Functional Magnetic Resonance Imaging of Tinnitus

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Functional Magnetic Resonance Imaging of Tinnitus

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"The beginning of knowledge is the discovery of something we do not understand."

— Frank Herbert (1920–1986)

"Education is what survives when what has been learned has been forgotten."

— B. S. Skinner (1904–1990)

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Introduction and outline of the Thesis

1.1 Outline of this thesis

Aims and scope

Tinnitus is a phenomena which, according to Wikipedia[1], can be described as:

> Tinnitus (pronounced /tɪˈnaɪtəs/ or /ˈtɪnɪtəs/ from the Latin word tinnītus meaning "ringing") is the perception of sound within the human ear in the absence of corresponding external sound [...]

This definition implies that tinnitus is some kind of phantom sound in the sense that it cannot be objectified by others. Furthermore, it appears that the perception of this sound takes place in the human ear. In this thesis, it is argued that this definition is not entirely correct and fails to describe that the central auditory system is presumably playing a major role in generating tinnitus.

According to another definition (U.S. National Library of Medicine, 2009), tinnitus may be described as

> A nonspecific symptom of hearing disorder characterized by the sensation of buzzing, ringing, clicking, pulsations, and other noises in the ear. Objective tinnitus refers to noises generated from within the ear or adjacent structures that can be heard by other individuals. The term subjective tinnitus is used when the sound is audible only to the affected individual.

This definition makes a distinction between objective and subjective tinnitus. Yet, the distinction between objective and subjective tinnitus (Møller, 2003; Lockwood *et al.*, 2002) is debatable (Jastreboff, 1990) in a sense that it is based on whether a sound can be detected or objectified by an external observer, rather than on the possible underlying mechanisms. In addition, it describes tinnitus as noises in the ear while often patients report it outside the ears (i.e. centrally in the head or lateralized outside the head).

Our definition of tinnitus is therefore different and describes it as:

> Tinnitus is an auditory sensation without the presence of an external acoustic stimulus.

Important is our definition is that tinnitus is by definition a percept. Whether it is generated in the peripheral auditory system ('in the ear'), the central auditory system or a combination of both is not essential in the definition. Also the distinction between objective or subjective is not made explicit. Tinnitus is similar to auditory hallucinations. Yet, these are two distinct phenomena which, respectively constitute meaningless sounds (e.g. buzzing, clicking or high-frequency tones) or meaningful sounds (e.g. music or voices) (Silbersweig and Stern, 1998; Griffiths, 2000; Møller, 2007).

[1] Wikipedia – http://en.wikipedia.org/wiki/Tinnitus; as of October 8, 2009

Although the exact mechanism of generation of tinnitus in humans is not known, a number of hypotheses based on data from animal models have lead to the idea that tinnitus is a disorder of the central auditory system. This disorder may be triggered by a peripheral cause (e.g. hearing loss), which in turn may lead to (plastic) changes in the central auditory system. Nevertheless, none of the proposed mechanisms has unequivocally been proven in humans. This thesis discusses the application of functional magnetic resonance imaging (fMRI) to study the central auditory system and tinnitus in humans and provides evidence that supports existing hypotheses.

fMRI is used as the main research method in the study of tinnitus since it offers the possibility to study the human brain in a non-invasive manner and is recognized as a tool to investigate the functions of the brain, especially for localizing functional changes. The main objective of this research project was to gain insight into functional changes in the auditory system and non-auditory areas, that may relate to the generation and perception of tinnitus.

This objective was pursued by means of a methodological approach, by designing studies comparing the neural responses in subjects without tinnitus to those in subjects with tinnitus. Therefore, the aims of the research project were:

i. to explore experimental designs tailored to study tinnitus in an fMRI environment.

ii. to study relevant groups of subjects with tinnitus, and to compare the functions of the brain in these groups with those in closely matched groups of subjects without tinnitus to gain more insight in changes that may underlie tinnitus.

For these purposes, a number of studies were designed and performed of which the main results obtained are presented in this thesis.

Outline

This thesis consists of a number of chapters:

Chapter 1 *Introduction* Chapter 1

This first chapter is meant as a general introduction to the auditory system. A short overview is given, describing the most important parts of the auditory system, ranging from the peripheral auditory system to the auditory cortex. In addition, an introduction to tinnitus is given. The final section of this chapter describes functional magnetic resonance imaging techniques and explains the indirect blood oxygen level dependent (BOLD) effect–a measure of neural activity.

Chapter 2 *Neural activity underlying tinnitus generation: Results from PET and fMRI* Chapter 2

Presents a systematic and comprehensive review of the functional imaging literature on tinnitus. An overview of experimental designs and neuroimaging methods that were previously used to study neural correlates of tinnitus is given. The main points of emphasis are that tinnitus is associated with central auditory activity and that also non-auditory regions of the brain are implicated in the sensation of habitual tinnitus, especially frontal cortex,

limbic regions and the cerebellum.

Chapter 3 **Chapter 3** *Functional imaging of unilateral tinnitus using fMRI*
Presents a study on sound evoked responses in the central auditory system. The major aim of this study is to determine tinnitus-related neural activity in the central auditory system. We investigate sound-evoked responses in subjects with unilateral tinnitus and compare those to subjects without tinnitus.

Chapter 4 **Chapter 4** *Unilateral tinnitus: changes in lateralization and connectivity measured with fMRI*
This chapter is an extension of chapter 3 and specifically investigates the lateralization of sound-evoked responses. Furthermore, it describes connectivity patterns between nuclei of the auditory pathway and the vermis of the cerebellum. The central idea is that activity in different parts of the brain that covary suggest that the neural processes underlying this activity may be interacting. This chapter describes normal sound-evoked responses, the lateralization of these responses, and the connectivity patterns between nuclei of the auditory pathway. Additionally, differences in neural activity between subjects with unilateral tinnitus and controls are described.

Chapter 5 **Chapter 5** *Neural correlates of human somatosensory interaction in tinnitus*
Is a chapter that investigates neural correlates of somatic tinnitus. In this form of tinnitus, somatic maneuvers elicit tinnitus or modulate the psychoacoustic attributes of tinnitus. Neural responses that underly these perceptual changes of the tinnitus are studied by using a maneuver that causes a change in the loudness of tinnitus: jaw protrusion. In addition, somatosensory and auditory integration are studied, which may form the neural basis for this perceptual change.

Chapter 6 **Chapter 6** *A diffusion tensor imaging study on the auditory system and tinnitus*
Explores the use of diffusion tensor imaging (DTI) to investigate the anatomical connectivity patterns between auditory and non-auditory areas in the brain. This chapter focusses on the structural integrity of white matter axons and compares several measures of connectivity between the auditory system and the limbic system in controls and subjects with tinnitus.

Chapter 7 **Chapter 7** *General discussion, conclusions and future perspectives*
Discusses and integrates the main outcomes of this thesis and their implications on further research.

1.2 From sound to neural signals

This introductory chapter is meant as a general introduction into the field of hearing research. It provides a brief overview of some topics in hearing research and the application of functional neuroimaging methods to this field. These first sections explain how sound can be described and how sound is translated into a neural signal—the basis for perception.

This section describes the auditory pathway and briefly explains the functions of the nuclei that are part of the auditory pathway (section 1.2). Furthermore, a short introduction on tinnitus is given, describing some basic aspects of tinnitus (section 1.3). The last section describes basic principles of functional magnetic resonance imaging (fMRI), the coupling between neural activity and fMRI signal intensity, and describes the main data processing steps (section 1.4).

Sound

In most cases, sound reaches us as fluctuations of atmospheric pressure (measured in Pa) over time. The characteristics of our hearing organ are such that we are only sensitive to a certain range of fluctuations. If the frequency of the fluctuations is between 20 Hz and 20 kHz, humans perceive it as sound.

Physically, a (constant) sound can be described in the temporal domain and in the frequency domain. In the temporal domain, a sound is characterized by a function of the air pressure over time (t) and can be described by a single sinusoidal if it is a pure tone, or as a summation of sinusoidal functions if it is a complex sound. In the frequency domain, sounds can be described by their frequency content, and correspond to a repeating period T in the time domain for a pure tone or a complex of repeating periods, each with its own amplitude, for a complex sound.

The primary characteristic of a sound is its sound pressure level (SPL). Sound pressure level is a logarithmic measure of the root-mean-square sound pressure of a sound (p_{rms}) relative to a reference value (p_{ref}). It is measured in decibels (dB) above a standard reference level.

$$SPL = 20 \cdot_{10} \log\left(\frac{p_{rms}}{p_{ref}}\right) \tag{1.1}$$

The commonly used reference sound pressure in air is $p_{ref} = 20\ \mu Pa$ (rms), which is usually considered the threshold of human hearing at a frequency of 1000 Hz (Yost, 2000). An intensity level is thus defined as the level compared to a reference level. An 20 dB increase in intensity corresponds to a 10-fold increase of pressure and a 60 dB increase in intensity corresponds to a 1000-fold increase of pressure.

Both the sound pressure level and the frequency are represented in the central auditory system. First, the sound pressure waves need to be transformed to electrical signals by the peripheral auditory system which is covered in the following section.

The peripheral auditory system

The peripheral hearing organ can be divided in three distinctive components that each serve different functions (see figure 1.1). These partitions correspond to the external, mid-

dle and inner ears. Sound is transmitted from the external environment to the inner ear through two conductive components of the peripheral auditory system.

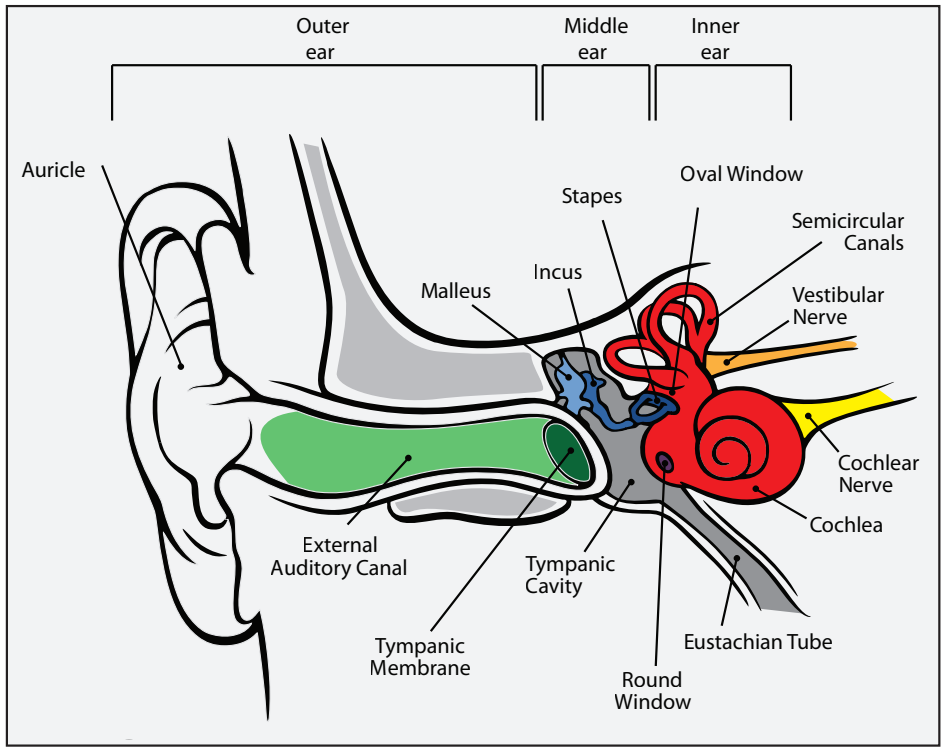

Figure 1.1 The peripheral auditory organ consists of three parts: the outer, middle and inner ears. From the outer ear, sound vibration reaches the tympanic membrane, which in turn moves the ossicles (malleus, incus and stapes) and causes fluid in the cochlea to vibrate. This in turn, causes vibration of the basilar membrane following deflection of hair cells triggering neural firing. (Adapted from: Chittka and Brockmann (2005))

The function of the outer ear is two-fold. First, sound is deflected inwards by the auricle and is focussed towards the tympanic membrane. Due to the structure of the auricle and ear canal, the sound intensity is amplified, especially in the range near 3 kHz (Yost, 2000) where the sensitivity of human hearing is best. Second, the sound is filtered due to the morphological structure of the auricle and thereby provides cues for vertical sound localization (Van Wanrooij and Van Opstal, 2004).

The middle ear provides at least two methods to bridge the mismatch in impedance between the atmospheric air (a low impedance medium) and the fluid in the inner ear (a high impedance medium). The first method is based on the difference in area between the tympanic membrane and the (much smaller) oval window, causing an amplification of the pressure on the tympanic membrane. The second method relates to the mechanic lever

action of the three connected ossicles amplifying the pressure even more. A reduction of the amplification may also occur due to an acoustic reflex. When presented with a high-intensity sound, the stapedius muscle and the tensor tympani muscle cause the ossicles to contract (Hüttenbrink, 1988). This acoustic reflex decreases the transmission of vibrational energy to the cochlea.

The sound pressure wave that has reached the tympanic membrane now enters the cochlea via the oval window and enters the fluid-filled compartments of the coiled cochlea. These compartments are separated by membranes of which the basilar membrane is crucial in sound detection. The mechanical properties of this basilar membrane are such that it is narrow and stiff at the basal end of the cochlea and wide and flexible at the apical end. This arrangement causes a gradual change in resonance frequency along the length of the membrane, decreasing in frequency towards the apex. Sounds of different frequencies thus have a different place of resonance along the basilar membrane, which is referred to as a tonotopic organization. The cochlea acts as a mechanical frequency analyzer, mapping the frequency content of the sound spatially onto the length of the basilar membrane, resulting in a frequency decomposition of the sound signal.

The organ of Corti is situated on top of the basilar membrane (figure 1.2) and consists of hair cells which are coupled to the tectorial membrane. Two types of hair cells exist that each have a distinctive organization and function. The inner hair cells (IHCs) form a single row of hair cells that protrude from the basilar membrane. In addition to the IHCs there are three rows of outer hair cells (OHCs) that are innervated by (efferent) central auditory system neurons. Sound causes mechanical vibration in the cochlea at a site of resonance. This movement causes deflection of the tectorial membrane relative to the basilar membrane and causes deflection of the stereocilia of the hair cells. This evokes neural discharges in some of the afferent fibers of the cochlear nerve. The OHCs display somatic electromotility, i.e. the mobility of the hair cells, which in turn influences the motion of the basilar membrane (Zheng *et al.*, 2000; Dallos, 2008). The OHCs thus function as an active acoustic amplifier with the ability of sharpening the frequency selectivity. One effect of this active amplification is the occurrence of spontaneous otoacoustic emissions that are presumed to relate to an instability of the feedback amplification system (Probst *et al.*, 1991).

The peripheral hearing organ can be affected in many ways which may lead to several types of hearing loss: conductive hearing loss, sensorineural hearing loss or a combination of these two. Conductive hearing loss results from disfunction of parts of the outer ear, the middle ear or a combination of these two, which can be characterized by a reduced signal transmission to the sensory hair cells.

Examples of conductive hearing loss include excessive ear wax blocking the auditory canal, perforation of the tympanic membrane and stiffening of the ossicle chain (sclerosis). Sensorineural hearing loss results from damage or dysfunction in the inner ear or the central auditory system. Loss or dysfunction of inner or outer hair cells cause sensorineural hearing loss. Noise trauma, ototoxic drugs and various diseases may cause sensorineural hearing loss.

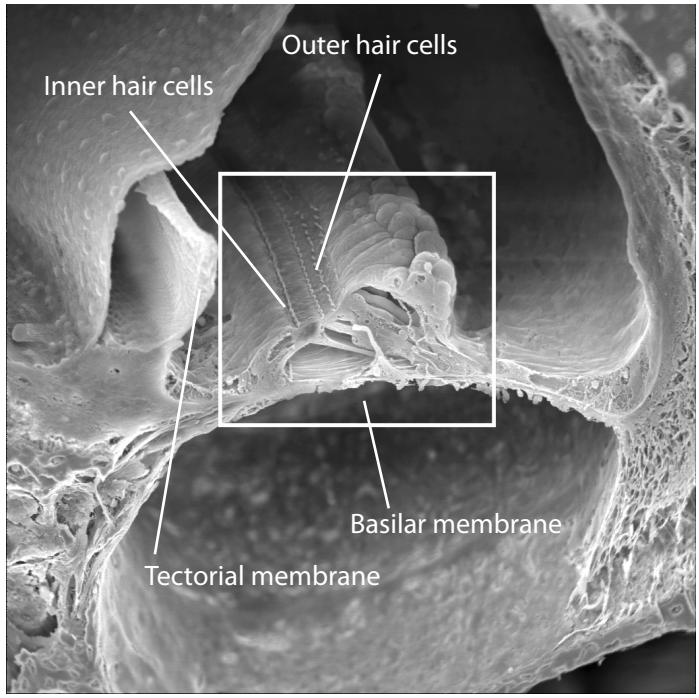

Figure 1.2 A cross section of the cochlea showing an electron microscopic picture of the organ of corti as indicated by the white box (Adapted from B. Kachar, NIDCD, NIH). In the boxed part, the two types of hair cells are visible. On the left side, a single-row of inner hair cells is visible while more to the right three rows of hair cells can be observed. The tectorial membrane is separated from the outer hair cell bundles due to the preparation techniques that were used.

In summary, the outer ear (1) receives sound (via pressure waves traveling through the air) and conducts it to the eardrum. It thereby translates air vibration in mechanical vibration. The middle ear performs (2) impedance matching between vibration in air and vibration in fluids and is capable of attenuation of loud sounds by the acoustical reflex. Finally, the inner ear functions (3) as a frequency analyzer and converts mechanical (fluid) vibration into electrochemical signals. The next section describes the path of the signals—the auditory pathway.

The auditory pathway

The organ of Corti, with its outer and inner hair cells is responsible for the conversion of mechanical vibration to electrical neural signals. Afferent fibers, sensory nerves carrying information from the periphery to the brainstem, constitute the auditory nerve (nVIII) and carry the information from the inner ear to the cochlear nucleus.

As a result of the tonotopic mapping of the cochlea, each nerve fiber is most sensitive to a particular frequency, its characteristic frequency. Information regarding the frequency of the stimulus is not only determined by the place along the basilar membrane that shows maximal resonance (i.e., place theory), but is also coded by the discharge rate (i.e., the temporal theory of frequency coding). Note that at frequencies above approximately 5000 Hz the phase-locking of the firing pattern to the stimulus is not possible anymore, since the discharge of auditory nerve fibers is limited to a minimum period of approximately 0.2 msec (called the refractory period).

Sound intensity is also preserved in the firing rate in auditory nerve fibers. It is assumed to be encoded by an change in the discharge rate of a single nerve fiber. In order to encode the 140 dB dynamic range of humans, information from multiple nerve fibers is used, combining information from low-, medium-, and high threshold fibers—each with an individual dynamic range of less than 35 dB (Ehret and Romand, 1997; Yost, 2000).

Figure 1.3 illustrates schematically the principal ascending auditory pathway. The auditory nerve terminates in one of the divisions of the (ipsilateral) cochlear nucleus, the anterior ventral cochlear nucleus (AVCN), the posterior ventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN). The frequency spectrum of the sound stimulus is preserved in the cochlear nucleus. The lower frequency axons innervate the lateral-ventral portions of the dorsal cochlear nucleus and the ventrolateral portions of the anteroventral cochlear nucleus. In contrast, the axons from the higher frequency organ of corti hair cells project to the dorsal portion of the anteroventral cochlear nucleus and the dorsal-medial portions of the dorsal cochlear nucleus.

The (AV)CN projects information bilaterally to the next nucleus in the auditory pathway: the superior olivary complex (SOC). Binaural processing takes place at this level – especially sound localization in the horizontal plane–by means of interaural time differences, processed by the medial superior olive (MSO) and interaural level differences, processed by the lateral superior olive (LSO).

The SOC, in turn, projects to the inferior colliculus (IC) via the lateral lemniscus (LL). The majority of the ascending fibers from LL project to IC. Parts of the ascending auditory pathways converge here. IC acts as an integrative (relay) station and is involved in the integration and relay of multimodal sensory perception, mainly startle reflex and vestibulo-ocular reflexes. Not only is there an indirect path from the CN via the SOC and LL but there are also direct connections from the CN and SOC. So, the CN and SOC both project to the IC.

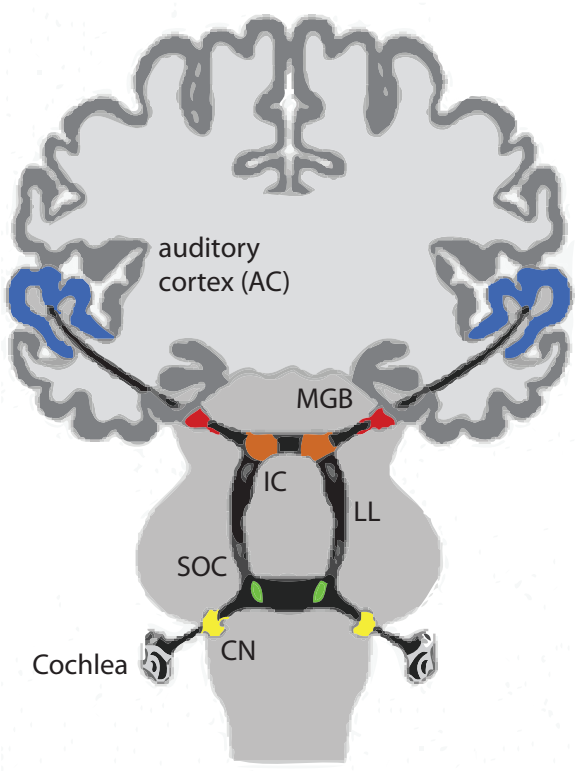

Figure 1.3 Schematic outline of the ascending auditory pathway. Fibers project from the inner hear cells in the cochlea to the cochlear nucleus (CN). From this point on the system is a binaural system. This auditory pathway projects to both, bilateral superior olivary complex (SOC) nuclei where horizontal sound localization takes place. Signals are transmitted via the lateral lemniscus (LL) to the inferior colliculus (IC). The IC not only receives information from this binaural pathway but also receives information from the contralateral and ipsilateral CN. The IC is the major auditory processing center of the midbrain and receives multimodal information. From the IC, signals are projected to the bilateral medial geniculate nuclei of the thalamus (MGB). From this point, signals are projected to the auditory cortex (AC) in the temporal lobes. (Adapted with kind permission from: C.Liberman and J.Melcher; Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston.)

