnitus are dominant in the right hemisphere. However, including the data from the subjects left-sided tinnitus in the above analysis may affect my results that demonstrate a dominancy of the right hemisphere, which would be the contralateral hemisphere in these cases. Future research should examine whether unilateral tinnitus perception involves cortical thickness alterations in the contralateral hemisphere.

The results of the current study indicate that high-resolution MRI, combined with computational advances in cortical thickness measurement techniques, are promising diagnostic tools. The use of semi-automated CTA, as implemented in Brain Voyager software, combined with the automated VBM technique, made the obtained results user-independent. Thus, these combined approaches reduced the errors that may arise due to observer corrections of anatomical variations in manual segmentation approaches. MRI-based cortical thickness analysis and VBM could objectively diagnose tinnitus. This would be an improvement over the current approach given that the only available tools for diagnosing subjective tinnitus are self-reported questionnaires. These results indicate the need for further studies that investigate cortical thickness in patients with tinnitus. Determining a recognisable pattern of cortical thickness in patients with tinnitus (as in Figure 6.3) may be possible if future studies address this issue. The results of these studies would certainly aid in the diagnosis and treatment of tinnitus.

Considering both the present and previous results of VBM studies of patients with tinnitus, which revealed GM reductions in different regions, it can be concluded

that tinnitus perception is associated with structural alterations in the brain. Tinnitus sufferers were observed to exhibit significant cortical thickness and GM volume reductions in regions that are associated with attention, memory, emotions and auditory functions. I believe that such structural deficits in these regions may be responsible for the functional deficits that are observed in the hearing-related, attentional and emotional symptoms of patients with tinnitus. The observed structural deficits may also represent an underlying mechanism of tinnitus pathogenesis. Volumetric studies that examine the volumes of subcortical structures, such as the amygdala and the hippocampus, or structural studies that investigate possible grey and white matter alterations are required to uncover the mechanisms of tinnitus pathogenesis. The present study did not perform these analyses, making it difficult to highlight conclusive findings regarding tinnitus perception from a neuroscience perspective.

7. Chapter 7. General Discussion, Conclusions and Future Work

7.1 Introduction

This thesis used functional and structural MRI to focus on the investigation of the neural substrates of tinnitus. The experimental work, described in chapters 4, 5, and 6, involves a progression in the understanding of the neural circuitry that is involved in tinnitus perception. The present results implicate anatomically and functionally specific cortical networks in tinnitus pathophysiology. The present chapter summarises the primary findings of this thesis and discusses possible future work on the basis of these results. For clarity, this chapter is divided into several sections that describe the following topics: the functional reorganisation associated with tinnitus perception, the structural alterations associated with tinnitus perception, the overlap between the functional and structural deficits, the limitations of this thesis, the conclusions, the clinical implications of this work, and suggested future directions.

7.2 Tinnitus perception involves functional reorganisation

In chapter 4, I investigated how tinnitus sufferers respond to unpleasant sounds as well as to pleasant and unpleasant visual stimuli. Using fMRI, the PFC, temporal lobe and limbic system were revealed to be involved in the pathophysiological mechanism of tinnitus perception. This conclusion was based on an observed increase in the BOLD signal in these regions in subjects with tinnitus compared to the controls. fMRI-detected hyperactivity is commonly believed to represent a functional reorganisation in neural activity and brain plasticity (Ungerleider 1995; Jäncke, Gaab et al. 2001). Therefore, I believe that tinnitus sufferers exhibit maladaptive neural plasticity in the PFC, the auditory region and the limbic system. Furthermore, such functional reorganisation in neural activity may be responsible for the negative associations of tinnitus perception. The elevated activity in these regions was determined to be positively correlated with HT in

subjects with tinnitus but not in controls. However, there was no significant difference in the HT between the two groups. Because no significant difference was observed between the two groups with respect to HT, it appears that there is a tendency for tinnitus-associated hearing deficits to affect the magnitude of neural reorganisation (expressed as the beta values of the BOLD signal). Tinnitus-related distress, as measured using the Newman THI score, was determined to be positively correlated with the mean fMRI BOLD signal in the right cingulate gyrus and the left superior temporal gyrus. These findings may confirm the role of the cingulate gyrus in tinnitus-related aversive emotions rather than tinnitus itself. The role of the cingulate gyrus in stress (Diorio, Viau et al. 1993), depression (Mayberg, Brannan et al. 1997) and anxiety (Sylvester, Corbetta et al. 2012) has been documented. The positive correlation in the left superior temporal gyrus may reflect the feedback loop between the limbic system and the auditory region in tinnitus. Enhancing the activation of the limbic system by viewing unpleasant images and listening to unpleasant sounds may amplify the auditory networks in tinnitus sufferers, which may, in turn, enhance tinnitus signal perception (Jastreboff and Hazell 2004). Future research on relatively undistressed and highly distressed subjects with tinnitus will aid in the identification of the neural networks involved in tinnitus-related negative associations. Neuromodulatory treatment approaches involving these causal networks could then be developed.