The IC comprises three major nuclei: the central nucleus (ICC), the external nucleus (ICX) and the pericentral nucleus. It provides the first level where horizontal and vertical sound localization are integrated and is also responsive to specific amplitude modulation frequencies, which might be responsible for detection of the pitch of a (complex) auditory signal. In addition, the IC is a multimodal nucleus, receiving input from the somatosensory system, via the spinal trigeminal system and the dorsal column nuclei (Zhou and Shore, 2006; Dehmel *et al.*, 2008), and it may play a role in somatosensory modulation of perceptual characteristics of tinnitus.

From the IC, connections pass to the bilateral medial geniculate body (MGB) of the thalamus. The thalamus is the major relay station for information to the cortex for almost all sensory systems, including the somatosensory system, the visual system (through the lateral geniculate body) and the auditory system. The MGB, in turn, projects to the auditory cortex (AC), which is located in the temporal lobe.

The auditory cortex

The primary destination of an auditory signal is –after several successive processing stages in the brainstem, midbrain and thalamus– a cortical area that corresponds to the auditory cortex. The auditory cortex is distributed over the upper part of the temporal lobe. Figure 1.4 shows the superior temporal surface with some distinct areas. It shows the transverse gyrus extending in the posteromedial to anterolateral direction which is called Heschl's gyrus (HG). The exact morphological features of the HG may vary between individuals and may also form a double or forked gyrus (Leonard *et al.*, 1998). Anterior to the HG is the transverse temporal gyrus that separates it from the planum polare (PP). The PP extends to the anterior tip of the temporal lobe, the temporal pole. Posterior to the HG is the planum temporal (PT), a triangular area that includes Wernicke's area, one of the most important functional areas for language. Note that this Wernicke's area is traditionally mostly functionally lateralized towards the left hemisphere.

The auditory cortex can be divided in several areas on the basis of the cell types, the cytoarchitecture. This division is based on the connectivity, neuro-chemical characteristics and cell morphology and composition of cell layers in the cortex, and follows the scheme according to Brodmann (1909). The auditory cortex can be divided in area 41 (BA 41, see figure 1.4B), which roughly coincides with the primary auditory cortex. Adjacent to this area is area 42 (BA 42), which is also known as the secondary auditory cortex. Surrounding these areas is area 22 (BA 22), the auditory association cortex. Although the architectonic location of the PAC does not always register with the morphological features of the cortex, mainly due to differences between subjects, it is approximately located in the medial two-thirds of the HG (Rademacher *et al.*, 2001); see figure 1.4A. Since there is no fixed nomenclature, the PAC may to a large degree also correspond to A1, and may largely overlap with three sub-areas: Te1.0, Te1.1 and Te1.2 (Morosan *et al.*, 2001).

Although there is evidence of a tonotopic mapping in the auditory cortex in non-human mammals (Ehret and Romand, 1997), the evidence for such a mapping in humans is sparse, and varies between several studies (Formisano *et al.*, 2003; Talavage *et al.*, 2004).

Relatively little is known about the functional differences between areas in the PAC regarding the processing of sound. The same holds for the surrounding (secondary) areas, often referred to as belt and parabelt areas.

Primary auditory areas presumably perform the processing of basic sound features like frequency and intensity level analysis (Hall *et al.*, 2001) while non-primary areas may play a role in spectrotemporally more complex sounds (Hall *et al.*, 2003; Langers *et al.*, 2003). It has been suggested that the cortical processing results in the re-encoding of incoming auditory signals into separate (parallel) streams. One of these streams seems involved in the identification of the (auditory) object –the 'what' pathway– while the other stream is engaged in the localization of the auditory object—the 'where' pathway (Alain *et al.*, 2001).

So, although much work has been done to characterize central auditory system processing stages, even the basic features of the representation of sound in the auditory brain (i.e. the auditory pathway from the periphery to the auditory cortex) remain to a large extent unknown.

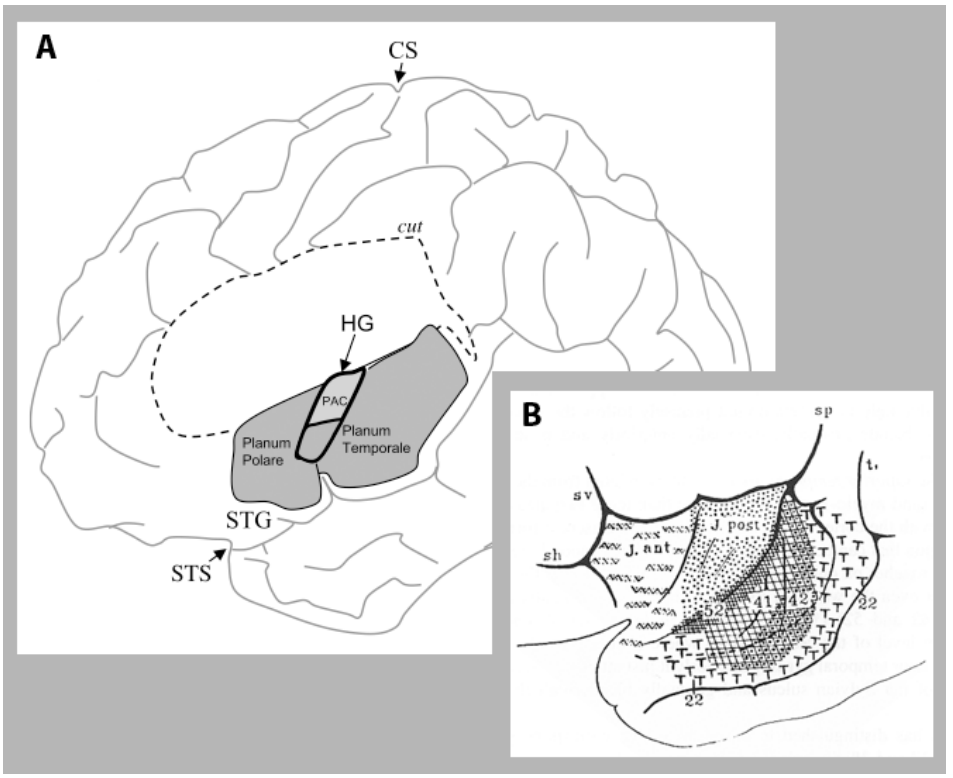

Figure 1.4 Panel **A**: Lateral view of the human auditory cortex exposing the superior temporal gyrus (STG). It shows Heschl's gyrus (HG), of which the medial two-third part corresponds to the primary auditory cortex (PAC). The areas surrounding the PAC include the planum polare and the planum temporale. The central sulcus (CS) and superior temporal sulcus (STS) are indicated as major anatomical landmarks; adapted from: Hall *et al.* (2003). Panel **B** shows the cytoarchitectonic organization of the same area as in panel (A), now according to Brodmann (1909). Indicated are the parainsular area (BA 25), the anterior or medial transverse temporal area (BA 41) and posterior or lateral temporal posterior area (BA 42). Surrounding these areas is the superior temporal area known as BA 22. The superior temporal sulcus is indicated as t1.

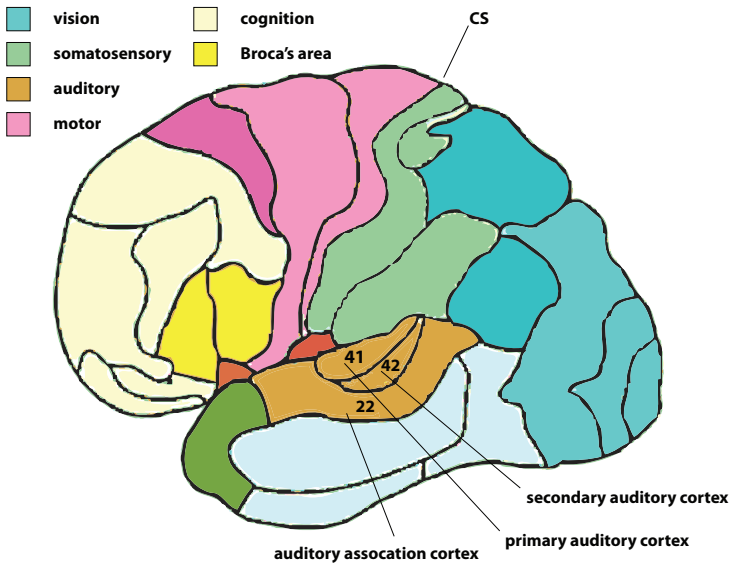

Figure 1.5 The lateral view of the cytoarchitectural areas in the brain according to Brodmann (1909). In addition to auditory areas (BA 41,42 and 22), areas that correspond with vision, motor function, somatosensory perception and cognition are depicted. Adapted from Mark Dubin, http://spot.colorado.edu/~dubin/talks/brodmann/brodmann.html

1.3 Tinnitus

The main theme of this thesis is tinnitus and its potential neural correlate. It is thus important to introduce tinnitus and explain some basic features of tinnitus. Tinnitus can be differentiated into subjective and objective tinnitus. In objective tinnitus, sound from the body leads to an auditory percept via normal hearing mechanisms, i.e. by stimulation of the hair cells in the inner ear. Consequently, objective tinnitus is not a true hearing disorder in the sense that the hearing organ is affected. Rather, normal perception of an abnormal sound source in the body (somatosound) causes the complaint. Typically, sources of objective tinnitus are of vascular or muscular origin. Due to vascular anomalies (Chandler, 1983), vibrations due to pulsatile blood flow near the middle or inner ear (Weissman and Hirsch, 2000; Liyanage *et al.*, 2006; Sonmez *et al.*, 2007) can become audible. Also, involuntary contraction of muscles in the middle ear (Abdul-Baqi, 2004; Howsam *et al.*, 2005) or in palatal tissue (Fox and Baer, 1991) may cause objective tinnitus. Objective tinnitus is rare and has been described only in case reports.

Subjective tinnitus is far more common than objective tinnitus. In contrast to objective tinnitus, there is no (overt) acoustic stimulus present in cases of subjective tinnitus. Yet, the distinction between objective and subjective tinnitus (Møller, 2003; Lockwood *et al.*, 2002) remains debatable (Jastreboff, 1990) in the sense that the definition is based on whether a somatosound can be detected or objectified by an external observer, rather than on the possible underlying mechanisms.

Almost all adults have experienced some form of tinnitus, mostly transient in nature, at some moments during their life. However, in 6–20% of the adults, tinnitus is chronic and for 1–3% tinnitus severely affects the quality of life. Tinnitus is more prevalent in men than in women and its prevalence increases with advancing age (Axelsson and Ringdahl (1989); Lockwood *et al.* (2002); see figure 1.6).

Subjective tinnitus has many different forms and varies in character and severity (Stouffer and Tyler, 1990). It can be perceived as an intermittent or a continuous sound (Lockwood *et al.*, 2002; Henry *et al.*, 2005) and can be perceived unilaterally, bilaterally or in the head (Axelsson and Ringdahl, 1989). Although subjects rate their tinnitus as very loud, the tinnitus is typically matched at levels of 5–10 dB sensation level (SL, i.e. the level compared to subjects' own threshold; (Vernon and Meikle, 2003)). In order to fully classify chronic subjective tinnitus, subjects need proper otological examination, audiological assessment and, in addition, need psychological profiling assessing the severity of the tinnitus and the accompanying distress and influence on the quality of life (Bartels, 2008).

Subjective tinnitus is often associated with peripheral hearing loss (Eggermont and Roberts, 2004; Nicolas-Puel *et al.*, 2006), although tinnitus with no or minor hearing loss has also been reported (Stouffer and Tyler, 1990; Jastreboff and Jastreboff, 2003). Many patients describe tinnitus as a sound in one or both ears. Therefore, it has been thought for many years that the tinnitus-related neural activity must also originate from a peripheral source, i.e. the cochlea.

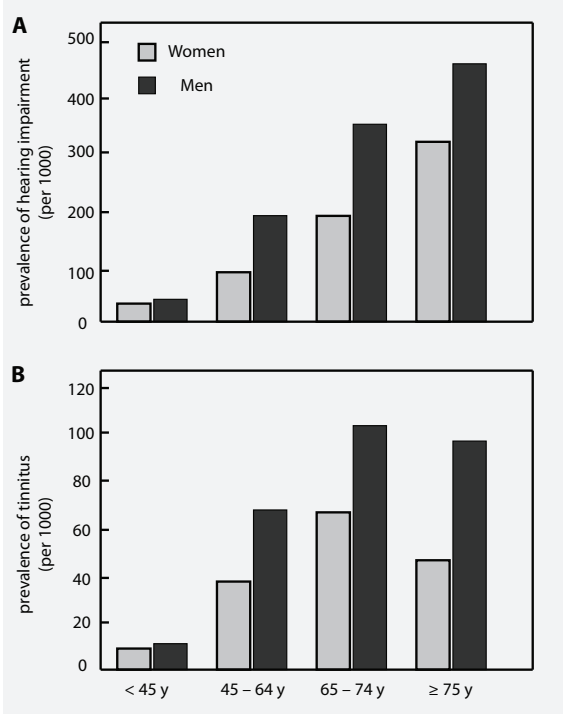

Figure 1.6 The prevalence of hearing impairment (panel A) and tinnitus (panel B); Adapted from: Lockwood *et al.* (2002)

Some clinical observations indicate however, that a peripheral origin of tinnitus cannot account for all forms of tinnitus. In patients that underwent sectioning of the eighth cranial nerve as part of retro-cochlear tumor surgery, tinnitus arose in 34% of the cases (Berliner *et al.*, 1992). Apparently, tinnitus may arise by disconnecting the cochlea from the brain. Sectioning of the eighth cranial nerve has also been applied in tinnitus patients in an effort to provide relief of the tinnitus. This was however not successful in 38–85% of cases (varying from 38% as reported by Barrs and Brackmann (1984) to 85% as reported by House and Brackmann (1981); reviewed earlier by Kaltenbach *et al.* (2005)). Clearly, in these cases, where the cochlea is disconnected from the brain, central mechanisms must be responsible for the tinnitus.

Changes in the central auditory system may be responsible for tinnitus. A popular hypothesis describes tinnitus as a change in the balance between excitatory and inhibitory input which may cause hyperactivity. The cochlea not only provides excitatory input to the cochlear nucleus but provides also abundant inhibitory input. Now, if the cochlea is impaired, both excitatory and inhibitory input to central auditory structures are reduced, but often inhibitory input is reduced more than the excitatory input (Kim *et al.*, 2004). This causes a shift in the balance between inhibition and excitation. Tinnitus is often associated with loss of hearing (due to injuries to inner and outer hair cells). Such injuries

now reduce the input to central auditory structures, causing disinhibition–a potential basis for neural hyperactivity (Eggermont, 2007b).

Causes of tinnitus are only in rare cases known and often relate to injuries to cochlear hair cells. Ototoxic agents such as certain antibiotics, salicylate and quinine, and intense sound may lead to tinnitus (Jastreboff and Sasaki, 1986; Jastreboff *et al.*, 1988; Kaltenbach, 2000; Eggermont and Kenmochi, 1998). Also, disorders of the central auditory system, such as meningitis and stroke, are known to cause tinnitus, accompanied by the disturbed perception of sound.

Tinnitus may also be influenced by the somatosensory modality (presumably via the so-called non-classical, or extralemniscal, auditory pathway; (Møller *et al.*, 1992)) and by changes in gaze (Cacace *et al.*, 1994a; Baguley *et al.*, 2006). Also, chemical substances, such as lidocaine are known to modulate characteristics of tinnitus (Melding *et al.*, 1978). These forms of modulation have been used in combination with functional imaging experiments as reviewed in chapter 2.

Summarizing, it should be noted that there is no single form of tinnitus and it is thus of great importance to distinguish several types of tinnitus since, in principle, each of these forms may have a different etiology and therapeutic approach.

1.4 Functional magnetic resonance imaging

Physics

Magnetic resonance imaging (MRI) techniques all exploit nuclear magnetic resonance and make use of a quantum mechanical property called nuclear spin. This spin characteristic can be, in a classical approach, regarded as the rotation of a particle around its own axis. Associated with this spin characteristic is a magnetic property and represents the angular momentum that charged rotating nuclei possess. When these nuclei are placed in a strong external magnetic field (B_0) they precess around the axis along the direction of the field (often called the $z-$axis) since the quantum mechanical restrictions prevent an exact alignment along the main field. The frequency of this precession is called the Larmor frequency and depends on the strength of the magnetic field.

The most abundant nuclei in the human body are the protons that form the hydrogen atom. When placed in an external field, they will align to the field, forming a distribution of either parallel or anti-parallel to the external field. Since the parallel alignment is energetically favorable, a greater fraction will align parallel. The net alignment of the nuclei together form a net steady state magnetization M_0. Note however that the overall behavior of large number of nuclei can be described in a classical fashion (Jezzard *et al.*, 2001) but the individual nuclei need a quantum-mechanical approach (Haacke *et al.*, 1999).

By means of 90° radio frequent (RF) pulses of the right frequency (i.e. the Larmor frequency, the resonance frequency that gives the most efficient energy transfer), the magnetic moment can be tilted into the transverse (xy)-plane. As a result, the component of the magnetization parallel to the applied magnetic field (i.e. the longitudinal magnetization, M_z) will decrease, and the component perpendicular to the field (the transverse magnetization, M_{xy}) will develop. A receiver can now detect the precession in the transversal plane.

Once the RF pulse ends, the return to the favored (parallel) state begins–called relaxation. First, the longitudinal component will grow back to its steady state magnetization by an exponential (longitudinal) relaxation process with a time constant T_1, involving spin-lattice interaction (see figure 1.7). For brain tissue, this T_1 time constant is of the order of 1 s.

Second, the transverse component will decrease to zero magnitude, characterized by two simultaneous complex effects. First, there are spin-spin interactions: interactions of individual spins that influence each other in such a way that the initial coherent phase becomes dephased. This dephasing is characterized by a time constant T_2. Furthermore, transverse spins also dephase due to inhomogeneities in the main magnetic field (B_0) resulting in a dispersion in Larmor frequencies, corresponding to a dispersion of the precession frequency. The combined effect of spin-spin relaxation and B_0-field inhomogeneities is characterized by a time constant T_2^*.

In summary, RF causes the longitudinal magnetization to flip to the transversal plane.

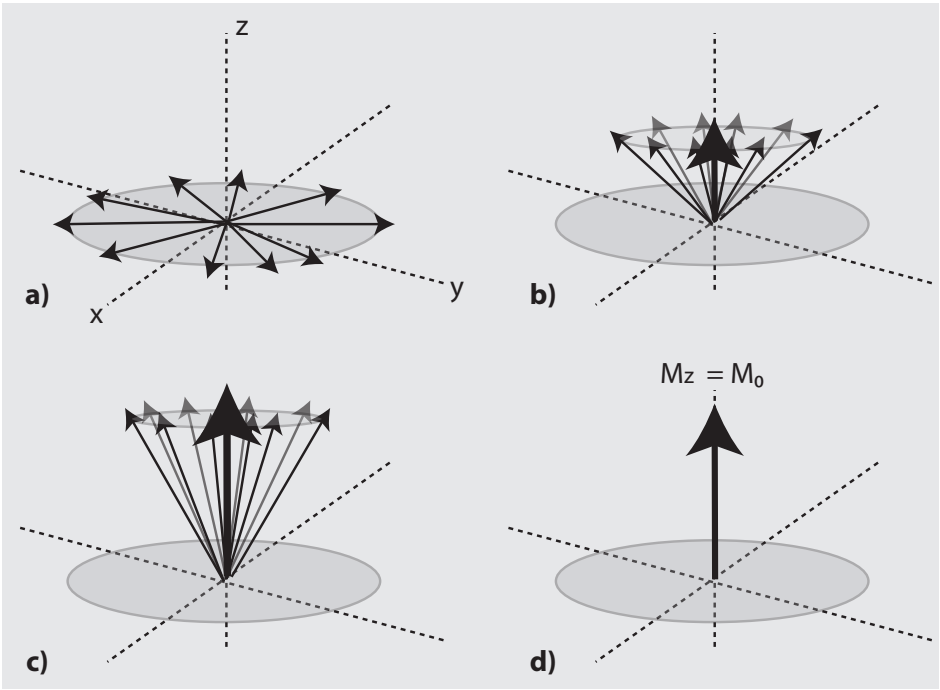

Figure 1.7 T_1 relaxation. After an 90° RF pulse has flipped all magnetization in the xy-plane (a), the magnetization relaxes back (b–d) to its equilibrium condition (d). Together they form a net steady state magnetization vector M_0. The longitudinal component slowly relaxes back according to an exponential relationship $M_z = M_0 \cdot \big(1 - \exp(-t/T_1)\big)$.

After the RF has stopped, the magnetization will relax back to the steady state magnetization. The magnetization will precess around the $z-$axis and will emit RF electromagnetic radiation and can be detected. These two types of relaxation, in addition to the number of protons in tissue, together comprise the contrast mechanisms in MR imaging.

Image formation

The Larmor frequency is essential in the detection of spin properties. The emission of RF from the rotating transverse magnetization is used to extract information about the location of the nuclei. Because the amount of RF is proportional to the density of protons (hydrogen nuclei), which differs between tissue types, anatomical images can be constructed by detecting the power of the RF that is emitted from a certain location. The magnitude can, however, not be determined directly, since signals from other locations that contain protons will interfere. By now adding gradients to the main magnetic field, a spatial distribution of signals, each with a different resonance Larmor precession frequency, can be detected. Thus a spatial variation in the magnetic field strength alters the resonance frequency and can be used to form images.

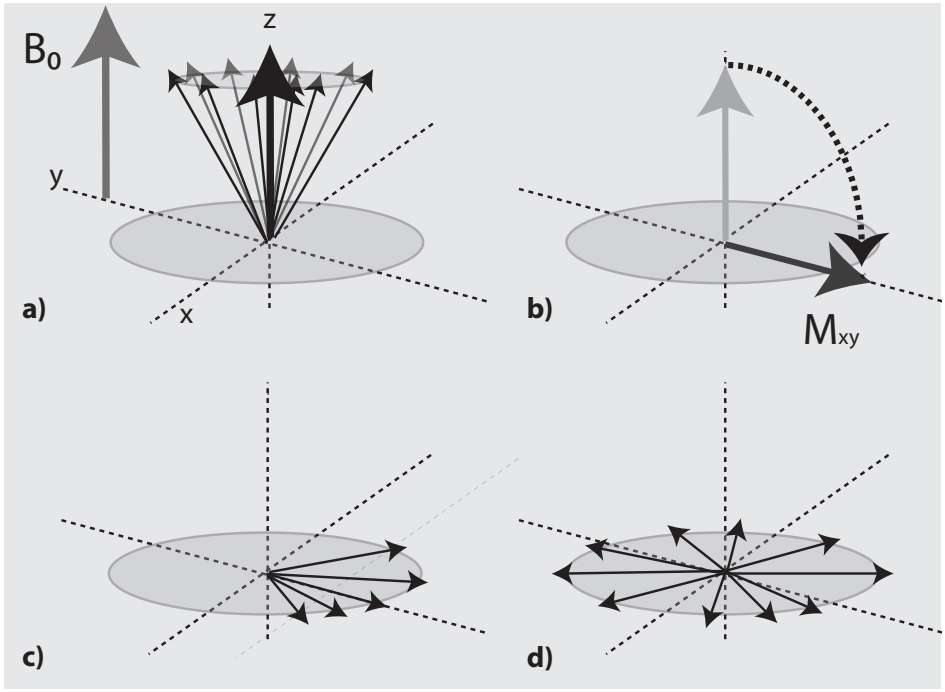

Figure 1.8 The application of an RF pulse and T_2 relaxation. After an 90° RF pulse has flipped all magnetization in the xy-plane (b), dephasing starts and decreases the transversal magnetization vector (c) to its equilibrium condition of a net transversal magnetization of zero (d). The transverse component of the magnetization decreases according to $M_{xy} = M_0 \cdot \exp(-t/T_2)$

In short, a pulse sequence contains the following items (Haacke *et al.*, 1999): First, the magnetization is given a chance to fully relax (figure 1.7d). Next, a 90° RF pulse is applied, flipping the magnetization into the transverse plane (figure 1.8b). The magnetization is the xy-plane precesses around the z-axis with the Larmor frequency that codes the location of the protons. The signal-emitting transverse magnetization will shrink (T_2 relaxation, figure 1.8c) and simultaneously, the longitudinal magnetization grows slowly back to its steady-state magnitude. The gradients will cause an additional dephasing, since protons at different locations will have different resonance frequencies, causing increased spin-spin interactions and lower T_2^* time constant. To recover signal losses, often another RF pulse is applied. This 180° pulse flips all magnetization 180°. This causes all spins with a phase lag to be turned into a phase lead and the magnetic moment refocusses again. This will, in turn, yield an RF pulse which can be detected—a spin echo.

For functional imaging of brain activity, a T_2^*-weighted sequence is most often used since it is sensitive to changes in the oxygen concentration in blood–a marker of neural

activity.

From neural activity to differences in $T2$

Functional MRI is an indirect method for measuring brain activation (Jezzard *et al.*, 2001). It does not measure electrical or magnetic activity that is generated by signal conduction mechanisms of neurons, like electro- and magneto-encephalography (EEG and MEG) or evoked potential (EP) methods. Rather, it measures changes in the magnetic properties of the blood. Figure 1.9 shows schematically the events that underly PET and fMRI signal intensity changes that may relate to task related changes.

Although there are some functional MR imaging methods that specifically measure changes in blood volume (VASO, vascular space occupancy; Lu *et al.* (2003)) or cerebral blood flow (Golay *et al.*, 2004; Petersen *et al.*, 2006), most fMRI methods make use of the blood oxygen level dependent (BOLD) contrast. This technique is based on the increase in signal intensity caused by an increase in oxygen concentration of blood (Ogawa *et al.*, 1990).

Synaptic activity in neurons, both excitatory and inhibitory, correspond to the consumption and increase in metabolic rate of oxygen (Logothetis *et al.*, 2001). The metabolic reserve within neurons and neighboring glia cells is limited and additional oxygen is needed to fulfill the oxygen need. As a response, vascular dilation takes place–the increase in diameter of blood vessels, which in turn leads to an increased cerebral blood volume (CBV) and cerebral blood flow (CBF). The corresponding increase in oxygen level now exceeds the need for it, causing an increase in oxygen-rich blood on the venous side of the neural activity. As a result, the ratio of deoxygenated hemoglobin to oxygenated hemoglobin will drop.

If oxygen is bound to hemoglobin (oxyhemoglobin), the ferrous core is diamagnetic similar to the surrounding (brain) tissue, causing hardly any disturbance of the local magnetic field homogeneity. Deoxyhemoglobin, on the contrary, is paramagnetic and differs strongly from the surrounding tissue and deforms the local magnetic field (susceptibility artifacts). This inhomogeneity now leads to a dephasing of the nuclear magnetic moments, reducing the net transverse magnetization. In summary, deoxygenated blood has a shorter T_2^* than oxygenated blood and forms the basis of the BOLD effect.

Regions of the brain that are active will show an increased CBV and CBF, leading to an increase of the local oxygenation level. This, in turn, will reduce the local field inhomogeneities, and will increase the T_2^*. If an MR imaging sequence is used that is sensitive to T_2^* changes, like with an echo planar imaging (EPI) sequence, this effect will show as a local increase in signal intensity and is called the hemodynamic response signal. By now performing acquisitions during two or more conditions of which one will act as a baseline and the other during some experimental condition (the performance of a certain task), the resulting difference in intensity can be detected and presumably related to the task that is contrasted to the baseline condition.

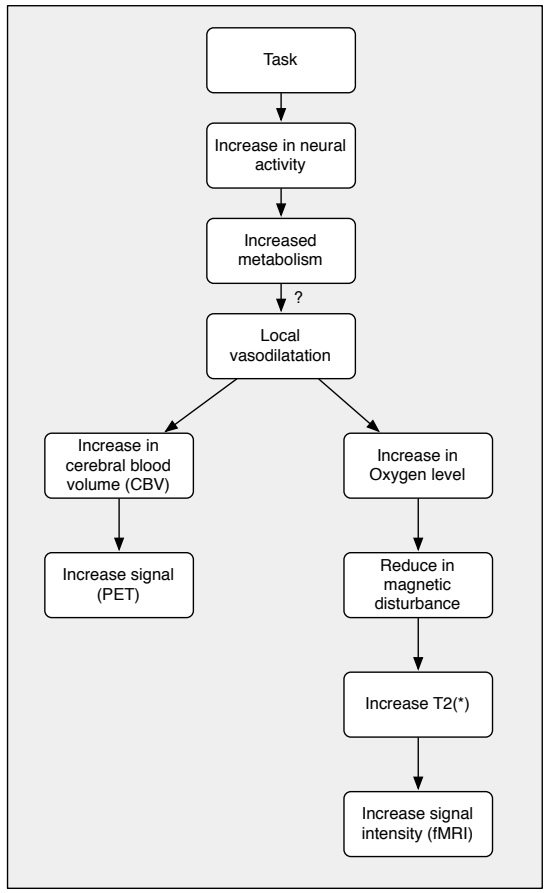

Figure 1.9 A flow chart that describes the events that underly BOLD fMRI signal contrast and PET signal contrast. An experimental task leads to a local increase in neural activity. This leads to increased metabolism for which oxygen is needed. As a consequence, vasodilation takes place leading to increased cerebral blood volume (CBV) and cerebral blood flow (CBF). This in turn can be measured with PET and MRI methods based on arterial spin labeling. The oxygen increase exceeds the actual need and forms an oxyhemoglobin overshoot. This leads to smaller difference in the magnetic disturbances with the surrounding tissue resulting in an increase of T_2^* which can be detected as an increase in signal intensity in the image. The exact neurovascular coupling remains partly unknown, which is depicted by the question mark (?).

From data-acquisition to statistical parameter maps and beyond

In a T_2^* weighted fMRI sequence the hemodynamic response amplitudes have typically a magnitude of only a few percent of the baseline signal level. Measurement noise and physiological fluctuations have a similar magnitude. As a consequence, signals can only be discriminated from noise by taking many acquisitions, and by applying statistical methods to determine which voxels in the brain contain significant contribution from the hemodynamic response.

Before actual signal detection can be performed, a number of (pre)processing steps are needed. Some of these steps are necessary while others may be omitted. The steps as presented here form a basis of processing steps that are considered standard. First, spatial realignment has to be performed to correct for subject movement, and involves estimating the six parameters of an affine, rigid-body transformation that minimizes the difference between each successive scan and a reference scan (usually the first of all scans acquired).

After realignment of the functional data (and optionally, the co-registration of the functional data and an anatomical image), the mean image of the series is used to estimate warping parameters that map onto a canonical standard anatomical space (e.g. Talairach and Tournoux (1988)). This is in most cases a 12-parameter affine transformation followed by non-linear deformations. The primary use of this stereotactic spatial normalization is to facilitate inter-subject averaging.

Next, the functional data can be smoothed by means of convolution with a Gaussian kernel. This improves the signal-to-noise ratio, while on the downside, it reduces the spatial resolution.

After these preprocessing steps, the acquired data may be analyzed on a voxel-by-voxel basis. Functional mapping studies generally use some form of statistical parameter mapping. Statistical parameter maps (SPMs) are images with values that are, under the null hypothesis, distributed according to a known probability density function, usually the Student's t or F-distribution. In general, a general linear model (GLM) is set-up that incorporates the expected time courses of the responses to each of the modeled conditions (X). Using (multiple) linear regression analysis, the amplitude of the coefficients (β) are fitted (Turner *et al.*, 1998).

Statistics are then performed on the regression coefficients to determine the significance of the response to each condition, or a linear combination of these (so-called contrasts that e.g. compare two responses against a baseline level). Analysis of variances (ANOVA) can be performed on the data and assesses whether inclusion of a certain condition (i.e. column in the model X) decreases the residual variance and thus describes part of the data. The resulting significance levels from individual voxels are combined into a SPM, which can be thresholded at a certain p-value (or, equivalently, a t or F-value). Thresholds can be chosen to restrict the statistically expected family-wise error (FWE) rate or the false discovery rate (FDR) below an acceptable level (e.g. 5 %).

Results from multiple subjects can be combined into an analysis on the group level. A fixed effects analysis assumes the effect of interest to be present in all subjects in equal fashion. This makes it very sensitive to activation but may also be vulnerable to outliers in the data. Moreover, given the assumptions underlying this analysis, it is not possible to make inferences regarding the significance of the detected effects in the population as a whole. A random effects analysis, on the contrary, does not assume equal activation patterns and allows the strength of effect to be different between subjects (i.e. the effect of each subject is treated as a random variable). This allows population inferences at the cost of sensitivity.

Although the data analysis in functional neuroimaging had been dominated by the use of multiple linear regression models, novel analysis methods have been introduced that are based on blind source separation techniques (Langers, 2009). Examples of these techniques are methods like principal component analysis (PCA), in combination with independent component analysis (ICA, Hyvarinen and Oja (2000)), which decompose functional neuroimaging data into components with a meaningful neurophysiologic interpretation in the absence of prior information about the experimental paradigm (or even in the absence of an experimental condition, so-called resting state experiments).

2

Neural activity underlying tinnitus generation: Results from PET and fMRI

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Abstract

Tinnitus is the percept of sound that is not related to an acoustic source outside the body. For many forms of tinnitus, mechanisms in the central nervous system are believed to play an important role in the pathology. Specifically, three mechanisms have been proposed to underlie tinnitus:

(1) changes in the level of spontaneous neural activity in the central auditory system,

(2) changes in the temporal pattern of neural activity, and

(3) reorganization of tonotopic maps.

The neuroimaging methods fMRI and PET measure signals that presumably reflect the firing rates of multiple neurons and are assumed to be sensitive to changes in the level of neural activity. There are two basic paradigms that have been applied in functional neuroimaging of tinnitus. Firstly, sound-evoked responses as well as steady state neural activity have been measured to compare tinnitus patients to healthy controls. Secondly, paradigms that involve modulation of tinnitus by a controlled stimulus allow for a within-subject comparison that identifies neural activity that may be correlated to the tinnitus percept. Even though there are many differences across studies, the general trend emerging from the neuroimaging studies reviewed, is that tinnitus in humans may correspond to enhanced neural activity across several centers of the central auditory system. Also, neural activity in non-auditory areas including the frontal areas, the limbic system and the cerebellum seems associated with the perception of tinnitus. These results indicate that in addition to the auditory system, non-auditory systems may represent a neural correlate of tinnitus. Although the currently published neuroimaging studies typically show a correspondence between tinnitus and enhanced neural activity, it will be important to perform future studies on subject groups that are closely matched for characteristics such as age, gender and hearing loss in order to rule out the contribution of these factors to the abnormalities specifically ascribed to tinnitus.

2.1 Introduction

Tinnitus definition and prevalence

Tinnitus is an auditory sensation without the presence of an external acoustic stimulus. Almost all adults have experienced some form of tinnitus, mostly transient in nature, at some moments during their life. However, in 6–20% of the adults, tinnitus is chronic and for 1–3% tinnitus severely affects the quality of life. Tinnitus is more prevalent in men than in women and its prevalence increases with advancing age (Axelsson and Ringdahl, 1989; Lockwood *et al.*, 2002).

Tinnitus can be differentiated into subjective and objective tinnitus. In objective tinnitus, sound from the body leads to an auditory percept via normal hearing mechanisms, i.e., by stimulation of the hair cells in the inner ear. Consequently, objective tinnitus is not a true hearing disorder in the sense that the hearing organ is affected. Rather, normal perception of an abnormal sound source in the body (somatosound) causes the complaint. Typically, sources of objective tinnitus are of vascular or muscular origin. Due to vascular anomalies (Chandler, 1983), vibrations due to pulsatile blood flow near the middle or inner ear (Weissman and Hirsch, 2000; Liyanage *et al.*, 2006; Sonmez *et al.*, 2007) can become audible. Also, involuntary contraction of muscles in the middle ear (Abdul-Baqi, 2004; Howsam *et al.*, 2005) or in palatal tissue (Fox and Baer, 1991) may cause objective tinnitus. Objective tinnitus is rare and has been described only in case reports.

Subjective tinnitus is far more common than objective tinnitus. In contrast to objective tinnitus, there is no (overt) acoustic stimulus present in cases of subjective tinnitus. Like any acoustic percept, tinnitus must be associated with activity of neurons in the central auditory system; abnormal tinnitus-related activity may arise from abnormal cellular mechanisms in neurons of the central auditory system, or may result from aberrant input from the cochlea or non-auditory structures.

The distinction between objective and subjective tinnitus (Møller, 2003; Lockwood *et al.*, 2002) is debatable (Jastreboff, 1990) in a sense that it is based on whether a somatosound can be detected or objectified by an external observer, rather than on the possible underlying mechanisms. As far as we can tell, all neuroimaging studies reviewed in this paper describe results for tinnitus where there is no objective sound source. In other words, this review is about subjective tinnitus.

Tinnitus and the central auditory system

Subjective tinnitus is often associated with peripheral hearing loss (Eggermont and Roberts, 2004; Nicolas-Puel *et al.*, 2006), although tinnitus with no or minor hearing loss has also been reported (Stouffer and Tyler, 1990; Jastreboff and Jastreboff, 2003). Many patients describe tinnitus as a sound in one or both ears. Therefore, it has been thought for many years that the tinnitus-related neural activity must also originate from a peripheral source, i.e., the cochlea.

Some clinical observations indicate however, that a peripheral origin of tinnitus cannot account for all forms of tinnitus. In patients that underwent sectioning of the eighth cranial nerve as part of retro-cochlear tumor surgery, tinnitus arose in 34% of the cases (Berliner *et al.*, 1992). Apparently, tinnitus may arise by disconnecting the cochlea from the brain. Sectioning of the eighth cranial nerve has also been applied in tinnitus patients in an effort to provide relief of the tinnitus. This was however not successful in 38–85% of cases (varying from 38% as reported by Barrs and Brackmann (1984) to 85% as reported by House and Brackmann (1981); reviewed earlier by Kaltenbach *et al.* (2005)). Clearly, in these cases, where the cochlea is disconnected from the brain, central mechanisms must be responsible for the tinnitus.

Evidence for changes in the firing pattern of neurons in the central auditory system as possible substrate of tinnitus is supported by research on tinnitus using animal models. Noise trauma and ototoxic drugs, which are known to cause peripheral hearing loss and tinnitus in humans, result in behavioral responses in animals that are consistent with the presence of tinnitus (reviewed in Eggermont and Roberts (2004)). These manipulations also result in changes of spontaneous neural activity in several auditory brain centers. For example, noise-induced trauma decreases spontaneous firing rates (SFRs) in the eighth cranial nerve and increases the SFRs at several levels in the auditory brainstem and cortex (Noreña and Eggermont, 2003; Kaltenbach *et al.*, 2004). Other possible neural correlates of tinnitus that have been investigated are changes in burst firing and neural synchrony (Noreña and Eggermont, 2003; Seki and Eggermont, 2003). Apparently, peripheral hearing loss results in a reduction of afferent input to the brainstem, which leads to changes in neural activity of the central auditory system, hereby causing tinnitus.

In addition to these possible changes in spontaneous neural activity, cortical tonotopic map reorganization has been recognized as possible neural correlate of tinnitus (Muhlnickel *et al.*, 1998; Seki and Eggermont, 2003; Eggermont, 2006). All of the above may occur as a consequence of an imposed imbalance between excitation and inhibition in the auditory pathway.

None of the proposed mechanisms has been proven unequivocally as a substrate of tinnitus in humans. Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) are imaging modalities that can be used to study neural activity in the human brain. Both techniques can assess some aspects of human brain activity and, hence, may identify mechanisms that underlie the generation of tinnitus in humans. This review focuses on the application of these two functional imaging methods and summarizes and discusses results of studies that use these methods to study tinnitus.

2.2 Functional imaging methods

Introduction

Functional imaging methods are used to study dynamic processes in the brain and localize brain areas involved in perception or cognition. Various methods are available that differ in spatial resolution, temporal resolution and their degree of invasiveness and can measure several important aspects of hypothesized tinnitus-related changes in neural activity.

Electroencephalography (EEG) and magnetoencephalography (MEG) are noninvasive methods that respectively measure the electrical and magnetic fields, resulting from (synchronized) firing of neurons. These techniques have a high temporal resolution ($\sim$ 1 ms) and a spatial resolution in the order of 1 mm. EEG and MEG can – given their high temporal resolution – give detailed insight in the temporal aspects of brain dynamics and may, for example, be used to assess possible tinnitus-related differences in neural synchrony (Seki and Eggermont, 2003; Noreña and Eggermont, 2003). In humans, power differences in the spectrum of the EEG and MEG signal in subjects with tinnitus compared to control subjects were reported (Weisz *et al.*, 2005a,b; Llinas *et al.*, 2005).

This review focuses on the results of studies that have used positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) in finding neural correlates of tinnitus in humans. Both methods measure signals that are only indirectly related to the magnitude of neural activity. A change of neuronal activity alters the local metabolism and perfusion of the brain (Raichle, 1998; Gusnard *et al.*, 2001; Raichle and Mintun, 2006). PET mainly measures a change in regional cerebral blood flow (rCBF), while most fMRI methods register a blood oxygen level dependent (BOLD) signal. In addition to BOLD-fMRI, other fMRI methods are available that are based on e.g., arterial spin labeling (Detre and Wang, 2002) or vascular space occupancy (Lu *et al.*, 2003). These methods, however, have not yet been used to assess tinnitus.

The most important information obtained from these techniques are the location, the extent and the magnitude of neural activity. Therefore, the question that may be addressed by the application of fMRI and PET is: which brain regions have an abnormal amount of neural activity in tinnitus subjects?

Positron emission tomography

PET imaging measures the regional cerebral blood flow (rCBF), using the uptake of a radioactive tracer injected in the blood circulation. An increase in neural activity causes the blood flow to increase regionally in response to a higher oxygen and glucose demand. The radioactive decay of the tracer results in the emission of photons, which are detected by the PET-scanner.

There are some limitations in using PET. By using radioactive tracers, ionization is induced in the human body, making it less suitable for repeated measurements of single subjects. A second limitation is the limited temporal resolution. The temporal resolution, which is determined by the half-life time of the employed tracer, is at best 2 min when

using labeled water ($H_2{}^{15}O$). Data is accumulated throughout this period and hence, no inferences can be made on a smaller timescale. A change of experimental condition within this period is not practically feasible. In addition, there is a limited spatial resolution due to the size of the detectors ($4 - 5$ mm). An additional inherent limitation to the spatial resolution is determined by the maximum free path of a positron before annihilation takes place, which varies from 2.4 mm (^{18}F) to 8.2 mm (^{15}O) in water (Weber *et al.*, 2003).

An important advantage of PET, especially for auditory research is that it is a silent imaging technique. Hence, interference of the scanner noise with the experimental design is minimized (Johnsrude *et al.*, 2002; Ruytjens *et al.*, 2006). Moreover, in contrast to fMRI, patients with implants containing metal (e.g., cochlear implants) can safely participate in PET studies. Finally, steady state measurements can be made using PET for which fMRI is not suitable (see 2.3).

Functional Magnetic Resonance Imaging

Functional MRI is another method to measure neural activity in the human brain. In short, hydrogen nuclei (protons) in the body display magnetic resonance behavior in the presence of the strong magnetic field of an MRI scanner. In MRI acquisitions, nuclei are exited by an electromagnetic pulse and their behavior after this pulse is characterized by two relaxation times: $T1$ and $T2/T2^*$. These time constants and the density of mobile protons are properties of the tissue and determine the local signal intensity. Differences in these properties determine the contrast in an MR image between various types of tissue.

Functional MRI relies on the difference in magnetic properties of oxygenated and deoxygenated blood. During an fMRI experiment, task-related increases in neural activity and metabolism lead to an increase in CBF. The local increase in available oxygen however exceeds the need for oxygen. As a result, the amount of oxygen in the blood increases in the area associated with the oxygen need. Hemoglobin contains a ferrous core that changes with respect to its magnetic properties when it binds to oxygen. The change in oxygenation level will therefore lead to a change in the magnetic susceptibility of blood, leading to a change in the MR signal (Ogawa *et al.*, 1990). The combination of increased rCBF accompanied with an increased blood oxygen level leads to a blood oxygen level dependent (BOLD) effect. This effect is used as contrast mechanism in functional MR imaging. Therefore, like PET, fMRI provides an indirect measure of neuronal activity.

A major limitation – especially in auditory research – is the acoustic noise produced by the scanner. During scanning, the MR scanner typically produces over 100 dB (SPL) of acoustic noise, making it difficult to segregate responses to experimental (auditory) stimuli from those to ambient scanner noise. A partial solution is the use of a sparse temporal sampling design (Hall *et al.*, 1999), where a silent gap is inserted between successive scans, giving enough 'silence' to present experimental stimuli to subjects and detect the response even with low sound pressure level stimuli (Langers *et al.*, 2007).