To investigate the relationship between the physical properties of tinnitus sounds and neurophysiological behaviour, I induced tinnitus-like perception in healthy controls. Although these sounds may not necessarily induce the same neurophysiological changes as actual tinnitus, I aimed to determine whether tinnitus-like conditions would induce different brain activation patterns compared

to pathological tinnitus. It appears that pathological tinnitus does not differ from induced tinnitus with respect to its neurophysiological mechanism. Mirz et al. (2000) performed a similar study and reported similar findings. Both Mirz's and the present study used a human model of tinnitus, whereas all the other available models are either behavioural or physiological animal models.

7.3 Tinnitus perception involves structural alterations

In chapter 5, I used DTI to examine the white matter and cortical connectivity in patients with tinnitus. I evaluated the FA and MD in the entire brain to investigate a possible disruption in WM connectivity in tinnitus sufferers. The results from this study appear to suggest that tinnitus perception involves WM pathways that connect the PFC with other regions, including the thalamus. Recently, Vanneste et al. (2011) reported a significant improvement in tinnitus-related distress when sufferers underwent transcranial magnetic stimulation of the PFC. Their findings, together with the current results, highlight the role of PFC connectivity in tinnitus perception. Reduced integrity of the WM that connects portions of the limbic system was also reported in chapter 5, confirming the role of the limbic system in tinnitus perception.

In chapter 6, I applied CTA and VBM to investigate whether GM alterations were associated with tinnitus perception. The temporal lobe and the limbic system exhibited cortical thickness reductions in tinnitus sufferers, and the PFC exhibited both GM and cortical thickness reductions. Reductions in cortical thickness in the right PAC, anterior cingulate and the cingulate gyrus were observed to be significantly correlated with HT in subjects with tinnitus but not in controls. Tinnitus-associated hearing deficits appear to result in significant neurodegeneration. Indeed, a strong association between hearing loss and tinnitus

perception has been reported in a large body of clinical and academic tinnitus studies (Davis and Refaie 2000). This association holds even when the tinnitus is mild, as it was in the sufferers who were examined in the present work. This correlation is believed to be due to changes in sensory inputs from the auditory cortex that may cause neural reorganisation and, consequently, a tinnitus signal (Eggermont and Roberts 2004). The clinical characteristics of tinnitus-related hearing loss differ from those of normal hearing. Individuals who perceive tinnitus with hearing loss reported a higher degree of annoyance and loudness of their tinnitus, and tinnitus-related negative associations were observed to worsen with severe hearing loss (Savastano 2008). Based on this latter finding, I assume that tinnitus, when combined with hearing deficits, may be characterised by different neuro-functional and neuro-structural properties than are observed in patients with normal hearing. This conclusion is based on the results presented in the chapters that describe the fMRI and CTA analyses, which indicate that tinnitus sufferers with higher HTs exhibit a greater degree of functional reorganisation and structural degeneration relative to individuals with normal hearing. In the tinnitus group, the cause of tinnitus and hearing deficits was related to age and noise exposure. In the control group, hearing deficits were related to age. However, correlation analyses between the structural changes and age did not reveal any significant correlation between the structural deficits and the age. Therefore, the functional and the structural reorganisation exhibited by subjects with tinnitus are suggested to be associated with the tinnitus and its associated hearing deficits rather than aging. Kaltenbach el al. (2004) reported hyperactivity in the DCN in animals that had been exposed to intense noise and this activity was correlated with the strength of the behavioural evidence of tinnitus. Noise-induced hearing loss is capable of

producing functional changes in the neurons. Willott and Lu (1982) reported increased neural excitability associated with alterations of the normal neural coding processes in the auditory system in animals that were exposed to intense noise.