In addition to the produced acoustic noise, there are a number of contraindications for MRI research in humans. These contraindications include the presence of metal implants

in the body. The fast switching of the magnetic fields in the MRI scanner may produce heat in the implant. Also, magnetic forces may cause dislocation of implants. These disadvantages make fMRI unsuitable for studies that aim to evaluate the effect of electrical implants for the treatment of tinnitus.

The main advantages of using fMRI compared to PET are the higher temporal resolution as well as the lack of ionizing radiation. This last point makes longitudinal studies of subjects possible. See Logothetis (2008) for a more in-depth review on fMRI.

2.3 Neuroimaging and tinnitus

Studies in animal models of tinnitus indicate that tinnitus may be related to abnormal spontaneous firing rates (SFRs) in auditory neural structures (Noreña and Eggermont, 2003; Seki and Eggermont, 2003). Unfortunately, some current neuroimaging techniques, especially fMRI, do not allow for the direct measurement of spontaneous firing rates. When using fMRI, there is an inherent signal from gray matter, white matter and cerebral spinal fluid depending on the imaging sequence used. These signals are based on tissue properties rather than a measure of neural activity like the uptake of oxygen ($[H_2{}^{15}O]$-PET) or glucose (FDG-PET) in PET imaging. The signal values as measured with fMRI can therefore not be quantified easily and thus, a value of an absolute baseline (a possible equivalent of spontaneous firing rates) cannot be determined.

Instead, fMRI relies mostly on the modulation of neural activity by some controlled experimental condition. Also PET, in combination with a tracer that has a short half-life time, can be used to measure differential activity. By measuring either rCBF with PET, or BOLD signals with fMRI in two (or more) conditions, differences between states (within single subjects) can be detected and may be used to assess neural activity (Ogawa *et al.*, 1990).

Several paradigms have been applied to assess neural correlates of tinnitus. One method employs sound stimuli and measures sound-evoked responses. Then, possible mechanisms related to tinnitus are inferred from the measured responses in the central auditory pathway. A second method relies on the ability of a subgroup of subjects with tinnitus to manipulate their tinnitus by somatic modulation. Examples discussed here are jaw protrusion and cutaneous-evoked tinnitus. A third method is rapid change of gaze or tonic lateral gaze causing or modulating tinnitus. The fourth method is based on pharmaceutical intervention that causes a temporal change of the tinnitus (e.g., lidocaine). Finally, in a subcategory of subjects, tinnitus is temporarily reduced following the offset of an external acoustical stimulus (Terry *et al.*, 1983; Roberts, 2007). This phenomenon is referred to as residual inhibition and may also be used as the basis of an experimental paradigm in functional imaging experiments. In all these paradigms neural activity is altered by the presentation of an external stimulus or by some manipulation that changes the perceptual characteristics of tinnitus. These may result in a measurable change in signal between experimental conditions.

In addition to this differential (within-subjects) method of measuring neural activity, PET imaging can be used to assess possible changes in steady state levels of neural activity. PET signals (i.e., rCBF) can be scaled to a standardized mean value for the whole brain (using e.g., grand mean scaling), enabling a between-subjects approach to assess possible tinnitus-related differences between subject groups.

Although conventional BOLD fMRI cannot easily be used to assess spontaneous neural activity (like SFRs), there are new potential methods developed that may assess baseline levels. One of these studies makes use of CO_2, saturating the BOLD response completely, therefore providing a 'ceiling'-level that might be used as a reference to assess baseline lev-

els of activity (Haller *et al.*, 2006). These techniques however have not yet been used to study tinnitus.

In this review, neuroimaging experiments on tinnitus are grouped on the basis of their experimental paradigm and discussed accordingly. It has become evident from these experiments that various brain areas play a role in tinnitus. In the discussion section, an overview will be given of these areas and their importance in tinnitus. Given the various definitions of (especially) cortical auditory areas we adopt the following nomenclature: The primary auditory cortex (PAC) corresponds to Brodmann area 41 (BA 41), the secondary auditory cortex corresponds to BA 42 and the auditory association cortex corresponds to BA 21, 22 and 38. For each study we interpret the results based on the Brodmann nomenclature regardless of the nomenclature used by the authors themselves. In many cases, the Brodmann areas were given but in some cases we had to translate the areas according to our nomenclature.

Table 2.1 gives a summary of the studies included in this review. For each study, we describe which imaging modality was used, which experimental design was used and how many subjects were included. In addition, the table shows whether subject groups were matched based on hearing levels and age. Table 2.2 gives a summary of reported effects on rCBF or BOLD signal of tinnitus related changes using various experimental paradigms. Each column corresponds to one type of paradigm. The symbols indicate several types of change in rCBF or BOLD signal that may correlate with tinnitus in several brain areas (represented by each row in the table).

Differences in sound-evoked neural activity as an attribute of tinnitus

Several studies measured sound-evoked activity in subjects with tinnitus and compared these responses to those in subjects without tinnitus. Both noise (either broadband or narrow-band noise) and music have been used as experimental stimuli. All studies on sound-evoked responses mentioned in this section made use of fMRI.

Melcher *et al.* (2000) examined sound-evoked activation to monaural and binaural noise stimuli. For the inferior colliculus (IC), a percentage signal change was calculated, comparing the sound-evoked response to a silent baseline condition. Compared to controls, lateralized tinnitus subjects showed an abnormal small signal change in the IC contralateral to the tinnitus percept, but not ipsilateral. Melcher *et al.* (2000) argued that tinnitus corresponds with abnormally elevated neural activity. When an external stimulus was presented, the hemodynamic response reached saturation, resulting in a reduced difference between the two conditions (i.e., sound on vs. sound off). This reduction would explain the low signal change in patients compared to controls.

In an unpublished conference abstract Melcher *et al.* (2005) put their previous results in a different perspective. In the IC of subjects with tinnitus they now measured an increased sound-evoked response compared to controls. To test the influence of ongoing background noise, a condition with background noise was included, by means of switching the helium pump back on. This caused a reduced response of the IC in subjects with

Table 2.1 Summary of the studies included in this review

number*	Reference	Imaging modality	Experimental design	Controls / Patients	Tinnitus	matching criteria **	
						Hearing loss	Age
1	Melcher et al. (2000)	fMRI 1.5T	sound-evoked	6 / 7	4 lateralized / 3 nonlateralized	y	y
2	Melcher et al. (2005)	fMRI 1.5T	sound-evoked	14 / 17	?	y	?
3	Lanting et al. (2008)	fMRI 3T	sound-evoked	12 / 10	10 lateralized	only lf ***	n
4	Smits et al. (2007)	fMRI 3T	sound-evoked	10 / 42	35 lateralized / 7 nonlateralized	n	n
5	Kovacs et al. (2006)	fMRI 3T	sound-evoked	13 / 2	2 lateralized	n	n
6	Lockwood et al. (1998)	PET $H_2{}^{15}O$	somatosensory modulation	6 / 4	4 lateralized	n	n
7	Cacace et al. (1999a)	fMRI 1.5T	somatosensory modulation	0 / 1	lateralized	****	-
8	Giraud et al. (1999)	PET $H_2{}^{15}O$	gaze-evoked tinnitus	0 / 4	4 lateralized (deafferentiated ear)	-	-
9	Lockwood et al. (2001)	PET $H_2{}^{15}O$	gaze-evoked tinnitus	7 / 8	8 lateralized (deafferentiated ear)	n	y
10	Staffen et al. (1999)	SPECT Xe^{133}	lidocaine	0 / 1	nonlateralized	-	-
11	Mirz et al. (1999)	PET $H_2{}^{15}O$	lidocaine	0 / 12	7 lateralized / 5 nonlateralized	-	-
12	Mirz et al. (2000a)	PET $H_2{}^{15}O$	lidocaine	0 / 8	4 lateralized / 4 nonlateralized	-	-
13	Andersson et al. (2000)	PET $H_2{}^{15}O$	lidocaine	0 / 1	nonlateralized	-	-
14	Reyes et al. (2002)	PET $H_2{}^{15}O$	lidocaine	3 / 9	3 lateralized / 6 nonlateralized	only lf ***	n
15	Plewnia et al. (2007)	PET $H_2{}^{15}O$	lidocaine	0 / 9	1 lateralized / 8 nonlateralized	-	-
16	Arnold et al. (1996)	PET FDG	steady state	14 / 11	8 unilateral / 2 bilateral	n	?
17	Wang et al. (2001)	PET FDG	steady state	10 / 11	8 lateralized / 3 nonlateralized	n	y
18	Langguth et al. (2006)	PET FDG	steady state	0 / 20	16 lateralized / 4 nonlateralized	-	-
19	Shulman et al. (1995)	SPECT Tc^{99}	steady state	0 / 2	?	-	-
20	Osaki et al. (2005)	PET $H_2{}^{15}O$	residual inhibition	0 / 3	3 nonlateralized	-	-

* corresponding to numbers appearing in table 2
** groups were matched according to criteria hearing loss and age; y: yes, n: no, ?: unknown; - : not applicable.
*** only matched at low-frequency (lf, 250 - 2000 Hz)
**** asymmetrical hearing loss

tinnitus, but not in subjects without tinnitus. So, the background sound produced by the scanner pump, may have led to a saturation of the neural response in subjects with tinnitus in initial experiments (Melcher *et al.*, 2000), explaining the reduced IC activity compared to controls.

In recent work sound-evoked responses were studied using a sparse sampling design (Lanting *et al.*, 2008). Stimuli consisted of monaural dynamic rippled broadband noise stimuli at two intensity levels (40 dB and 70 dB SPL). Responses were measured at the level of the primary and secondary auditory cortex combined and the IC of subjects with unilateral tinnitus and near-normal hearing. These were compared with those of subjects without tinnitus. Results showed increased sound-evoked responses, a reduced response lateralization (i.e., stimuli presented to the contralateral and ipsilateral ear gave roughly the same signal change) and a disturbed intensity level dependency in subjects with tinnitus compared to subjects without tinnitus at the level of the IC.

Smits *et al.* (2007) used binaurally presented music in a block design and compared responses in subjects with tinnitus to those of subjects without tinnitus. Controls showed

Table 2.2 Effect on rCBF or BOLD signals using various experimental paradigms. Each paradigm shows presumable tinnitus-related changes in rCBF or BOLD signals within subjects (somatosensory modulation, gaze-evoked tinnitus, lidocaine and residual inhibition) or differences in rCBF or BOLD signals between groups of subjects (sound-evoked responses and steady state metabolism). The symbols indicate changes in rCBF or BOLD signals for several brain areas corresponding to the paradigm that was used. The numbers in the table refer to the cited authors as shown in the right column and correspond to the numbers in table 2.1.

Area	Paradigm						Reference
	Sound-evoked responses	Somatosensory modulation	Gaze evoked tinnitus	Lidocaine	Residual inhibition	Steady-state metabolism	
Frontal lobe				↓[11-13]		↑[19]	1 Melcher et al. (2000)
Limbic system	⇑[6]			↓[12,15]		↑[19]	2 Melcher et al. (2005)
Auditory association cortex		∝[6]	∝[8]	↓[11-13,15] ∝[14]	↑[20]	↑[18,19] asymmetry[17]	3 Lanting et al. (2008)
Secondary auditory cortex	asymmetry[4,5]			↓[13]			4 Smits et al. (2007)
Primary auditory cortex	asymmetry[4,5] ⇑[6,11]	∝[6,7]		↓[10,13]		↑[18,19] asymmetry[16,17]	5 Kovacs et al. (2006)
Thalamus	asymmetry[4]	∝[6]					6 Lockwood et al. (1998)
Inferior colliculus	⇓[1] ⇑[2,3] asymmetry[4]						7 Cacace et al. (1999a)
Lower brainstem			∝[9]				8 Giraud et al. (1999)
Cerebellum			∝[9]		↓[20]		9 Lockwood et al. (2001)
							10 Staffen et al. (1999)
							11 Mirz et al. (1999)
							12 Mirz et al. (2000a)
							13 Andersson et al. (2000)
							14 Reyes et al. (2002)
							15 Plewnia et al. (2007)
							16 Arnold et al. (1996)
							17 Wang et al. (2001)
							18 Langguth et al. (2006)
							19 Shulman et al. (1995)
							20 Osaki et al. (2005)

Legend	
⇑	*Increased* response to sound in tinnitus subjects
⇓	*Decreased* response to sound in tinnitus subjects
↑	*Increased* rCBF or BOLD corresponding to *decreased* tinnitus
↓	*Decreased* rCBF or BOLD corresponding to *decreased* tinnitus
∝	*Increased* and *decreased* rCBF or BOLD corresponding to *increased* and *decreased* tinnitus, respectively.
↑	*Increased* rCBF signal in tinnitus subjects
Asymmetry	Abnormal *asymmetry* in rCBF or BOLD signal

a leftward lateralization of the PAC (i.e., a predominant left auditory cortex response to sound stimuli). In subjects with bilateral tinnitus however, the sound-evoked response was symmetrical, while the response was lateralized ipsilateral to the side of perceived tinnitus in the PAC. The same pattern, although not statistically significant, was observed in the medial geniculate body (MGB). Kovacs *et al.* (2006) showed a similar cortical asymmetry in two subjects with unilateral tinnitus (i.e., a smaller sound-evoked response in the cortex contralateral to the tinnitus). Both studies however, did no match their subject groups on hearing levels (normal hearing controls and subjects with tinnitus with hearing losses up to 100 dB). This lack of hearing-level matched groups may have confounded results of both studies, making it difficult to attribute the findings purely to tinnitus.

The papers (Melcher *et al.*, 2000, 2005; Lanting *et al.*, 2008) appear to be contradictory at first sight: in contrast to Melcher *et al.* (2000) who reported decreased responses in the IC of subjects with tinnitus, the other two studies showed increased responses. A methodological difference may account for these differences. While Lanting *et al.* (2008) applied a sparse imaging protocol, in Melcher *et al.* (2000) images were acquired continuously with high levels of background noise. Therefore, this latter experiment was performed in a relatively noisy environment and may have caused the IC to respond excessively to the scanner noise. Similarly, the sound of the scanner helium pump may cause significant levels of ambient sound, which may reduce the hemodynamic response to the experimental sound stimuli (Melcher et al., 2005).

Thus, Melcher *et al.* (2000), Melcher *et al.* (2005) and Lanting *et al.* (2008) are consistent with the interpretation that the IC of subjects with tinnitus displays a disproportionate response to sound, either ambient or experimentally controlled.

Lanting *et al.* (2008) did not find a difference in the auditory cortices between subjects with tinnitus and controls. This may be a consequence of that fact that they analyzed the auditory cortices as single ROIs, without making a distinction between primary and association areas within each auditory cortex.

Although these sound-evoked responses seem elevated in subjects with tinnitus, another previously unconsidered factor may also play a role. Hyperacusis which is defined as an abnormal sensitivity to sound, may also lead to increased sound-evoked responses and is often coinciding with hearing loss and tinnitus (Møller, 2006c; Jastreboff and Jastreboff, 2003).

Somatosensory modulation of tinnitus

A second group of functional imaging experiments on tinnitus makes use of the characteristic ability that a subset of subjects with tinnitus appear to have. This is the ability to modulate their tinnitus by some somatic manipulations. Modulation of tinnitus can be achieved by somatosensory interactions like forceful head and neck muscle contraction (Levine, 1999; Levine *et al.*, 2003; Abel and Levine, 2004; Levine *et al.*, 2008) and oral-facial movements (OFMs) like jaw clenching of jaw protrusion (Chole and Parker, 1992; Rubinstein, 1993; Pinchoff *et al.*, 1998). The effect of these manipulations on the tinnitus

may express itself as a loudness change, a change in pitch, or both.

Most studies on somatosensory modulation mentioned here have used PET as the imaging modality whereas only one study on cutaneous evoked tinnitus used fMRI. Other somatosensory manipulations, like movements of the head and neck are known to modulate tinnitus (Levine *et al.*, 2003) but are mostly incompatible with imaging studies due to motion restrictions.

Oral-facial Movements

A subset of subjects with tinnitus, varying from about a third of the patient population (Cacace, 2003) to 85% (Pinchoff *et al.*, 1998), can change the loudness of the perceived tinnitus by OFMs.

Lockwood *et al.* (1998) used $[H_2{}^{15}O]$-PET to map brain regions in subjects with the ability to alter the loudness of their unilateral tinnitus, and compared their responses to those of subjects without tinnitus. In the tinnitus subjects, the loudness of the tinnitus was either increased (in two subjects) or decreased (in two subjects) by OFMs (jaw clenching). A change of the tinnitus loudness was accompanied by a corresponding change in rCBF in the left PAC and auditory association cortex (Brodmann area (BA) 41 and 21) contralateral to the ear in which tinnitus was perceived upon oral-facial movements: a reduction of the tinnitus resulted in a decrease in rCBF, and an increase of the tinnitus resulted in an increase of the rCBF. Interestingly, monaural cochlear stimulation evoked a bilateral response in the auditory cortical regions. Thus, the lateralization in response to a monaural sound differed conspicuously from that of monaural tinnitus. Not only cortical areas but also the right thalamus including the MGB showed rCBF changes upon OFMs and loudness changes of the tinnitus.

In addition, the authors noticed in subjects with tinnitus compared to controls an increased sound-evoked rCBF in the left PAC as well as an increased sound-evoked rCBF in the limbic system (left hemisphere hippocampus). Although these results suggest abnormal auditory processing in tinnitus subjects, the differences might have been related to differences in age and hearing levels between the subject groups. The subjects with tinnitus had high-frequency hearing losses varying from 30–70 dB while the control group had normal hearing levels. Recent findings of Shore *et al.* (2008) showed that in animals somatosensory input to the auditory system may be enhanced after noise-induced hearing loss. This result underlines the importance of matching of subject groups on characteristics other than the tinnitus. It suggests that the differences as reported by Lockwood *et al.* (1998) might reflect changes due to hearing loss rather than purely tinnitus-related neural changes.

Age differences between groups in general also may lead to differences in measured signals (either CBF or BOLD effect). D'Esposito *et al.* (2003) point out that normal aging, which involves possible vascular changes, may lead to changes in the measured signals which may confound the results if groups are not properly matched.

The last confounding factor may be attributed to gender differences. Gender differences were found showing differences in the primary auditory cortex between males and

females in silent lip reading (Ruytjens *et al.*, 2007a) as well as processing of noise stimuli (Ruytjens *et al.*, 2007b). Subject groups should thus be matched on gender to prevent misinterpretation.

Cutaneous-evoked tinnitus

A rare type of somatosensory interaction in tinnitus is cutaneous-evoked tinnitus (Cacace *et al.*, 1999b). Cacace *et al.* (1999a) described one subject with tonal tinnitus elicited by stroking a region on the backside of the hand, and another subject with tinnitus elicited by touching the fingertip regions of one hand. The latter subject, also having a moderate severe to severe hearing loss in the left ear while having normal threshold at the right ear, was included in an fMRI experiment. A repetitive finger tapping task, eliciting tinnitus, was used while performing fMRI acquisitions. In addition to somatosensory cortical areas, an area in the PAC contralateral to the hand triggering the tinnitus was activated. A control experiment using the other hand (which did not elicit tinnitus) was also performed, but no changes in activity of the auditory cortex were found. Apparently, finger tapping with the hand contralateral to the tinnitus specifically modulated neural activity in the PAC that is specifically related to the tinnitus percept. Asymmetrical hearing levels could however be a confounding factor in this study.

Gaze-evoked tinnitus

In gaze-evoked tinnitus, subjects can change characteristics of their tinnitus by rapidly changing gaze or by lateral gaze. Both forms may occur after posterior fossa surgery for gross total excision of space-occupying lesions (mostly vestibular schwannomas of the cerebellopontine angle), often accompanied with complete unilateral loss of the auditory nerve (Cacace *et al.*, 1994b, 1999b; Coad *et al.*, 2001; Baguley *et al.*, 2006). The neural mechanism of this phenomenon remains unknown although complete deafferentation of auditory input seems the most common initiator of gaze-evoked modulation of tinnitus.

Giraud *et al.* (1999) performed a study in subjects with gaze-evoked tinnitus (following profound hearing loss due to the removal of a large tumor) who reported a change in loudness following gaze manipulations (rapidly changing gaze) in the horizontal plane (left–right) and not in the vertical plane (up–down). By contrasting horizontal gaze with vertical gaze they demonstrated in a $[H_2{}^{15}O]$-PET design that (changes in) tinnitus corresponded to changes of the rCBF bilaterally in auditory association areas (BA 21, 22) but not in the PAC The absence of PAC involvement (i.e., changes in rCBF corresponding to changes in perception of tinnitus) might be explained by pathways that project directly from the MGB to auditory association areas, providing a bypass of the PAC (Møller *et al.*, 1992; Silbersweig and Stern, 1998). The activity of the auditory association cortex thus might reflect subcortical processing of aberrant neural signals that modulate the percept of tinnitus. This study did not include a control group, which might have disentangled the complex rCBF changes into components that are similar between the groups (and may be normal responses related to changes in gaze) while the differences between groups could reflect tinnitus related rCBF changes.

Lockwood *et al.* (2001) investigated gaze-evoked tinnitus in a PET design where horizontal (far) lateral gaze induced a loudness change (increase) and central fixation did not. rCBF changes were compared to those in control subjects without tinnitus. Subjects developed gaze-evoked tinnitus after posterior fossa surgery to remove an acoustic neuroma. This surgery was accompanied with complete unilateral loss of the auditory nerve. Gaze-evoked tinnitus was associated with rCBF changes in the lateral pontine tegmentum – a region including the vestibular and cochlear nuclei (CN). In this area, the measured response in subjects with tinnitus was larger than those in the control subjects. It is however difficult to segregate possible tinnitus related activity from hearing loss, since the groups had different hearing levels (in this study only age and sex were matched). In addition, an area in the cerebellum (vermis) was associated with lateral gaze (i.e., lateral gaze contrasted with central fixation). These areas have been reported to control eye movements like saccades and gaze holding (Glasauer, 2003), supporting the hypothesis that crosstalk between the auditory system and the system controlling eye movement might play a role in gaze-evoked tinnitus.

Thus, based on these two reports, is remains unclear what the underlying mechanism of gaze-evoked tinnitus is and whether there is a simple neural correlate of tinnitus. The auditory brainstem (Lockwood *et al.*, 2001) and especially the auditory association cortex (Giraud *et al.*, 1999) show tinnitus related changes in neural activity.

Lidocaine as modulator of tinnitus

Lidocaine may cause temporary relief of tinnitus when administered intravenously (Melding *et al.*, 1978; Darlington and Smith, 2007). It is a local anesthetic and anti-arrhythmic agent and has both central and peripheral sites of action. Lidocaine affects various molecular channels and receptors in the auditory system (Trellakis *et al.*, 2007), which may explain its effect on tinnitus.

Several neuroimaging studies reported correlation between local rCBF changes and modulation of tinnitus due to lidocaine. Note, however, that lidocaine has dose-dependent effects on the vascular system. It is associated with vasoconstriction in low dose ranges and vasodilatation in high dose ranges (Johns *et al.*, 1985). The neurovascular coupling relates fractional changes in CBF proportionally to fractional changes in oxygen consumption (Buxton and Frank, 1997) and hence, BOLD signals. Vasodilatation in turn, induces a larger blood flow and hence, larger CBF values and BOLD signal. Local (intracortical) injection of lidocaine on the other hand causes inhibition of multi unit neural activity as well as a reduction in stimulus driven modulation of neural activity as measured with BOLD fMRI (Rauch *et al.*, 2008) Thus it is important to keep in mind that lidocaine may impose global changes in CBF and BOLD effect when administered systemically (causing a dose-dependent vascular change) while it may reduce rCBF and regional BOLD effects when injected locally.

Staffen *et al.* (1999) measured rCBF in one subject with chronic tinnitus using single positron emission tomography (SPECT), a technique similar to PET imaging. Regional CBF was determined by inhalation of xenon-133 before and after suppression of tinni-

tus. Lidocaine was used to suppress tinnitus and caused a decrease of global perfusion and reduced rCBF. Effects were stronger in the right auditory cortex compared to the left auditory cortex, thereby reducing left–right asymmetries (existing prior to the lidocaine administration). This lidocaine-induced change in asymmetry in the auditory cortex was not observed in one subject without tinnitus. Although lidocaine may have induced global changes in perfusion (rather than tinnitus-specific changes) as mentioned by the authors, it cannot directly explain the reduction in left–right asymmetry in the auditory cortex compared to one control subject. This last point may indicate a correlation with tinnitus. Note however that there was no change in global CBF in the control subject indicating that the reported effects might not be reliable.

Lidocaine and masking sounds were used in a PET design showing a reduction in rCBF following lidocaine administration (Mirz *et al.*, 1999). Lidocaine administration induced a reduction in rCBF of the right middle frontal gyrus and auditory association cortex (BA 21) when compared to baseline, regardless of the side of the perceived tinnitus. Masking sound on the tinnitus-affected ear(s) showed a decrease of the PET signal from these regions. In addition, there was an increase of rCBF in the left PAC (BA 41) compared to a baseline condition. The authors concluded that lidocaine and masking sounds affect neural activity at different anatomical locations and might involve different mechanisms. This conclusion was however based on a population of subjects with tinnitus without comparing the results to those measured in a control group. It thus remains questionable if the reported changes are indeed solely tinnitus-related.

Mirz *et al.* (2000a) later showed that administration of lidocaine resulted in a decrease of rCBF in the superior frontal gyrus, the middle frontal gyrus and associative auditory regions in the right hemisphere, as well as a decrease in parts of the limbic system (amygdala, anterior cingulate gyrus) in the left hemisphere (Morgane and Mokler, 2006). The authors concluded based on these results that, in addition to auditory areas, areas associated with emotion and attention play a role in tinnitus. Again, no subjects without tinnitus were included for comparison.

A case of a subject with bilateral tinnitus (left dominant) was studied with $[H_2{}^{15}O]$-PET (Andersson *et al.*, 2000). Results not only showed a decrease of rCBF in the left PAC, SAC en AAC, but also a right lateralized decrease in frontal paralimbic areas (BA 47, 49, and 15), following administration of lidocaine. Sound stimulation resulted in bilateral activation of auditory areas. They concluded, based on the changes in auditory areas and paralimbic areas, that tinnitus perception is mediated through auditory attention and emotional processing.

Reyes *et al.* (2002) showed that rCBF changes occurred in the right auditory association area (BA 21 and 22) after lidocaine administration (accompanied with a change in tinnitus loudness), using a single blind, placebo controlled $[H_2{}^{15}O]$-PET design. The effects of lidocaine were assessed by subtracting the placebo effects from the lidocaine-induced effects. General effects of lidocaine (assessed by subtracting a rest-condition from the lidocaine condition) were an increase in rCBF of the bilateral basal ganglia, cingulate gyrus and the left thalamus. A decrease was observed in the Rolandic fissure. Interest-

ingly, lidocaine could not only cause relief (four subjects), but also an increase in loudness (four subjects), or no change in loudness (one subject).

In a $[H_2{}^{15}O]$-PET study, Plewnia *et al.* (2007) also showed a decreased rCBF in the left AAC after lidocaine administration. In addition, they found a reduced rCBF in the right gyrus angularis (BA 39) and the posterior cingulate gyrus (BA 31) of the limbic system. Only patients with a tinnitus loudness reduction after a bolus injection of lidocaine were included in this study. The auditory association cortex was further used as a target for repetitive transcranial magnetic stimulation (rTMS). A dose-dependent decrease in the tinnitus loudness (as measured using a visual analog scale) was observed, i.e., the longer rTMS was performed, the larger the reduction of the loudness of tinnitus. Whether the influence of lidocaine was solely attributed to tinnitus remains however questionable since no control group was used to assess the global effect of lidocaine.

In summary, most studies indicate involvement of the right auditory association cortex (BA 21, 22 and 38) responding to a lidocaine-induced change in the loudness of the tinnitus (Staffen *et al.*, 1999; Mirz *et al.*, 1999, 2000a; Reyes *et al.*, 2002; Plewnia *et al.*, 2007). Although most studies showed that lidocaine induced a decrease in loudness of the tinnitus (Staffen *et al.*, 1999; Mirz *et al.*, 1999, 2000a; Plewnia *et al.*, 2007) and a corresponding reduction of the rCBF in the auditory association cortex, an increase in loudness was also observed (Reyes *et al.*, 2002). Increase in the loudness of the tinnitus also corresponded to an increase in the rCBF in the auditory association cortex. Notably, several studies report changes of neural activity in the non-auditory areas like the limbic system (amygdala and cingulate gyrus) and paralimbic areas that may correspond to a lidocaine-induced change of the loudness of the tinnitus or may correspond to decrease in perceived annoyance, mediated through lidocaine (Mirz *et al.*, 2000a; Andersson *et al.*, 2000; Plewnia *et al.*, 2007).

With the exception of the study of Reyes *et al.* (2002), none of the other studies included controls or used a placebo-controlled design to assess global effects of lidocaine. This is a serious issue and might hamper the interpretation of the results. Nevertheless, global effects of lidocaine would presumably have symmetrical effects on CBF values while many studies report changes only in the right auditory association cortex. Whether these changes really correspond to a correlate of tinnitus remains debatable.

Steady state measurements

Steady state metabolic activity in cortical areas can be assessed using radioactive labeled glucose. This approach makes use of ^{18}F-deoxyglucose (FDG), which can be used in a PET design. Locally enhanced brain activity may lead to enhanced glucose uptake and can be detected by the PET scanner as a local increase of radioactive decay. Due to the relatively long half-life time of ^{18}F (110 min), measurements within one subject using different experimental conditions are not feasible. Rather, only differences between groups can be measured excluding the direct need for manipulating the perceptual characteristics of tinnitus.

Arnold *et al.* (1996) were the first to make use of FDG-PET to detect changes in metabolic activity and compared measurements of subjects with tinnitus with those of subjects without tinnitus. Results showed a stronger asymmetry in the auditory cortex activity in subjects with tinnitus compared to subjects without tinnitus. Nine subjects with tinnitus showed larger metabolic activity in the left PAC whereas one showed larger activity in the right PAC. These asymmetries might also be due to the tinnitus location (6 left, 2 right, and 2 centrally) and the possible asymmetry in hearing-loss of the tinnitus subjects, making it difficult to attribute asymmetries in metabolic activity with respect to tinnitus.

Wang *et al.* (2001) repeated this measurement and calculated a symmetry index for the auditory cortex for each subject. Results showed that glucose metabolism in the auditory cortex of subjects with tinnitus was asymmetric between the left and right auditory cortices, with that of the left being higher than that of the right. Note that this was independent of the localization of the perceived tinnitus (4 left, 4 right and 3 centrally). It is not clear whether both groups had a matching degree of hearing loss and whether this was symmetrical. The asymmetry-indices of subjects with tinnitus were significantly higher than those of the control group and in close agreement with Arnold *et al.* (1996).

Langguth *et al.* (2006) found asymmetrical activity in the PAC of subjects with tinnitus (17 lateralized to the left and 3 to the right). This was not correlated with the tinnitus location (9 left, 7 right, and 4 centrally). Patients had no to moderately severe, symmetrical hearing loss. No control group was used, making it hard to attribute findings to tinnitus, since cortical activity is not always entirely symmetrical. Also, Langguth *et al.* (2006) found a correlation between the reduction of tinnitus by rTMS focused at the temporal lobe with the increased rCBF, and the corresponding PET signal strength. This suggests that rTMS can specifically suppress neural activity that is related to the tinnitus percept.

In addition to these FDG-PET studies, Shulman *et al.* (1995) used SPECT imaging of the brain with technetium-99 m labeling (Tc-HMPAO). In two subjects, significant regional abnormalities in cerebral perfusion bilateral of temporal, frontal, parietal, hippocampal and amygdala regions were demonstrated as compared with normative technetium-SPECT of brain data. No control group was used. Chronologically, this is one of the first imaging results to link the limbic system to tinnitus.

In summary, most studies using steady state measurements report an increased asymmetry in metabolic activity between the left and right PAC in subjects with tinnitus (Arnold *et al.*, 1996; Wang *et al.*, 2001; Langguth *et al.*, 2006). The left PAC shows in almost all cases an increase in metabolic activity as compared to right side (but not all, see cf. Langguth *et al.* (2006)) suggesting that the asymmetry is related to the tinnitus. Interestingly, this seems not to be dependent on the lateralization of the tinnitus. In addition, steady state measurements show functional changes in other areas like the limbic system in tinnitus (Shulman *et al.*, 1995).

Residual inhibition

Residual inhibition is a transient suppression of tinnitus after auditory stimulation (Terry *et al.*, 1983; Roberts, 2007). Osaki *et al.* (2005) made use of this phenomenon and studied three subjects who experienced bilateral tinnitus that was suppressed while their cochlear implant was turned on. After 5-10 min of use of the cochlear implant, residual inhibition was achieved that lasted for 5-10 min. During residual inhibition, the auditory association cortex (BA 21 and 38) showed an increase in rCBF. In contrast, when tinnitus re-emerged, an increase in rCBF in the right cerebellum was observed. rCBF changes in these subjects were compared with those of six subjects with a cochlear implant but without tinnitus. In these subjects, no changes were observed. Thus, residual inhibition of tinnitus was associated with in change in neural activity in the auditory association cortex and cerebellum.

2.4 Discussion

It is obvious that tinnitus, like any other percept, must be related to some pattern of neural activity in the central nervous system. It seems logical to assume that in tinnitus the pathological activity specifically involves one or more auditory brain areas. The neuroimaging literature reviewed here is generally consistent with this view, although a comprehensive view of the neural activity that underlies tinnitus is still lacking.

Tinnitus is often associated with changes in spontaneous neural activity in the auditory pathway (Kaltenbach, 2000; Eggermont, 2007b). One of the proposed changes is a change in the spontaneous firing rates (SFRs) of auditory neurons that may be responsible for tinnitus. Since an increased (stimulus-driven) firing rate in auditory neurons typically corresponds to the presence of sound source, an increased spontaneous firing rate could also lead to an auditory percept, i.e., tinnitus.

Alternatively, the temporal pattern of spontaneous neural activity could change by e.g., increased synchrony of activity across auditory neurons (Seki and Eggermont, 2003; Eggermont, 2007b). In general, an increased firing rate or increased synchrony of neural activity could be generated by an external acoustical source. Hence, changes in neural synchrony may also be perceived as tinnitus.

The third candidate in the triad of changes that may underlie tinnitus is a reorganization of the tonotopic map in auditory neurons in the central auditory system. Although such changes themselves may not directly correspond to tinnitus, they may contribute to abnormal neural activity. For example, cortical reorganization may lead to the overrepresentation of frequencies at the edge of a peripheral hearing loss (Rajan and Irvine, 1998; Eggermont, 2006). In other words, eighth-nerve or lower-brainstem neurons that are tuned to an edge frequency could be excessively projected to a region of the auditory cortex.

The neuroimaging modalities discussed in this review (PET and fMRI) are expected to be sensitive to change in overall neuronal activity and additionally may reveal changes in the cortical tonotopic maps if a suitable paradigm is used (Talavage *et al.*, 2004). Hence, these techniques may possibly not identify all changes in neural activity that may relate to tinnitus. Specifically, fMRI and PET will not be able to identify changes in the timing of neural activity at a timescale smaller than about 2 s. Note that this represents a higher temporal resolution than a common TR of 10 s in auditory fMRI Hall *et al.* (1999) would suggest. This resolution can be obtained by adding jitter to the onset of a stimulus condition, hereby changing the relative timing of the onset of a condition within a fixed TR.

Thus, changes in synchronous neural activity, as suggested by Seki and Eggermont (2003) and Eggermont (2007b) may not be apparent in fMRI or PET data. Nevertheless, some of the neuroimaging results, assessing changes in the magnitude of activity, are very suggestive when interpreted in conjunction with results from animal studies. Table 2 shows the effects on rCBF or BOLD signal of tinnitus related changes using various experimental paradigms.

Below, we discuss the results for the various brain areas and, where possible, compare these to results from animal models of tinnitus. For reasons of clarity, the discussion is organized by brain area. However, obviously the neural activity in any brain area is not independent of that in other parts of the brain. For example, the neural activity in inferior colliculus in the brainstem will be determined by inherent collicular mechanisms, but also by input from the lower brainstem, the thalamus and the cortex. So, a particular change that is associated with tinnitus would not necessarily reflect a specific role of the inferior colliculus in tinnitus. The change may simply reflect abnormal function of connected brain areas. Since the connectivity analysis of the central auditory system has only applied in a few cases (Goncalves *et al.*, 2001; Langers *et al.*, 2005b; Upadhyay *et al.*, 2008), all of which are not related to tinnitus, a discussion on the functional connectivity of connected auditory brain areas is not really possible in relation to tinnitus. Therefore, a discussion of neuroimaging results on a per-brain-area basis seems to be appropriate.

Lower brainstem

In humans, exposure to loud sounds may produce hearing loss and tinnitus. Several studies in animals show that hearing loss caused by exposure to loud sounds results in an increase of neural activity in the CN. These changes are present in the dorsal CN (Kaltenbach *et al.*, 1998, 2000; Zhang *et al.*, 2006), and in the ventral CN (Brozoski *et al.*, 2007). Enhanced SFRs in the CN presumably result in enhanced activity in other auditory brain areas including the cortex, which may then cause tinnitus.

Imaging studies in humans only occasionally describe details of the lower brainstem. This may be due to a number of factors. The first factor is the poor spatial resolution (which is about 2–5 mm, depending on the technique that is used) compared to the nuclei that are imaged. This results in only 3–4 voxels corresponding to the CN (Hawley *et al.*, 2005). A second factor is the poor signal-to-noise ratio that is typically obtained when imaging the lower brainstem. At present, there is no imaging study that shows enhanced spontaneous activity in the CN in tinnitus patients, which would correspond to enhanced neural activity described in animals.

The CN may well be the nexus of somatosensory modulation of tinnitus. In guinea pigs, both the ventral and dorsal CN receive somatosensory input via the trigeminal ganglion (Shore *et al.*, 2008; Dehmel *et al.*, 2008). Another source of multisensory interaction involves projections of the dorsal column nuclei and to the CN (Itoh *et al.*, 1987). These anatomic and functional connections between the somatosensory and the central auditory system may underlie the influence of somatic modulation on tinnitus that is frequently described by patients with tinnitus (Levine, 1999; Levine *et al.*, 2003). Somatosensory-based treatment modalities might be useful for a tinnitus subgroup that exhibit somatosensory modulation (Levine *et al.*, 2007)

Lockwood *et al.* (2001) showed a change in rCBF in the CN accompanying the perceptual change of tinnitus by lateral gaze. An increase of tinnitus loudness was correlated to an increase of the rCBF in the CN. Currently, this is the only neuroimaging study that

describes results in the CN of tinnitus patients.

So, at present, one neuroimaging study of modulation of tinnitus by gaze indirectly suggests changes of neural activity of the CN in tinnitus, although it is not clear to what extent the observed effects are directly related to tinnitus or that the effects are related to differences in hearing loss between groups. Neuroimaging evidence for enhanced spontaneous activity of the CN in tinnitus is currently lacking.

Inferior colliculus

For the inferior colliculus (IC), both animal data and human fMRI provide some insight in the neural mechanisms related to tinnitus. Chinchillas with noise trauma and behavioral evidence of tinnitus, show increased spontaneous activity (SFRs) and enhanced sound-evoked responses in the IC (Salvi *et al.*, 1990, 2000; Wang *et al.*, 2002; Brozoski *et al.*, 2007). The enhanced neural activity, again, may correspond to tinnitus may also reflect reduced effectiveness of inhibitory neural circuits, which has also been suggested in tinnitus (Eggermont, 2005; Møller, 2006b)

The human IC is a structure that can be easily identified on an MR image. Its neural response is typically well detectable in auditory fMRI experiments using a sparse sampling design (Langers *et al.*, 2005b). The small size of the IC at standard imaging resolution does not allow for the identification of functional substructures. Rather, the activity of the IC is usually expressed as a single region-of-interest response. The IC is typically not identifiable in functional PET studies, presumably because of its small size.

While current functional MRI paradigms cannot identify changes in SFRs, an abnormal sound-evoked response has been found in tinnitus patients. Although one initial study reported a different result (Melcher *et al.*, 2000), two recent studies from independent groups (Melcher *et al.*, 2005; Lanting *et al.*, 2008) show an increased sound-evoked response in subjects with tinnitus with nearly normal hearing. In addition, a disturbed lateralization of activity was observed (Smits *et al.*, 2007; Kovacs *et al.*, 2006), although in these studies the subjects groups had no matching hearing levels (normal hearing controls and subjects with tinnitus with hearing losses up to 100 dB). This may have confounded results, making it difficult to attribute the findings to tinnitus.

Thus, the animal and human data suggest that enhanced sound-evoked responses of the IC are characteristics of both tinnitus and hearing loss. This abnormal sound-evoked activity may be caused by pathology that is inherent to the IC. Alternatively, it could result from abnormal neural input from a lower or a higher part of the auditory pathway. It is currently unclear whether the enhanced activity is at all related to tinnitus. It might also reflect hyperacusis, a common complaint of tinnitus patients, which is also believed to be related to enhanced activity of the central auditory system (Formby *et al.*, 2003; Møller, 2006c). Nevertheless, it is possible that the abnormal IC responses observed in tinnitus patients are somehow related to the tinnitus percept. The difference observed between tinnitus patients and controls, both with near-normal hearing, is an indication that central auditory processing in the brain stem is abnormal in patients with tinnitus.

Thalamus

The extensive bottom–up (afferent) and top–down (efferent) connections between the medial geniculate body of the thalamus (MGB) and the auditory cortex suggest a key role of the thalamus in auditory perception. Connections between the cortex and the thalamus are believed to contribute to the steady-state brain rhythms that can be observed in EEG and MEG signals. These brain rhythms seem abnormal in patients with tinnitus (Weisz *et al.*, 2005b,a; Llinas *et al.*, 2005) and may indicate pathology in the cortico-thalamic loops although subjects were not always matched on their hearing levels in these studies which may act as a confound. Salicilate-induced changes in spontaneous activity (SFR) in the MGB (Basta *et al.*, 2008) underline the role the MGB may play in tinnitus.

Somatosensory modulation of tinnitus by oral-facial movements (OFMs) showed a correlation between rCBF changes in the right MGB and tinnitus loudness (Lockwood *et al.*, 1998) and could very well be mediated through pathways projecting to nuclei of the thalamus (Møller *et al.*, 1992). Interestingly, recent findings of Shore *et al.* (2008) showed that in animals somatosensory input to the auditory system may be enhanced after noise-induced hearing loss. Thus, the effects as reported by Lockwood *et al.* (1998) might reflect changes due to hearing loss rather than tinnitus. The role of the MGB in tinnitus is thus marginally demonstrated.

Primary auditory cortex

The auditory cortex is important in sound perception, although the auditory system exhibits some capacity to, for example, discriminate frequencies after bilateral ablation of cortical auditory areas (Goldberg and Neff, 1961). Nevertheless, the human PAC (often described as BA41) invariably responds to acoustic stimulation of the ear (Elliott, 1994; Johnsrude *et al.*, 2002; Binder *et al.*, 1994; Belin *et al.*, 1999). Moreover, the PAC is associated with auditory hallucinations in patients with schizophrenia (Dierks *et al.*, 1999). Consequently, it seems very likely to assume that neural activity in the PAC plays some role in all human sound perception, including tinnitus. Hence, tinnitus is almost certainly related to some aspect of neural activity in the PAC.

In cats, the SFRs of neurons in the PAC was increased after noise trauma (Noreña and Eggermont, 2003). Also, an increase in synchrony of neural activity was observed (Seki and Eggermont, 2003). Additional neural plasticity was observed following acoustic (pure-tone) trauma, which resulted in a change in the cortical tonotopic map (Komiya and Eggermont, 2000; Eggermont, 2006). Recently, salicylate-induced tinnitus in rats was found to increase FDG activity in the auditory cortex using a micro PET imaging technique (Paul *et al.*, 2009). Together, these data show that induced hearing loss causes changes in both the level of activity and the synchrony between neurons of the PAC.

If tinnitus in humans also corresponds to a change in activity in PAC, one would expect this to lead to measurable effects in neuroimaging studies. The positive correlation between tinnitus loudness and rCBF, that was shown in experiments where tinnitus was modulated by somatosensory excitation (Lockwood *et al.*, 1998; Cacace *et al.*, 1999a,b) or lidocaine (Andersson *et al.*, 2000)) do suggest a direct coupling between tinnitus and PAC

activity. In addition, some steady state metabolism studies showed an increase of rCBF (Shulman *et al.*, 1995) or an increased asymmetry (Arnold *et al.*, 1996; Wang *et al.*, 2001; Langguth *et al.*, 2006) in the PAC of subjects with tinnitus, as compared to non-tinnitus subjects. Similar asymmetries between both hemispheres have also been reported using fMRI (Smits *et al.*, 2007; Kovacs *et al.*, 2006).