7.4 Structural and functional overlap

Considering all of the above results, it should be noted that the observed functional and structural changes that are associated with tinnitus perception overlap in three primary brain regions: the PFC, the temporal lobe (including the auditory cortex) and the limbic system. The observed physiological alterations are characterised by an increased fMRI BOLD signal and may indicate underlying structural lesions, which manifest as cortical thickness reductions and disrupted WM integrity. The reduced integrity of the WM tracts that connect the PFC, the temporal lobe and the limbic system with other cortical and neocortical structures, together with the observed reduced cortical thickness and the reduced GM volume in the PFC, may indicate a primary lesion that underlies tinnitus pathogenesis. A consequence of such deficits may cause a secondary defect that is characterised by abnormal functionality in these regions. Another explanation for the observed overlap in the functional and structural reorganisation is that the hyperactivation in these regions may reflect an early neuropathological symptom of tinnitus that subsequently develops into structural neurodegeneration.

A reduction in gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, was demonstrated to produce cortical hyperexcitability (Gu, Li et al. 2005). This neural hyperexcitability may account for the increased BOLD signal and may precede the observed structural deficits. It is also possible that the reduction in the GABA levels is a causal factor in tinnitus. Reduced GABA levels may cause

neuronal hyperactivity that both manifests as internal sounds and induces neurodegenerative effects. The role GABA in clinical depression and mood disorders, which are characterised by similar symptoms as those associated with tinnitus, has been demonstrated in several studies (Brambilla, Perez et al. 2003; Hayley, Poulter et al. 2005). An animal model study of tinnitus (Middleton, Kiritani et al. 2011) demonstrated an increase in DCN neuronal activity due to GABAergic inhibition. Based on this study, GABAergic inhibition appears to be an early biomarker of tinnitus pathophysiology. In addition to this biomarker, brain structural and functional differences also exist in this population, including abnormal activity, as was observed in the fMRI study; a loss of WM integrity, as was observed in the DTI study; and a loss of GM volume, as was observed in the CTA and VBM studies.

Whether the structural or the functional characteristics of tinnitus pathophysiology manifest first is unknown. It is important to understand the role of neuroplasticity in tinnitus perception. Uncovering such a role will aid in the identification of a cure for tinnitus or prevent tinnitus from becoming disruptive. With regard to adaptive neuroplasticity, which is critical for memory and learning processes, an increase in the GM volume in motor-related brain regions was reported in healthy controls following a brief motor skills training protocol. This effect was observed to be associated with a decreased fMRI BOLD signal (Hamzei, Glauche et al. 2012). Conversely, an increased BOLD signal was determined to be associated with disrupted WM integrity in cognition-related brain regions in MS patients (Rocca, Valsasina et al. 2009). The observed increase in the fMRI BOLD signal in this previous study was considered to represent the contributions of WM structural deficits to the maladaptive neuroplasticity that is associated with MS.

These findings demonstrate the coupling between the structural and the functional brain changes that are involved in adaptive and maladaptive neuroplasticity. Studies of humans and computational models of neuronal networks have suggested that functional networks in the brain are based on structural connectivity. Any change in neuronal connectivity is believed to result in functional reorganisation, the purpose of which is to maintain homeostasis of the neural circuits. Hence, structural changes in the brain are considered to predict the emergence of functional reorganisation (Bullmore and Sporns 2009; Honey, Sporns et al. 2009). On that basis, it can be suggested that tinnitus pathogenesis is associated with structural deficits that are characterised by reduced GM volume, reduced cortical thickness and disrupted WM integrity. The hyperactivity that was exhibited by tinnitus sufferers in the present fMRI study may represent a maladaptive response to structural deficits. This response would subsequently manifest as a tinnitus signal.

Recently, in a VBM study of tinnitus sufferers with hearing loss compared to healthy controls, it was reported that the tinnitus patients exhibited an increased GM volume in the superior and middle temporal gyri and a decreased GM volume in the superior frontal gyrus, the occipital lobe and the hypothalamus (Boyen, Langers et al. 2012). However, no differences in the GM volume were observed when the comparison involved patients with both tinnitus and hearing impairment and individuals with hearing impairment only (Boyen, Langers et al. 2012). Husain et al. (2011) performed a DTI and VBM study of three groups: tinnitus sufferers with hearing loss, those with hearing loss but not tinnitus and normal controls. These authors reported a GM volume reduction in the bilateral superior and medial frontal gyri in those with hearing loss alone relative to those with both