One study however showed no difference between sound-evoked responses of subjects with tinnitus and those of controls in the auditory cortex (Lanting *et al.*, 2008). This demonstrates that either, there is no change in neural activity, that any tinnitus-related changes are not measurable with fMRI, or the slight age and/or hearing differences between tinnitus and control subjects prevented any difference from being seen. Note that in this study a large ROI was taken combining both the PAC and secondary auditory areas. The measured responses thus have contributions from both areas rather than from the PAC exclusively. In addition to this, there is one study on gaze-evoked tinnitus showing the absence of PAC activity related to tinnitus (Giraud *et al.*, 1999). Instead, the auditory association cortex did show a tinnitus related difference in activity.

Many of the studies described have some serious limitations making it difficult to segregate possible tinnitus-related activity from other confounds like the lack of a control group or a control group that was improperly matched to the tinnitus subjects with respect to e.g., hearing levels, age and gender. Nevertheless, the body of results (except two) strongly suggests that tinnitus may be associated with increased neural activity in the PAC.

Secondary auditory cortex and auditory association cortex

Both animal and human studies suggest an involvement of the secondary auditory cortex in tinnitus. In cats, administration of salicylate and quinine (known to induce tinnitus in humans) was reported to result in an increase in the spontaneous firing rate in the secondary auditory cortex (Eggermont and Kenmochi, 1998).

Some of the human imaging studies, mentioned in the previous section concerning the PAC, also showed that the secondary auditory cortex (Andersson *et al.*, 2000) or the auditory association cortex (Lockwood *et al.*, 1998; Langguth *et al.*, 2006; Shulman *et al.*, 1995; Wang *et al.*, 2001) were related to tinnitus.

Interestingly, one study on gaze-evoked tinnitus showed responses in the auditory association cortex, but not in the PAC (Giraud *et al.*, 1999). The apparent bypass of the PAC could also be observed in other studies that used modulation of the perceptual characteristics of tinnitus like lidocaine (Mirz *et al.*, 1999, 2000a; Reyes *et al.*, 2002) and residual inhibition (Osaki *et al.*, 2005). This bypass suggests involvement of the non-classical auditory pathway (Møller *et al.*, 1992), which directly projects from the MGB to the auditory association cortex. Of course, the fact that no responses in the PAC were measured, does not necessarily mean that the PAC was uninvolved with tinnitus.

In summary, some studies on modulation of tinnitus by lidocaine, lateral gaze or residual inhibition, show association with the perceptual changes of the secondary auditory cortex but not the PAC. On the other hand, there are studies described here that actually

find association of the primary, secondary auditory cortex and auditory association cortex with tinnitus. Thus, many studies show that the secondary auditory cortex and the auditory association cortex behave differently in patients with tinnitus while this behavior in the PAC is not always clearly observed.

Limbic system and the frontal lobe

The limbic system is participating in many aspects of life involving and regulating motivation, mood, and emotion (Dalgleish, 2004). It consists of many subsystems (Morgane and Mokler, 2006) of which the hippocampus, the amygdaloid complex, the cingulate gyrus and the prefrontal cortex are important parts. Typical complaints attached to tinnitus, such as problems with sleep, anxiety, depression, and emotions such as fear, indicate the association of the limbic system with tinnitus (Jastreboff, 1990). Several cognitive therapies for tinnitus presumably affect the interaction between frontal, limbic and auditory brain areas. By reducing or altering the emotional content of the tinnitus percept by habituation (Jastreboff and Jastreboff, 2003; Jastreboff, 2007), many subjects with tinnitus can find relief from their complaints. With this approach the percept of tinnitus may not be altered, but its emotional attributes are.

In humans, the connections of the limbic system with tinnitus have not often been shown in imaging results. The hippocampus showed increased rCBF in steady state measurements in subjects with tinnitus (Shulman *et al.*, 1995) and showed a sound-evoked response whereas it did not in controls (Lockwood *et al.*, 1998).

A role for the amygdala in tinnitus was also suggested in steady state measurements in two subjects with tinnitus (Shulman *et al.*, 1995). The use of lidocaine has revealed that the decrease of loudness in subjects with tinnitus was accompanied by a decrease in rCBF in the left amygdala and anterior cingulate gyrus (Mirz *et al.*, 2000a). A lidocaine-induced decrease of rCBF was also observed at the posterior cingulate gyrus (Plewnia *et al.*, 2007). Not many studies did actually include a proper control group or made use of placebo-controlled design (e.g., lidocaine vs. placebo), thus limiting the interpretation of these studies. Only one study mentioned global effects of lidocaine and shows increased rCBF of the pons, midbrain and left and right basal ganglia as well as cingulate gyrus in response to lidocaine administration (Reyes *et al.*, 2002).

In addition to the limbic system, the frontal lobe shows also involvement in tinnitus. Lobotomy of the frontal lobe may decrease the annoyance of tinnitus but leaves the perceived loudness unchanged (Beard, 1965). Apparently, the frontal lobe is associated with the emotional response to tinnitus. Involvement of the right middle frontal gyrus was observed as a lidocaine induced rCBF decrease accompanied by a reduction in tinnitus loudness (Mirz *et al.*, 1999).

In summary, several studies that show association of the limbic system and frontal lobe with tinnitus that are based on the modulation of tinnitus by lidocaine. These results could be based on a global effect of lidocaine on rCBF (Reyes *et al.*, 2002). Nevertheless, results are consistent across studies and suggest that the limbic system and the frontal

lobe are associated with tinnitus. Yet, the mechanisms behind these brain systems and the influence on tinnitus remain unknown.

Cerebellum

The cerebellum is involved primarily in planning of motor actions, motor control, and motor learning. In addition, it was proposed that the cerebellum might also be associated with higher-order functions (Schmahmann, 1991). This view is not without controversy (Glickstein and Doron, 2008), as most higher-order processes co-occur with eye movement control (Glasauer, 2003). Experimental paradigms that involve a higher-order task often involve eye-movements. Thus, the cerebellum activity that is associated with a task may in fact reflect the eye movement motor control, rather than higher-order processing related to the task.

Nevertheless, auditory sensory processing in the cerebellum has been reported. Fifteen studies reporting neural correlates of passive and active listening were summarized in a meta-analysis (Petacchi *et al.*, 2005) and a general role of the cerebellum in auditory processing was found. Indeed, from an animal study in cats, anatomical connections between the CN and parts of the cerebellum were shown to exist (Huang *et al.*, 1982) forming an anatomical basis for auditory sensory input into the cerebellum.

Evidence of the participation of the cerebellum in tinnitus is sparse. In rats with noise-induced tinnitus, elevated neural activity was observed in the paraflocculus of the cerebellum (Brozoski *et al.*, 2007). In humans, this area has also been shown to be active in subjects without tinnitus in response to sine-wave tones (Lockwood *et al.*, 1999).

The association of the cerebellum with tinnitus has been discussed in only a few studies. In addition to the vermis, involved in integrating head and eye position in combination with vestibular signals (Lockwood *et al.*, 2001), the right cerebellum was also reported in tinnitus and showed a decreased rCBF during residual inhibition (Osaki *et al.*, 2005). Although not directly related to tinnitus, aversive sounds mimicking tinnitus presented to subjects without tinnitus also showed rCBF changes in the cerebellum (Mirz *et al.*, 2000b).

All evidence put together, the association of the cerebellum with tinnitus is not substantially supported by the current neuroimaging studies.

2.5 Conclusion

A number of fMRI and PET imaging studies aimed to identify the neural correlates of tinnitus. Both imaging modalities depend on the hemodynamic response to neural activity. They may identify changes in local neural activity that result from induced modulation of tinnitus and, in some cases, may identify abnormal steady-state activity associated with tinnitus.

PET and fMRI have a limited spatial ($\sim$ mm) and temporal ($\sim$ seconds) resolution. This limits the use of these methods to the investigation of the rather slow hemodynamic responses that can be identified in brain areas, summarizing responses of a large number of neurons. In addition, these methods only measure the strength of activity. Subtle changes in e.g., neural synchrony that have also been suggested to relate to tinnitus (Eggermont, 2007a) presumably remain unnoticed when the brain is studied with PET or fMRI.

The studies presented here suggest abnormal neural activity in tinnitus patients at several levels in the brain. Specifically, cortical and sub-cortical auditory brain areas show a correlation between blood flow and tinnitus loudness. However, in many cases, it is unclear to what extent the abnormalities truly relate to tinnitus. Some aspects may also be related to hearing loss or hyperacusis, rather than tinnitus. Also, differences between subject groups may have been confounded to differences in matching criteria between groups (e.g., hearing levels and age).

The observation that tinnitus corresponds to abnormal neural activity in auditory brain areas is not very surprising. After all, tinnitus is the abnormal percept of sound. The question remains as to how the abnormalities emerge. To what extent does the abnormal activity in the auditory cortex, which presumably has a close correspondence to the tinnitus percept, reflect an inherent abnormality of the cortex? In other words, does it reflect pathology of the cortex or is it a consequence of an abnormal interaction with subcortical brain areas and possibly limbic or frontal regions. And to what extent does the abnormality simply reflect the consequence of peripheral hearing loss? These questions remain to be answered and their answer may be key in understanding the pathology of tinnitus.

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3

Functional imaging of unilateral tinnitus using fMRI

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Abstract

This article shows that the inferior colliculus plays a key role in unilateral subjective tinnitus. The major aim of this study was to determine tinnitus-related neural activity in the central auditory system of unilateral tinnitus subjects and compare this to control subjects without tinnitus. Functional MRI was performed in 10 patients (5 m) with unilateral tinnitus (5 left-sided, 5 right-sided) and 12 healthy subjects (6 m); both groups had normal hearing or mild hearing loss. Functional MRI experiments were performed using a 3T Philips Intera Scanner. Auditory stimuli were presented left or right and consisted of dynamically rippled broadband noise with a sound pressure level of 40 or 70 dB SPL. The responses of the inferior colliculus and the auditory cortex to the stimuli were measured. The response to sound in the inferior colliculus is elevated in tinnitus patients compared to controls without tinnitus.

3.1 Introduction

Tinnitus is an auditory sensation without the presence of an external acoustic stimulus. Almost all adults have experienced some form of tinnitus, mostly transient in nature. However, in 6–20% of the adults, tinnitus is chronic and for 1–3% tinnitus severely affects the quality of life. Tinnitus is more prevalent in men than women and the prevalence increases with advancing age (Axelsson and Ringdahl, 1989; Lockwood *et al.*, 2002).

Tinnitus can be differentiated into subjective and objective tinnitus. For objective tinnitus there is some auditory source inside the body. Possible sources of objective tinnitus commonly have a vascular or muscular origin. Due to a vascular anomaly, vibrations of pulsatile blood flow near the middle or inner ear (Liyanage *et al.*, 2006; Sonmez *et al.*, 2007) can become an acoustic source. Also contractions or spasms of the tympanic membrane (Abdul-Baqi, 2004) or stapedius muscle may cause clicking and hereby act as a sound-generating source.

With subjective tinnitus however, there is no acoustic stimulus present. Common forms of sensorineural hearing loss, such as presbyacusis or noise-induced hearing loss, may be associated with subjective tinnitus.

The sensorineural processes that underlie the perception of objective and subjective tinnitus must be quite different. In objective tinnitus, sound generated in the body is transduced in the inner ear. It stimulates the hair cells in the cochlea, which subsequently leads to a neural response. In contrast, in subjective tinnitus there is no sound to stimulate the cochlea.

There is a relation between subjective tinnitus and hearing loss (Eggermont and Roberts, 2004). Many (but not all) patients with subjective tinnitus have some form of hearing loss. Since the hearing loss usually has a peripheral origin, it has been thought for many years that the tinnitus activity must also originate from a peripheral source, e.g., the cochlea. However, many observations indicate that this view cannot be correct for all forms of tinnitus. In patients that underwent sectioning of the eight cranial nerve as part of retrocochlear tumor surgery, tinnitus arises in 50% of the cases (Berliner *et al.*, 1992) while sectioning of the eighth cranial nerve in tinnitus patients did not provide relief of the tinnitus in 38–85% of cases (House and Brackmann, 1981) (reviewed by Kaltenbach *et al.* (2005)). In these cases, tinnitus cannot originate from the cochlea. Consequently, mechanisms in the central auditory system must be responsible for these forms of tinnitus.

In animals with induced hearing loss, spontaneous neural activity increases at several levels in the auditory pathway (Noreña and Eggermont, 2003; Kaltenbach, 2006) and/or neural activity across neuronal populations may increase synchronicity (Noreña and Eggermont, 2003; Seki and Eggermont, 2003). Apparently, peripheral hearing loss can result in plastic changes in the balance of excitation and inhibition in the central auditory system. These findings suggest that, although tinnitus is associated with peripheral hearing loss, it appears not to originate from the cochlea. Rather, the central auditory system plays a key role in tinnitus.

If the central auditory system of tinnitus-patients functions differently from that in normal hearing subjects, it would be conceivable that also the response to sound of the brain is different in tinnitus. In this study we investigated the response of the auditory cortex and the inferior colliculus to monaural broadband stimulation. The response of the brain centers was measured using functional magnetic resonance imaging (fMRI).

3.2 Materials and Methods

Subjects

Ten patients with unilateral tinnitus and twelve subjects without tinnitus were recruited at the University Medical Center Groningen (UMCG), all with no neurological and psychiatric history. All subjects were investigated by an audiologist using standard pure tone audiometry (250–8000 Hz). The mean audiogram per subject group is shown in figure 3.1. In the patient group, the tinnitus percept was assessed by matching the frequency with an external tone or noise band at the non-tinnitus side. Details of the subject characteristics are shown in table 3.2. The handedness of all subjects was assessed by using a translated version of the Edinburgh inventory (Oldfield, 1971). Of the patients, 9 were right-handed and 1 ambidextrous. Of the subjects without tinnitus, 10 were right-handed, 1 left-handed and 1 ambidextrous. The study was approved by the local medical ethics committee and written informed consent was obtained for each participant.

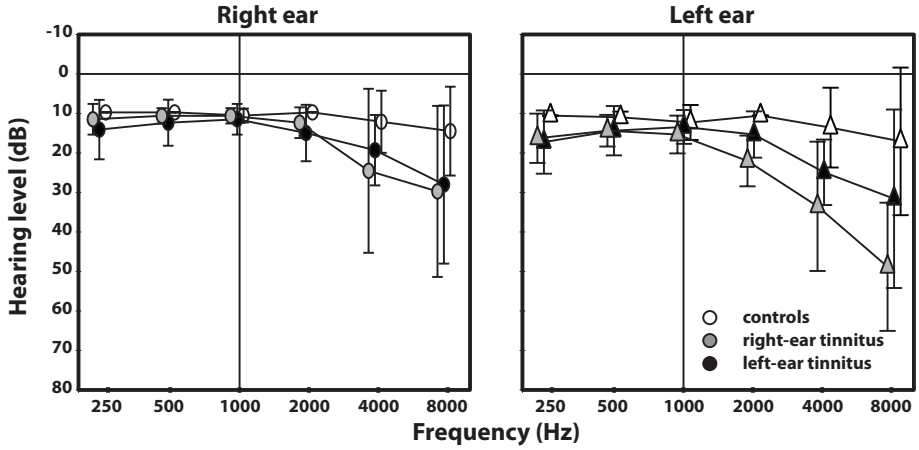

Figure 3.1 Subject group hearing thresholds. Hearing thresholds for controls and tinnitus patients were measured using pure tone audiometry. The error bars indicate the group standard deviation around the mean.

MRI Protocol

All imaging experiments were performed on a 3T MRI system (Philips Intera, Philips Medical Systems, Best, The Netherlands) with an eight-channel phased-array headcoil (SENSE headcoil).

A T1-weighted fast-field echo scan was acquired for anatomical orientation (TR 11.12 ms; TE 4.6 ms; flip-angle 15°; matrix 256 × 256 × 9; voxel-size 1.0 × 1.0 × 2.0 mm^3). An imaging volume was positioned on this scan such that it contained the left and right cochlear nuclei (CN), superior olivary complex (SOC), inferior colliculi (IC), medial geniculate nuclei (MG) and both temporal lobes containing the auditory cortices (AC). The volume was aligned to the brainstem on a midsaggital view. The data was ac-

Table 3.1 Subject characteristics

Characteristics	Controls ($n = 12$)	Left-sided tinnitus ($n = 5$)	Right-sided tinnitus ($n = 5$)
Age (years)			
average	32.5	48.4	53.2
range	24–59	37–61	30–67
Gender			
Male	6 (50%)	3 (60%)	2 (40%)
Tinnitus			
average pitch (Hz)	–	8600	7800
Range	–	6000–11000	1000–14000

quired using coronal oriented slices. The functional scans consisted of 2179-ms single-shot T2*-sensitive echo planar imaging (EPI) sequences with 41 2-mm thick slices (TR 10 s; TE 22 ms; flip-angle 30°; matrix 128 × 128, field of view 224 mm, SENSE reduction factor 2.7). The influence of acoustic scanner noise was reduced using a sparse sampling strategy (Hall *et al.*, 1999) in which auditory stimuli were presented during a 7.8-s gap of scanner silence between two successive acquisitions. For each subject three runs of 51 acquisitions were performed.

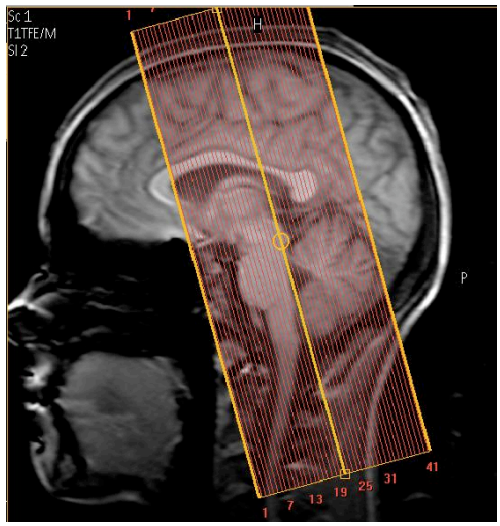

Figure 3.2 Positioning of the functional scans on a midsaggital plane of the T1-weighted scan. The volume, indicated by the orange box, containing 42 slices of 2 mm slice thickness was positioned parallel to the brainstem, such that the IC was captured by the middle slice.

An additional 3D T1-weighted fast-field echo scan (TR 25 ms; TE 4.6 ms; flip-angle 30°; matrix 256 × 256 × 160; voxel-size 0.94 × 0.94 × 1.0 mm^3) was acquired with the same orientation as the functional scans to serve as anatomical reference.

Stimulus and paradigm

Auditory stimuli were delivered by either an MR-compatible electrostatic audio system (S-001Mk2 and SRM-00, Stax Ltd., for the first patient and the first 5 control subjects) or by a MR compatible electrodynamic system (MR Confon GmbH, Baumgart *et al.* (1998)). These systems were driven by a PC setup equipped with a digital-analogue card (National Instruments 6052E, National Instruments Corporation, Austin, TX), Labview (National Instruments Corporation, Austin, TX) and Matlab 6.5 (The Mathworks Inc.) which generated dynamic rippled noise.

These rippled noise stimuli consist of temporally and spectrally modulated noise (Langers *et al.*, 2003). The stimuli had a frequency-range of 125–8000 Hz with a spectral modulation density of 1 cycle per octave, a temporal modulation frequency of 2 cycles per second and a modulation amplitude of 80%. The rippled noise stimuli were presented immediately when MR acquisition started and ended before the next acquisition. All stimuli had a duration of 7.5 s.

Stimuli were presented at 0, 40 and 70 dB (SPL) either at the right or left ear. The stimuli were presented in a cyclic randomized order. Each stimulus condition (five in total) was presented ten times per functional run except for the 'silent' condition (i.e., 0 dB bilaterally), which was presented eleven times. Subjects were instructed to respond by left or right button presses with the right thumb whenever they perceived an audible stimulus in the left or right ear, respectively. This was done to monitor the subjects' attention to sound stimuli during acquisition.

Preprocessing

MR images were analyzed using Matlab 6.5 (The Mathworks Inc.) and SPM5 (Functional Imaging Laboratory, The Wellcome Department of Imaging Neuroscience, London, UK). The functional images were realigned and spatially coregistered with the high-resolution anatomical image. Images were thresholded to omit voxels outside the brain.

Based on the high-resolution anatomical images, a customized normalization template was made using Voxel Based Morphometry methods using the anatomical data of the first 13 subjects. The functional images were spatially normalized to this template based on the gray-matter segment of the anatomical image and were spatially smoothed with an isotropic 5-mm Gaussian kernel resulting in a voxel-size of $2.0 \times 2.0 \times 2.0$ mm^3.

Regression Analysis

A general linear model was set up to analyze the relative contribution of each sound condition to the measured response. The model included four covariates of interest (β_i), one constant factor to model the mean per session (Y_0) and a linear factor to correct for linear drift in the scanner signal. The model was applied to the data of all individual voxels and a significance level for each sound condition was determined separate by using t-tests to visualize the localization, the level and extent of activation in individual subjects. The combined effect of all sound stimuli to the measured response was assessed by an F-test.

Region of interest analysis

For each subject four large regions of interest (ROI) were drawn for the auditory cortex (AC), and the inferior colliculus (IC), both left and right, based on anatomical atlases.

Within each region of interest the 10% of the voxels that responded most strongly according to the t-test per sound condition were selected and the coefficients from the linear regression (β_i) were averaged. A percent signal change (S_i) compared to the silence condition was calculated for each sound condition, based on the regression coefficients as indicated in equation (3.1).

$$S_i = 100 \times \frac{\beta_i}{Y_0} \tag{3.1}$$

For each subject we calculated this percent signal change for both the left and right auditory cortex (AC) and inferior colliculus (IC) and determined if there were statistically significant differences ($p < 0.05$) between subject groups, between loudness levels and lateralization. This analysis was done by using a repeated measures ANOVA method within SPSS 13.

3.3 Results

Region of Interest Analysis

All measured responses were analyzed using repeated measures ANOVA with SPSS 13. In this analysis the loudness dependency, the lateralization and subject group were main effects that were tested. The loudness dependency was, for example, determined over both ears and all subject groups. The results were visualized using box-plots.