tinnitus and hearing loss. When the authors compared the GM volumes of the subjects with tinnitus and normal subjects, no significant difference were observed. Husain et al. (2011) also reported a reduction in FA in several WM bundles, including the superior longitudinal fasciculus, the inferior fronto-occipital fasciculus and the anterior thalamic radiations, in subjects with hearing loss relative to subjects with no hearing loss. These authors suggested that the perception of internal noise (i.e., tinnitus) may be associated with less severe neuro-structural changes compared to when hearing loss is present. Leaver et al. (2011; Leaver, Seydell-Greenwald et al. 2012) reported GM volume reductions in the ventromedial PFC and increased gyrification of the dorsomedial PFC in a group of tinnitus sufferers whose hearing losses ranged from slight to moderate. These changes were not correlated with the degree of hearing loss but, rather, correlated with certain consequences of the tinnitus, such as depression and anxiety scores. Schneider et al. (2009) reported a reduction in the GM volume of the medial aspect of Heschel's gyrus in a group of tinnitus sufferers, and the degree of the volume reduction was dependent on the degree of hearing loss. Tinnitus is more complex than internal sound perception, and its negative consequences may have substantial effects on the structure and function of the brain.

7.4 Limitations

As in any study, the fMRI experiment has limitations in addition to the relatively small sample of participants with tinnitus. Specifically, the contribution of the scanner noise to the auditory stimuli, even though this noise was diminished by the use of earplugs, may have increased the BOLD signal. Furthermore, the scanner noise may have enhanced sufferers' perception of tinnitus sounds and

increased their irritation. Hence, the limbic system may have exhibited a higher degree of activity in the scanner. Heartbeat motion-related artefacts were highly possible. I used the standard post-processing techniques that are available in the software, but this effort does not guarantee that the artefacts were completely eliminated. These artefacts may appear as a displacement in the BOLD signal (Slabu and Duifhuis 2008). Despite these known limitations, fMRI experiments are considered the gold standard for investigating brain function.

A limitation of the CTA may have been the automated algorithm. This algorithm relies on an automatic tissue classification technique that divides the MRI images into GM, WM, and CSF. Although this automatic tissue classification has been validated, it is possible that a false WM layer can be improperly inserted into the GM layer when calculating the cortical thickness. Nonetheless, the estimation of the cortical thickness measures in our healthy control group was within the range of those reported by other published studies of healthy adults (Kabani, Le Goualher et al. 2001). A similar limitation exists for VBM. Most DTI studies, including the present analysis, are incapable of precisely determining WM architecture when different fibre populations pass through one voxel. In this case, the fractional anisotropy is reduced and becomes close to the GM or CSF values. Another limitation of all DTI studies, including the present study, is its high sensitivity to any physiological motion, which may interfere with the detected water motion and affect the accuracy of the results. I attempted to overcome this potential complication by reducing the scanning time to approximately 9 minutes; however, any motion with a magnitude of 5-10 μm (which is the range of water molecule motion in the fibres during the MR scan) will affect the results (Mori

and Zhang 2006). DTI, despite its limitations, is the only imaging modality that is capable of providing information regarding WM architecture in vivo.

Although I avoided the limitations of other tinnitus studies, such as the heterogeneity of the tinnitus sufferers with respect to hearing status and age (Leaver, Renier et al. 2011), it was not possible to avoid the laterality of tinnitus perception. Three of 15 of the subjects with tinnitus who participated in this work had unilateral tinnitus. Perceiving tinnitus on one side only may have a different neural basis than bilateral tinnitus. The comparison of the neural basis of unilateral versus bilateral tinnitus within this thesis was very limited by the small number of subjects with unilateral tinnitus.

7.5 Clinical implications

One obstacle to identifying a cure for tinnitus is our lack of understanding of its pathophysiological mechanisms. Although many functional and a small number of structural imaging studies have been performed, there is no consensus regarding how, where or why tinnitus begins. This thesis identified functional and structural abnormalities that are exhibited by tinnitus sufferers using functional and structural MRI analyses. Together with previous functional (Mirz, Gjedde et al. 2000) and structural (Leaver, Renier et al. 2011) studies, the results of this thesis emphasise the involvement of the limbic system, the PFC and the auditory cortex in tinnitus pathogenesis. Such findings improve our understanding of the pathological mechanism of tinnitus. In most cases, tinnitus is experienced subjectively and cannot be diagnosed objectively, which constitutes another obstacle to understanding and curing this disorder. Functional and structural MRI analyses may be promising objective diagnostic tools, as it was possible to use

these techniques to distinguish tinnitus sufferers from the normal subjects. This result was conclusively demonstrated in the CTA study.