The Auditory Cortex

The box-plots in figure 3.3 show the results of the measured responses in the left and right auditory cortex, for all subject groups. The responses to sound stimuli ranged from 0.1–3%.

Clearly visible is a loudness dependency, that is, a stimulus of 70 dB (SPL) yielded a statistically significant ($p < 0.05$) larger response than a stimulus of 40 dB (SPL). This loudness dependency was present for all three groups (controls, left-sided tinnitus and right-sided tinnitus). There was also a statistically significant ($p < 0.05$) lateralization; contralateral stimuli yielded larger responses than ipsilateral stimuli. This holds for all three groups (controls, left-sided tinnitus and right-sided tinnitus).

There was, however, no statistically significant difference between the amplitudes of the responses of the subject groups for both left and right auditory cortex. The responses measured in the auditory cortex in controls did not differ significantly from those measured in both patient groups (left-sided tinnitus and right-sided tinnitus).

The Inferior Colliculus

Figure 3.4 shows the results for the left and right inferior colliculus (IC), for all three subject groups. Compared to the auditory cortex, the measured responses in the inferior

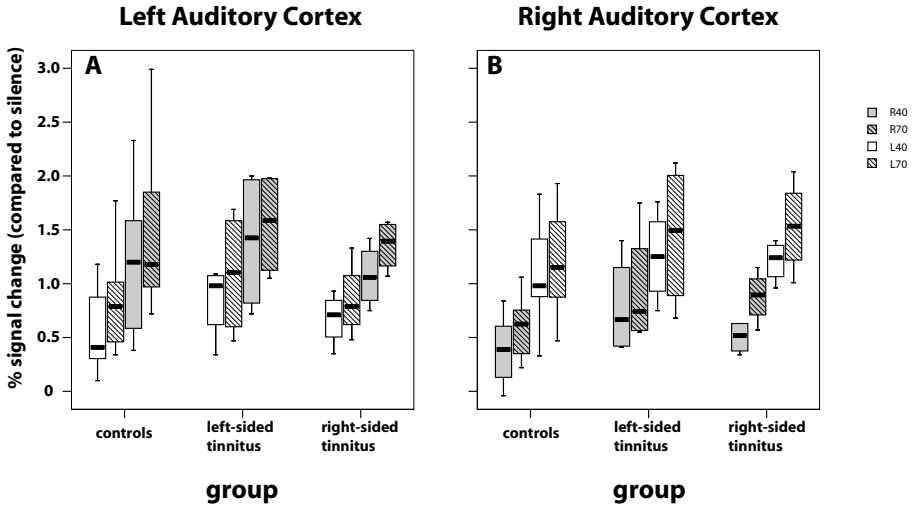

Figure 3.3 The percent signal change measured in the left (A) and right (B) auditory cortex for three subject groups shown as box-plots (showing smallest observation, 25^{th}, 50^{th} and 75^{th} percentile, and largest observation). For each group, four responses are shown: responses to stimuli of 40 and 70 dB (SPL), respectively, presented at the left ear (L40 and L70) and the right ear (R40 and R70).

colliculus (IC) were lower in magnitude: 0.15–1.5%, whereas the responses in the auditory cortex (AC) were up to 3%. In the control group, the similar staircase-like pattern as at cortical level is visible. It shows a loudness dependency, where 70 dB stimuli give a larger response than 40 dB stimuli. In this group there is also a clear lateralization towards the contralateral side, i.e., contralateral stimuli give larger responses than ipsilateral stimuli.

For the two patient groups however, the responses in the IC differed significantly ($p < 0.05$) from the control group. Firstly, the median responses to the sound stimuli were larger in both patient groups (0.4–0.8%) when compared to the control group (0.2–0.6%), for each stimulus.

Also, the lateralization of the responses was disturbed in the patient groups. When a sound was presented at the side where the tinnitus was perceived, the fMRI response did increase with increasing loudness, but was elevated when compared to control subjects. For a sound presented at the side opposite to the perceived tinnitus, the 40 and 70 dB stimuli gave the same amount of signal change (i.e., no increasing response with increasing loudness).

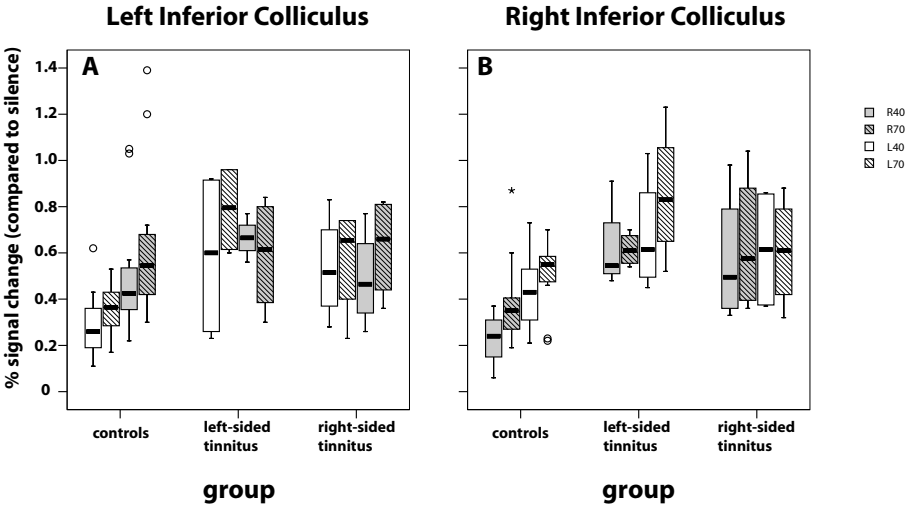

Figure 3.4 The percent signal change measured in the left (A) and right (B) inferior colliculus for three subject groups shown as box-plots, as in figure 3.3. Outliers are depicted as separate points.

3.4 Discussion

In this study we investigated the response to broadband auditory stimuli in the auditory cortex and inferior colliculus of normal hearing subjects and tinnitus patients using functional MR imaging. We used a sparse sampling paradigm (Hall *et al.*, 1999) to minimize interaction between the auditory stimuli and the background scanner noise.

In the auditory cortex of all subject groups we found a lateralization effect, i.e., contralateral stimuli gave a larger response than ipsilateral stimuli. We also found a loudness dependency, i.e., stimuli of 70 dB (SPL) gave a larger response than stimuli of 40 dB (SPL). In the control group we found a functional asymmetry as described earlier (Devlin *et al.*, 2003). The responses in the auditory cortex in the control group were higher in the left hemisphere than in the right hemisphere. In the patient groups this is not as clear due to the limited group size. There were no statistically significant group differences observed in the auditory cortex: no tinnitus related differences were observed.

The responses measured in the inferior colliculi showed a different pattern. These were on average lower in amplitude than measured in the auditory cortex. The control group showed a response similar to the auditory cortex; there was both a lateralization effect and a loudness dependency. In tinnitus patients, the responses were significantly different from the control group. Firstly, the response was significantly larger in both

patient groups compared to the control group. Secondly, the loudness dependency was different in the inferior colliculus opposite to the tinnitus percept. When a sound was presented at the tinnitus ear, the response was larger for a louder stimulus. However, when a sound was presented at the non-tinnitus ear, the response amplitude did not show a loudness dependency. Thus, we found a clear difference between tinnitus patients and normal hearing controls regarding the response of the inferior colliculi. Both the responses to stimulation of the tinnitus ear and stimulation of the non-tinnitus ear were different from that in normal hearing controls.

In the literature, various animal studies with noise-induced tinnitus report an increased spontaneous neural activity at the level of the (dorsal) cochlear nucleus (Kaltenbach, 2006) and the inferior colliculus. Only a few studies describe the effect of auditory stimuli on the neural activity in "tinnitus animals". In chinchillas with induced noise trauma-and possibly tinnitus-citetSalvi:1990bv have shown increased compound action potentials in the inferior colliculus in response to an auditory stimulus. Firstly, the slope of the amplitude level functions was steeper than normal after the noise trauma. Secondly, at frequencies below the induced hearing loss, the maximum response amplitude increased to a threefold of the normal response. Their explanation was a change in gain setting in the central auditory pathway. This gain setting can be up or down regulated to compensate for a decrease or increase of neural activity from the cochlea. Our data fit the findings of Salvi *et al.* (1990) very well, since we also found increased responses to sound stimuli at the inferior colliculus, in tinnitus patients compared to control subjects.

Melcher *et al.* (2000) also performed fMRI on patients with unilateral tinnitus. In contrast to our results, they showed a decrease of the response in the inferior colliculus contralateral to the tinnitus percept. Their explanation is twofold. Firstly, if tinnitus is accompanied by increased neural activity in silence and if neural activity is bound to a maximum, the neural activity can be driven into saturation when presenting an additional auditory stimulus. When two stimulus conditions are compared (i.e., silence vs. stimulus), a decreased level of activity can be found in areas in the brain linked with tinnitus compared to the unaffected areas. A second explanation was described as physiological masking of the tinnitus related activity. In this model, the neural activity related to tinnitus is decreased or masked by an external auditory stimulus. It is not possible distinguishing between these two explanations since they predict the same fMRI result: a decreased response signal.

The results of Melcher *et al.* (2000) appear to contradict our results. However, the different findings may be due to differences in the experimental procedure. The MRI signal could be significantly influenced by the acoustic noise of the scanner. In order to minimize this effect, we used a sparse imaging strategy (Hall *et al.*, 1999) with a repetition time (TR) of 10 s with 8 s of silence. Melcher *et al.* (2000) however, used a variable TR of 2 s with substantial noise produced by the scanner, which presumably affected the measured responses of the inferior colliculus.

We show that in tinnitus patients, the inferior colliculus produces an enlarged response. Possibly, the tinnitus subjects in the study of Melcher *et al.* (2000) also show an enlarged response to the substantial scanner noise. This may have saturated the inferior colliculus, resulting in only a small additional response when stimulated with sound from the headphones. Thus, our experiments and those by Melcher *et al.* (2000) are both consistent with the view that in tinnitus patients the inferior colliculus is easily saturated.

Recently, Smits *et al.* (2007) investigated the lateralization of activity in the auditory pathway of control subjects and patients with uni- and bilateral tinnitus using fMRI. This was achieved by comparing the spatial extent of activation (i.e., number of voxels activated due to auditory stimuli) in the left hemisphere with the extent in the right hemisphere for nuclei of the auditory pathway. They found activation lateralized towards the tinnitus side in auditory cortex and inferior colliculus of patients with right-sided tinnitus and the medial geniculate body of patients with left-sided tinnitus. In addition, controls showed a lateralization to the left AC. They interpret their results on patients with right-sided tinnitus as being in agreement with Melcher *et al.* (2000), who showed a lower activation of the right inferior colliculus in these patients. Similar to Melcher *et al.* (2000), Smits *et al.* (2007) did not use a (sufficiently) sparse imaging paradigm. As explained above, this accounts for the observed effects.

The enhanced activity in the inferior colliculus of tinnitus patients may be due to a change in the balance of excitation and inhibition. Reduced inhibition could explain the enhanced response, and may be responsible for the tinnitus our subjects experience. With this work we have succeeded in identifying a neural correlate of tinnitus measured with fMRI and locating it in the auditory pathway. Future work has to provide an insight into the response in the complete auditory pathway in tinnitus patients.

3.5 Acknowledgements

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4

Unilateral tinnitus: changes in lateralization and connectivity measured with fMRI

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Abstract

Tinnitus is a percept of sound that is not related to an acoustic source outside the body. For many forms of tinnitus, mechanisms in the central nervous system are believed to play a role in the pathology. In this work we specifically assessed possible neural correlates of unilateral tinnitus. We used functional magnetic resonance imaging (fMRI) to investigate possible changes in neural activity between controls, subjects with left-sided tinnitus and subjects with right-sided tinnitus. We measured sound-evoked responses and assessed potential differences between the groups in the level of activity, the lateralization of the responses and connectivity patterns between auditory nuclei. The lateralization of the tinnitus percept was not represented in the measured fMRI activity in the auditory pathway. We showed that the vermis of the cerebellum responded to sound in subjects with tinnitus. In contrast, no cerebellar response was observed in controls subjects. Additionally, we showed that the lateralization at the level of the right primary auditory cortex (PAC) and right inferior colliculus (IC) was significantly lower in subjects with tinnitus than in controls. The abnormal lateralization of the IC in subjects with tinnitus was also reflected in changes in connectivity patterns between the IC and the medial geniculate body (MGB) in subjects with tinnitus. These findings are shown to be consistent with the hypothesis that tinnitus may be related to reduced inhibitory effectiveness in the central auditory system. Also, they suggest the potential involvement of the vermis of the cerebellum in tinnitus.

4.1 Introduction

Tinnitus is an auditory sensation without the presence of an external acoustic stimulus. A number of neural mechanisms that might underlie tinnitus have been proposed: changes in the spontaneous firing rates (SFR) of neurons in the auditory system (Noreña and Eggermont, 2003; Kaltenbach *et al.*, 2004), changes in burst firing and neural synchrony (Noreña and Eggermont, 2003; Seki and Eggermont, 2003), and tonotopic map reorganization have been recognized as possible neural correlates of tinnitus (Muhlnickel *et al.*, 1998; Seki and Eggermont, 2003; Eggermont, 2006). All of these mechanisms may occur as a consequence of an imbalance between excitation and inhibition in the auditory pathway as may be caused by hearing loss (Eggermont and Roberts, 2004). None of the proposed mechanisms have, however, been proven as a substrate of tinnitus in humans.

Functional imaging methods have been applied to study neural correlates of tinnitus (for a review, see Adjamian *et al.* (2009); Lanting *et al.* (2009)). These methods essentially measure the hemodynamic response in the brain that results from local brain activity. fMRI in subjects with unilateral tinnitus has been shown to give deviant response lateralization and response levels in tinnitus patients, although the results were not consistent across the studies (Melcher *et al.*, 2000; Smits *et al.*, 2007; Lanting *et al.*, 2008; Melcher *et al.*, 2009).

The goal of our study was to investigate neural correlates of tinnitus in humans. It is an extension on our previous work (Lanting *et al.*, 2008). We used fMRI to characterize sound-evoked responses in various brain centers, and examined response lateralization and connectivity in a group of subjects without tinnitus and compared the findings to those

in subjects with unilateral tinnitus. Connectivity was quantified by correlation measures, where the responses in various brain areas were related to each other (Horwitz, 2003). We performed this study using monaural auditory stimuli, systematically varying intensity and stimulus side.

4.2 Materials and methods

Subjects

Thirteen subjects with unilateral tinnitus were recruited at the University Medical Center Groningen, all without known neurological and psychiatric history. Additionally, sixteen subjects without tinnitus were recruited. All subjects were selected to have near-normal and symmetrical hearing. Hearing thresholds were obtained using standard pure-tone audiometry at the octave frequencies from 250 to 8000 Hz.

In the patient groups, the perceived tinnitus frequency and loudness level were determined by a matching procedure. The frequency matching was performed with an external tone presented at the non-tinnitus ear at a comfortable level. The loudness level was then determined by adjusting the level of this tone to match the tinnitus loudness. In addition, the handedness of each subject was determined using a translated version of the Edinburgh inventory (Oldfield, 1971). Details of the subject characteristics are shown in table 4.1. The study was approved by the local medical ethics committee and written informed consent was obtained for each participant.

Table 4.1 Subject characteristics

Characteristics	Controls ($n = 16$)	Left-sided tinnitus ($n = 7$)	Right-sided tinnitus ($n = 6$)
Age (years)			
average	39.1	46.5	52.8
standard deviation	16.6	8.1	13.1
range	23–76	40–62	31–76
Gender			
male	8 (50%)	4 (57%)	4 (67%)
Tinnitus			
average pitch (Hz)	–	7100	8600
range (Hz)	–	750–14000	6000–11000
average loudness (dB SL)	–	15	23
range (dB SL)	–	10–25	10–55
Handedness			
right handed	14 (88%)	6 (86%)	5 (83%)

Acoustic stimulation and paradigm

Auditory stimuli were delivered by a MR compatible electrodynamic system (MR Confon GmbH, Baumgart *et al.* (1998)). This system was driven by a PC setup equipped with a

digital-analogue card (National Instruments 6052E, National Instruments Corporation, Austin, TX) controlled by Labview 6.1 (National Instruments Corporation, Austin, TX). The auditory stimuli were generated off-line using Matlab 6.5 (The Mathworks Inc., Natick, MA) and consisted of temporally and spectrally modulated broadband 'rippled' noise (Langers *et al.*, 2003). The stimuli had a frequency-range of 125–8000 Hz with a spectral modulation density of 1 cycle per octave, a temporal modulation frequency of 2 cycles per second and a modulation-amplitude of 80%. The rippled noise stimuli were presented immediately when MR acquisition started and ended before the next acquisition. All stimuli were 9.5 s in duration. Stimuli were presented at 40 and 70 dB (SPL) either at the left or right ear. The stimuli were presented in a cyclic randomized order. Each condition (four in total) was presented ten times per functional run, the 'silent' condition (i.e., no stimulus) was presented eleven times. Subjects were instructed to respond by left or right button presses with the right thumb whenever they perceived an audible stimulus in the left or right ear, respectively. This was done to monitor the subjects' attention to sound stimuli during acquisition.

MRI Protocol

All imaging experiments were performed on a 3T MRI system (Philips Intera, Philips Medical Systems, Best, The Netherlands) with an eight-channel phased-array head coil (SENSE head coil). A T1-weighted fast-field echo scan was acquired for anatomical orientation (TR 11.1 ms; TE 4.6 ms; flip-angle 15°; matrix 256 × 256 × 9; voxel-size 1.0 × 1.0 × 2.0 mm^3). The functional scans consisted of 2179-ms single-shot T_2*-sensitive echo planar imaging (EPI) sequences with 41 2-mm thick slices (TR 10 s; TE 22 ms; flip-angle 30°; matrix 128 × 128, field of view 224 mm, SENSE reduction factor 2.7) and were acquired using a coronal orientation, aligned to the brainstem when viewed on a midsagittal cross-section. The influence of acoustic scanner noise was reduced using a sparse sampling strategy (Hall *et al.*, 1999; Langers *et al.*, 2005a) in which auditory stimuli were presented during a 7.8-s gap of scanner silence between the end of each acquisition and the successive one. For each subject three runs of 51 acquisitions were performed. An additional 3D T1-weighted fast-field echo scan (TR 25 ms; TE 4.6 ms; flip-angle 30°; matrix 256 × 256 × 160; voxel-size 0.94 × 0.94 × 1.0 mm^3) was acquired with the same orientation as the functional scans to serve as anatomical reference.

Data analysis

MR images were analyzed using Matlab 7.1 (R14) (The Mathworks Inc., Natick, MA) and SPM5 (Functional Imaging Laboratory, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/). The functional images were corrected for motion using realignment of all images to the first acquired volume of each subject and were spatially coregistered with the T1-weighted high-resolution anatomical image. The high-resolution anatomical image was segmented in grey matter, white matter and cerebral spinal fluid (CSF) segments. The grey-matter segment of the anatomical image was normalized to a custom normalization template (for more details, see Lanting *et al.* (2008)) and the resulting transformation parameters were also applied to the functional data. The normalized functional data were spatially smoothed using an isotropic

Gaussian kernel with a full width at half maximum of 4 mm, to improve signal-to-noise ratio characteristics while retaining the ability to discern small auditory structures (e.g., the brainstem nuclei). Functional images were interpolated to voxel dimensions of 2.0 × 2.0× 2.0 mm^3.

A general linear model was set up for each subject to analyze the relative contribution of each condition to the measured response. The linear regression model included four covariates of interest, one for each stimulus condition; one constant factor to model the mean and a linear term to correct for linear drift in the scanner signal. The model was applied to the data of each individual voxel and four contrast images were created, one for each condition (i.e., left 40 dB vs. baseline, left 70 dB vs. baseline, right 40 dB vs. baseline and right 70 dB vs. baseline; all levels measured in SPL). These contrast images were used to obtain statistical parametric maps (SPMs). One F-statistic SPM was made, equally weighting all four conditions where sound was presented. At a later stage, the estimated regression coefficients were used to calculate a percent signal change for each condition for each subject.

A random-effects analysis expresses the typical characteristics of the population and it assesses the statistical significance of the measured responses by comparing the mean value to the variability across subjects (Friston *et al.*, 1999). In the present study we used the four (single-subject) contrast images in a second level random-effects analysis based on a flexible factorial design in SPM5. In this design three factors were defined. One factor was a subject factor; one factor was defined as a group factor (i.e., controls, subjects with tinnitus perceived on the left side and subjects with tinnitus perceived on the right side) and one factor was defined as a within-subjects stimulus factor (i.e., the contrast images created at single subject level). Inferences on group level were performed using an omnibus F-test on the summary statistics.

Region of interest analysis

In addition to the second level random-effects analysis we performed a region of interest (ROI) analysis, assessing sound-evoked responses in 10 anatomical areas comprising (part of) the auditory pathway and one area in the vermis of the cerebellum. The left and right primary auditory cortices were defined as the combination of the TE1.0, TE 1.1 and TE 1.2 areas defined by the SPM5 Anatomy toolbox (Morosan *et al.*, 2001; Rademacher *et al.*, 2001; Eickhoff *et al.*, 2005, 2007). For the left and right auditory association cortices (AAC) we used the left and right superior temporal gyrus as defined by Brodmann (BA 22) based on the AAL template in MRIcron (`http://www.sph.sc.edu/comd/rorden/mricron/`). Both the ROIs of the primary and association cortices were normalized to match our anatomical template in order to have a corresponding image space. The left and right medial geniculate body of the thalamus (MGB), the left and right inferior colliculi (IC), the left and right cochlear nuclei (CN), and the ROI consisting of the vermis of the cerebellum were manually drawn based on an anatomical atlas (Woolsey, 2003; Martin, 2003).

A repeated measures ANOVA was performed for each ROI separately, using the mean percent signal change within each ROI for each experimental condition and for each subject. Two main factors were defined: (1) subject group and (2) stimulus condition (left 40 dB (L40), left 70 dB (L70), right 40 dB (R40) and right 70 dB (R70); all levels in dB SPL). In addition, the interaction between these two main factors was assessed (group x

stimulus).