7.6 Conclusion

The work that is presented in this thesis highlights the neural substrates of tinnitus using a multi-analytical imaging approach. I used functional and structural MRI techniques to investigate the neural activity that characterises tinnitus. Using different analytical approaches, three primary brain regions were revealed to be involved in tinnitus perception: the PFC, the temporal lobe (including the auditory cortex), and the limbic system. The fMRI portion of this thesis revealed an increased BOLD signal in these regions, which is commonly accepted to reflect increased neuronal activity. These findings support the neurophysiological model of tinnitus that was suggested by Jastreboff (1990), who hypothesised that the tinnitus signal functions as an unpleasant stimulus to which the limbic system responds. The continuous tinnitus signals cause the limbic and the autonomic nervous systems to become hyperexcitable. This hyperexcitability, as described in the fMRI chapter of this thesis, may explain the negative associations that characterise tinnitus, such as irritation, anxiety and sleep disturbances.

The other portion of this thesis focused on the structural brain changes that may be associated with tinnitus. These analyses revealed the involvement of brain regions that were similar to those that were identified in the fMRI study. The structural investigation approach was divided into three different studies: DTI, CTA and VBM. I investigated possible changes in the brain GM using CTA and VBM. WM integrity and cortical connectivity were examined using DTI and CTA, respectively. The brain regions involved in auditory functions, emotions, memory and attention have been revealed to contribute to tinnitus pathophysiology. I

propose that the structural deficits that were observed in the brain GM and WM, as well as the observed disruptions in cortical connectivity, may be the neural bases for the functional reorganisations that are described in the fMRI chapter. These structural changes may be very early predictors of subsequent functional reorganisation. That is, tinnitus may begin with structural deficits, such as a reduction in the brain GM or WM volumes. These structural lesions may have several causes, such as inner ear damage due to noise exposure. The subsequent functional reorganisation may be the brain's effort to compensate for those structural deficits. This process illustrates the maladaptive neuroplasticity that underlies tinnitus pathogenesis.

This thesis also highlights the contribution of tinnitus-related hearing loss to the observed functional and structural reorganisation of the brain. This effect was demonstrated by the observed significant correlations between changes the fMRI BOLD signal and both cortical thickness and HTs in subjects with tinnitus. These findings reveal the role of hearing status in tinnitus on the neuroplasticity that underlies tinnitus pathogenesis. It appears that there is a positive association between hearing deficits in tinnitus and structural and functional changes in the brain. This result suggests that a focus on hearing loss treatment may prevent or reduce the maladaptive neural reorganisation and neurodegeneration that occurs during the development of tinnitus.

Generally, this thesis observed that tinnitus-related abnormalities cause distress. Although tinnitus is incurable, advances in the understanding of its physiological mechanisms, including the findings that are presented in this thesis, may aid in the identification of an effective tinnitus treatment.

7.7 Directions for future work

A logical continuation of this thesis is to include larger tinnitus populations with different hearing statuses, including normal, mild, moderate, and severe hearing loss. Most of the participants with tinnitus in this thesis had normal hearing, although a subset had hearing deficits. Therefore, these subjects represent the tinnitus population with normal hearing, which constitutes a fraction of tinnitus sufferers. The correlation between hearing thresholds and the observed functional and structural alterations suggests that future studies should examine the effect of tinnitus-related hearing loss on brain plasticity.

Other future work should investigate why certain individuals can cope with tinnitus whereas others cannot and seek medical assistance. Is the brain emotional circuit involved in both groups or is this circuit only involved in individuals who are unable to cope with their tinnitus? The subjects with tinnitus who were included in this thesis all sought medical assistance for their tinnitus. The neural substrates of tinnitus in individuals who successfully manage their tinnitus or who are not bothered by it should be examined. The identification of the neural substrates of tinnitus-related negative emotions may aid in the development of treatments that improve sufferers' quality of life.

Recently, repetitive transcranial magnetic stimulation (rTMS) was reported to improve tinnitus-related distress (Kleinjung, Eichhammer et al. 2005). The majority of these studies rely on patients' self-evaluation of their tinnitus. Conducting functional and structural MRI studies pre- and post-rTMS will provide an objective evaluation of this technique as a treatment to improve tinnitus symptoms. Notably, such a study would unmask the maladaptive neural mechanism of tinnitus and the neuromodulatory mechanisms that suppress

tinnitus. Once these latter mechanisms are identified, new effective treatments that eliminate tinnitus may be developed.

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