Lateralization

Since we use monaural stimuli, it was possible to assess the preferred stimulus lateralization of the auditory nuclei. For each ROI of each subject, the mean response to left (L) and right ear (R) stimuli was calculated, averaging the response to the 40 and 70 dB (SPL) stimuli at each ear. From these values a lateralization index was obtained, defined as

$$LI = \frac{L - R}{|L| + |R|} \tag{4.1}$$

Connectivity analysis

For connectivity analyses, we used the Pearson correlation and (conditional) partial correlation to assess, respectively, functional and effective connectivity (Friston, 1994). Our model consisted of the following ten auditory regions: the left and right CN, IC, MGB, PAC and AAC. In addition, the vermis of the cerebellum was included as the eleventh ROI. The mean of all voxels within each ROI was calculated for each point in time (i.e., scan). The obtained fMRI time courses of these ROIs were transformed to zero mean and unit variance for each subject. These arrays were concatenated over subjects resulting in a matrix $\boldsymbol{X1}$ of 11 time courses of 2448 elements in time (16 subjects $\times$ 153 time points) for the control group and a matrix $\boldsymbol{X2}$ of 11 time courses with each 1989 elements in time (13 subjects $\times$ 153 time points) for the patient group. For each group the covariance matrix $\boldsymbol{\Sigma}$ was calculated (which is the same as the Pearson cross-correlations since the signals were standardized) and from these we obtained the partial correlation coefficient matrix $\boldsymbol{\Pi}$ (following Marrelec *et al.* (2006, 2007)). Each partial correlation coefficient Π_{ij} in the matrix $\boldsymbol{\Pi}$ represents a measure of the interaction between the time courses of two regions (i and j) in the network, i.e., the correlation that cannot be accounted for by the influence of any other ROI in the network.

To assess whether differences in correlations between groups were significant, we used non-parametric permutation testing (Good, 2002). First, we calculated for both correlation measures (Pearson and partial correlation) the observed difference in correlation coefficients between the subject groups. Then, we randomly permuted the assignment of subjects to the two groups (retaining the original group sizes) and calculated the difference between correlation coefficients for each permutation. We performed this 5000 times and obtained a reference distribution of differences in (partial) correlation coefficients for each connection. To assess whether the observed difference in correlation exceeded the significance level of $p = 0.05$, we calculated the proportion p of sampled permutations where the absolute difference was greater than, or equal to, the observed difference.

4.3 Results

Audiometry

Pure tone audiometry (250-8000 Hz) was performed prior to the functional imaging sessions. The mean audiogram and the standard deviation around the mean are displayed

per group in figure 1. For the control group, the mean hearing threshold was 14 ± 11 dB hearing level (HL), where the average was determined over both ears and over frequencies of 250 – 8000 Hz. The subject group with left-ear tinnitus has average hearing thresholds at 19 ± 14 dB HL and for the group with right-ear tinnitus this was 19 ± 13 dB HL. For the frequency-range of 250-2000 Hz, the groups had comparable hearing thresholds (average hearing thresholds were 11 ± 4 dB for the controls and 13 ± 5 and 14 ± 6 dB HL for respectively the groups with left-sided tinnitus and right-sided tinnitus).

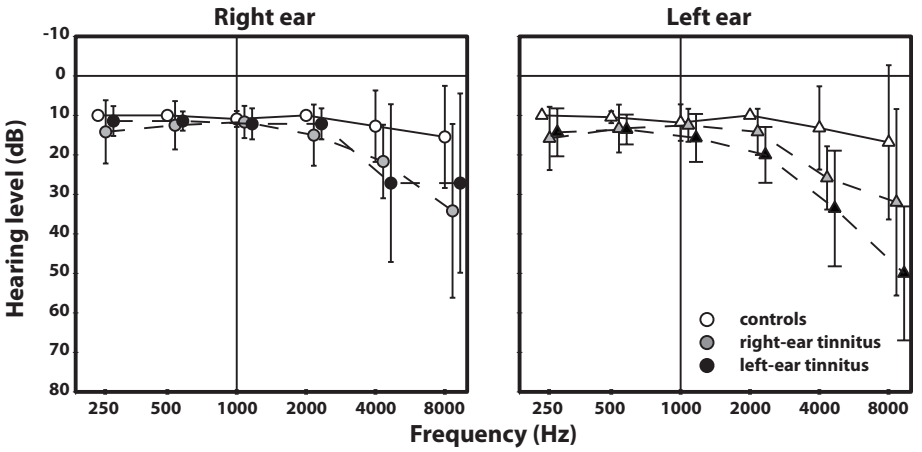

Figure 4.1 Hearing thresholds for the right and the left ear for the three subject groups. The solid line represents the hearing thresholds of the control group and the two dashed lines represent the hearing thresholds of the two groups with unilateral tinnitus. The error bars indicate the standard deviation around the mean.

Statistical parameter mapping

The significance of the BOLD responses to auditory stimuli that were presented to the left and right ear was visualized by means of F-test statistical parametric maps (SPM) pooling all subjects ($n = 29$) together. Figure 4.3 shows cross-sections of the brain in gray-scale with a color-coded overlay showing significant responses to sound (pooling all 4 conditions) based on an omnibus F-test ($F > 8.34$, $p < 0.05$ FDR, pooled over all subjects). It clearly shows significant responses in the CN, the IC, the MGB and the bilateral auditory cortices. When contrasting the subject groups, no clear differences were observed, with the exception of the vermis of the cerebellum (not shown in this figure). No significant differences between the two patient groups were observed. Apparently, the lateralization of the tinnitus did not cause the response strength or location to be different between both patient groups. Based on this finding we decided to pool the patient data. From here on, we only compare responses between controls and all patients with tinnitus.

Region of interest analysis

Based on the information from the Anatomy toolbox and anatomical atlases, we performed ROI analyses on 10 ROIs in the auditory pathway and the vermis of the cerebellum. This last ROI was chosen since it responded differently between the controls and patient group. Table 2 shows the size of each ROI, measured in voxels (of $2 \times 2 \times 2$ mm^3).

Table 4.2 Size of each ROI, measured in number of voxels (the size of each voxel is $2 \times 2 \times 2$ mm^3).

ROI	left hemisphere	left hemisphere
Auditory association cortex (AAC)	1339	1569
Primary auditory cortex (PAC)	469	563
Medial geniculate body (MGB)	53	63
Inferior colliculus (IC)	29	33
Cochlear nucleus (CN)	63	52
Cerebellum vermis	287	

As an example, figure 4.2 shows a statistical distribution of the responses to the four experimental conditions in the left primary auditory cortex. The figure shows the distribution of voxels as function of the percent signal change for the four conditions, for one subject. It shows that, based on the intensity level (40 and 70 dB SPL) and the stimulus presentation side (right ear and left ear), the histograms are shifted compared to each other. Based on the omnibus F-test (equally weighing all conditions), the 10% most active voxels in each ROI were used in the analysis and the coefficients from the linear regression were averaged. A percent signal change was calculated, averaging the regression coefficients within the region that responded most strongly and comparing it to the regression coefficient describing the baseline level of activity for the same area.

The box plots in figure 4.4 show the responses for controls and subjects with tinnitus for the different ROIs. For each ROI, it shows the measured percent signal changes for the four experimental conditions, L40, L70, R40 and R70–all compared to baseline. In addition, the mean value for each subject group is shown.

The left hemisphere nuclei are displayed on the left side of the figure and the right hemisphere nuclei are displayed on the right side. In the middle, there are cross-sections of the brain (either coronal, transversal, or both), showing each ROI in a yellow color overlay combining both the left and right hemisphere ROI in one picture.

Apparent from all nuclei of the auditory pathway, except for the MGB, is the sound intensity dependency, i.e., the 70 dB (SPL) stimuli yielded a larger response than the 40 dB (SPL) stimuli. In addition to the sound intensity dependency, there is a preferred stimulus lateralization. With the exception of the CN, the auditory pathway is lateralized towards the contralateral ear; in other words, there is a stronger response to contralateral stimuli than to ipsilateral stimuli.

Repeated measures ANOVA analysis was performed with the factor 'condition' as repeated measure and 'group' as independent variable. In addition to these main factors,

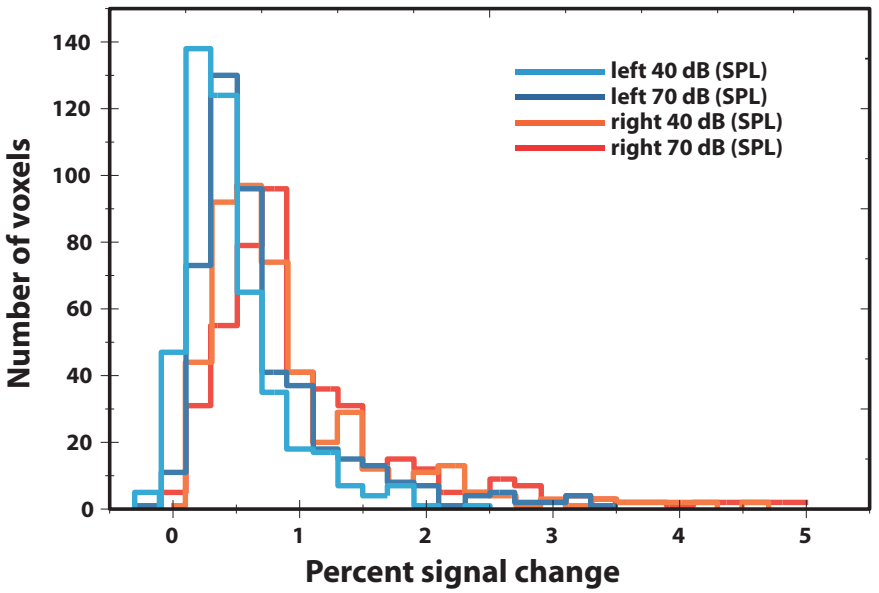

Figure 4.2 An example of the distribution of voxel percent signal changes in a ROI. The responses to four conditions are shown for the left primary auditory cortex of one subject. The number of voxels as function of the percent signal change is shown, indicating a sound intensity dependency and lateralization (i.e. the mean of 70 dB SPL > mean 40 dB SPL and mean right > mean left)

their interaction was also assessed for potential differences in responses between controls and patients. The factor 'condition' was significant in all ROIs with the exception of the vermis of the cerebellum ($p < 0.05$ for the CN, IC, MGB, and PAC; $p < 0.001$ for the AAC). This indicates that there were significant differences between experimental conditions for ROIs in the auditory pathway. The vermis of the cerebellum did not respond differently between experimental conditions.

For the factor 'group', the vermis of the cerebellum was the only ROI that showed a significant difference ($p = 0.005$) between controls and patients with tinnitus. For all conditions, patients showed a larger response than controls in the vermis of the cerebellum, which is clearly visible in figure 4.4.

Finally, there were two significant interactions. The right PAC showed a significant interaction of group · condition ($p = 0.0003$). Patients, on average, showed a smaller difference between the ipsilateral (right-ear) stimuli and the contralateral (left-ear) stimuli than the controls. The same pattern could be observed in the right IC, showing a significant interaction group · condition ($p = 0.0002$). Again, the difference between the ipsilateral stimuli and contralateral stimuli was smaller in patients than in controls.

In addition to these ROI analyses, we specifically assessed the sound-evoked activity in the IC using the same methods as in a recent study of Melcher *et al.* (2009). These authors selected for both the left and right IC the voxel with the lowest p-value based on

the contrast binaural sound vs. baseline and quantified the percent signal change of this voxel. They averaged the response of the left and right IC and plotted the results for each subject separately.

In order to compare results we averaged the responses of the voxel with the highest T-value according to the contrast sound vs. baseline for the left and right IC for all four conditions (i.e. we averaged eight percent signal changes to obtain one value per subject). The results were plotted in figure 4.11 and shows that the subject groups of the study of Melcher *et al.* (2009) have statistically different sound-evoked responses in the IC while our subject groups do not show this difference.

Lateralization

Figure 4.5 shows for each nucleus the corresponding mean lateralization index for each subject group. The lateralization indices are shown for the left hemisphere nuclei (blue) and the right hemisphere nuclei (red) for the auditory pathway (AAC, PAC, MGB, IC and CN) and the cerebellum. A value of +1 indicates a response to left-ear stimuli only, whereas a value of -1 indicates a response to right-ear stimuli only. The ipsilateral lateralization of the CN and the contralateral lateralization of the IC, MGB, PAC and AAC are clearly visible, although the level of lateralization varies between nuclei. The PAC, the AAC and the IC were strongly contralaterally lateralized whereas the MGB was lateralized more weakly. The vermis of the cerebellum, in contrast, did not show any lateralization (which can also be observed from figure 4.4). Interestingly, in almost all nuclei, the lateralization index was closer to zero in patients compared to controls, although this only reaches significance in the right hemisphere nuclei (excluding the CN) using a repeated measures ANOVA ($p = 0.04$). The lateralization index of left hemisphere nuclei did not significantly differ between subject groups, although the same trend can be observed. When looking at individual nuclei, significant differences were observed in the right PAC ($p < 0.02$) and right IC ($p < 0.001$). In these nuclei, the lateralization index was significantly lower in subjects with tinnitus compared to controls.

Connectivity analysis

We calculated the Pearson correlation coefficient (see figure 4.6**A**) and the partial correlation coefficient (see figure 4.6**B**) between all nuclei that were included in the ROI analysis as a measure for, respectively, functional and effective connectivity.

The strongest Pearson correlations were observed between the left and right nucleus at each level of the auditory pathway; coefficients varied between 0.27 for the left and right PAC and 0.68 for the left and right MGB in the control group. When looking at successive levels in the auditory pathway in controls, the ipsilateral PAC and AAC were highly correlated with each other with a correlation coefficient of 0.67 for the left hemisphere and 0.65 for the right hemisphere. The ipsilateral PAC and MGB showed a smaller correlation, varying between 0.33 (left) and 0.36 (right). Between the ipsilateral MGB and IC, the correlations were 0.49 (left) and 0.52 (right).

Interestingly, not only successive connections showed moderate or high correlation. The left and right CN also correlated to a large degree with the right MGB with correlation coefficients of 0.58 (left CN) and 0.49 (right CN). In addition, the left CN and the left MGB also showed a strong correlation of 0.58. Finally, the vermis of the cerebellum was correlated with the AAC with correlation coefficients of 0.22 (left AAC) and 0.18 (right AAC). The group of patients showed a similar pattern of correlations.

The partial correlation coefficients as shown in figure 4.6B, were lower than the Pearson correlation, partly since much of the correlation was task related (e.g., sound-evoked fMRI responses). The partial correlation is the remaining correlation that cannot be accounted for by other nuclei or by task-related effects. Evident from the partial correlations is that the strongest correlation appeared between the left and right hemisphere nuclei. This holds for the AAC, the MGB, the IC and even the CN, whereas the left and right PAC showed a lower partial correlation. The AAC and the ipsilateral PAC were also strongly partially correlated (partial correlation values of 0.59 (left) and 0.53 (right) in the control group). For both subject groups the connection between the left CN and left MGB also showed high partial correlation coefficients of 0.25 for the controls and 0.29 for the patient group. The left AAC and the vermis of the cerebellum showed a partial correlation coefficient of 0.13 in the controls and 0.24 in the patient group, indicating that the cerebellum appears to have an effective connection with the AAC.

Figure 4.7 shows significance maps for the differences between the controls and patients for both the Pearson correlation and the partial correlation. The color-coded values indicate the significance level of the observed difference between the groups displayed on a logarithmic scale, e.g., a significance level of $p = 0.01$ corresponds to a value of $-\log_{10}(p) = 2$. These significance levels were determined using permutation testing by comparing the observed difference with the distribution of possible differences based on 5000 permutations.

Two examples are shown in figure 4.8. Figure 4.8**A** shows the distribution of the possible differences in the Pearson correlation coefficient of the connection between the right IC and the right PAC. The observed difference in Pearson correlation coefficients, calculated as the correlation coefficient of that connection of patients (0.00) minus that of the controls (0.26), is marked by the bold red line, and is located in the tail of the distribution and corresponds to a significance level of p = 0.0004. This shows that the controls had a significantly higher Pearson correlation between the right IC and the right PAC than the patients (see also figure 4.7).

Figure 4.8**B** shows the distribution of differences in the partial correlation coefficient of the connection between the right IC and the left MGB. The observed difference was significantly different from zero ($p = 0.027$) and indicates that patients have a stronger partial correlation (0.16) between the two nuclei than those of the controls (-0.02; see also figure 4.7).

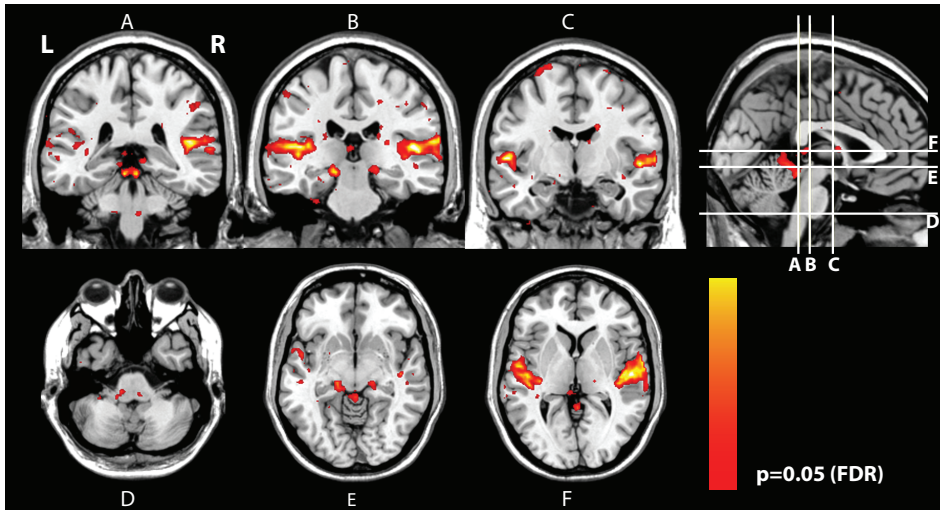

Figure 4.3 Coronal and transversal cross-sections of the human brain in gray-scale with a color-coded overlay showing significant responses to sound. The red-yellow color-coded areas indicate areas with a significant response to sound stimuli (omnibus F-test, $F > 8.34$, $p < 0.05$ FDR, pooled over all subjects). Evident from this figure is the auditory pathway, showing the CN (A and D), the IC (A and E), the MGB (B and E) and the auditory cortices (A, B, C, and F).

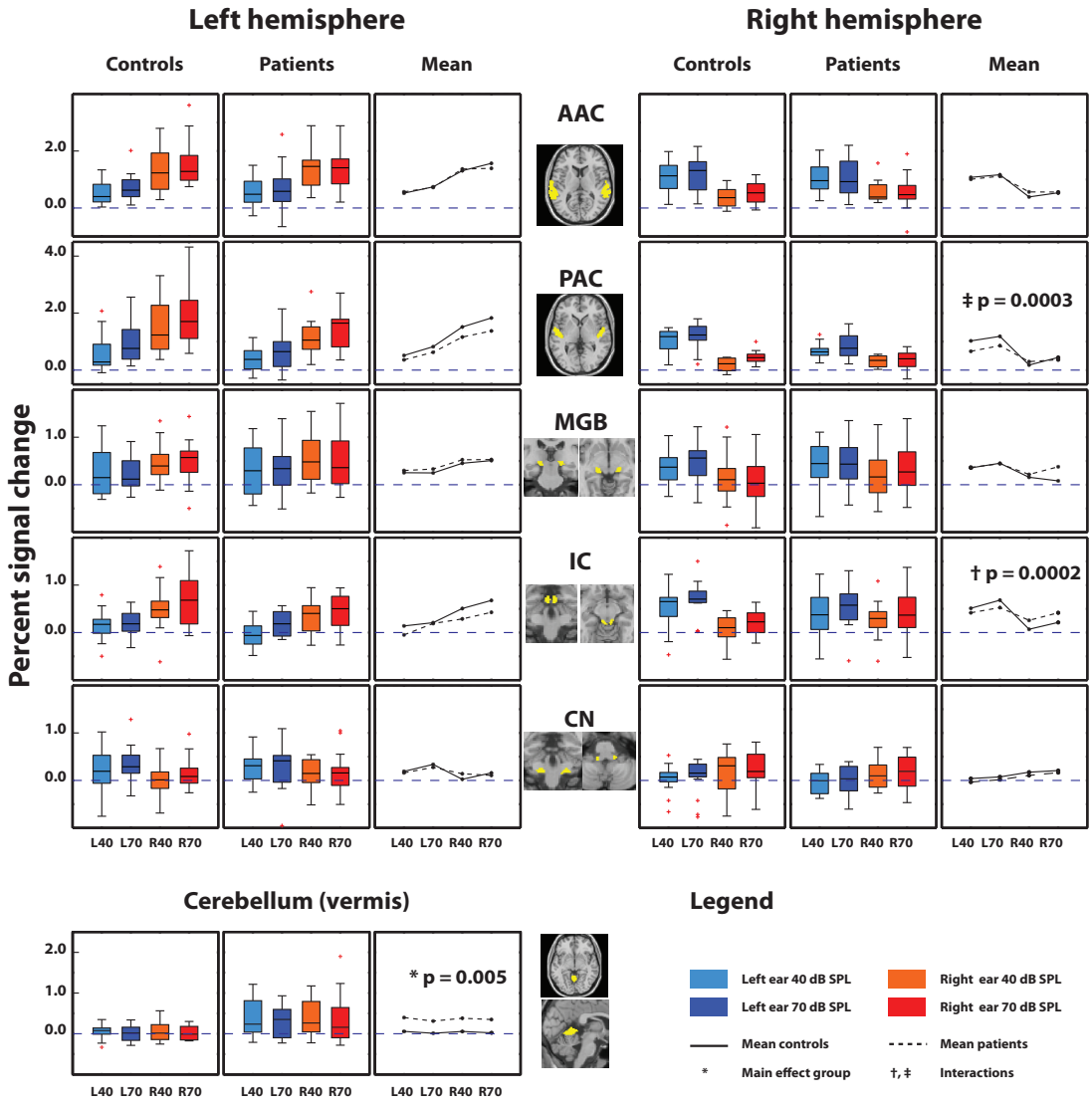

Figure 4.4 The percent signal changes measured in each ROIs of both the left and right hemisphere (AAC, PAC, MGB, IC and CN) and the vermis of the cerebellum for both subject groups. For each ROI, the responses to the four experimental conditions are shown as box plots for each group separately (the first and 4^{th} column shows the responses of the controls whereas the 2^{nd} and 5^{th} column show the responses of the patients with tinnitus). In addition, for each group, the mean per condition is visualized in the plot next to the box plots (the 3^{rd} and 6^{th} column, respectively).

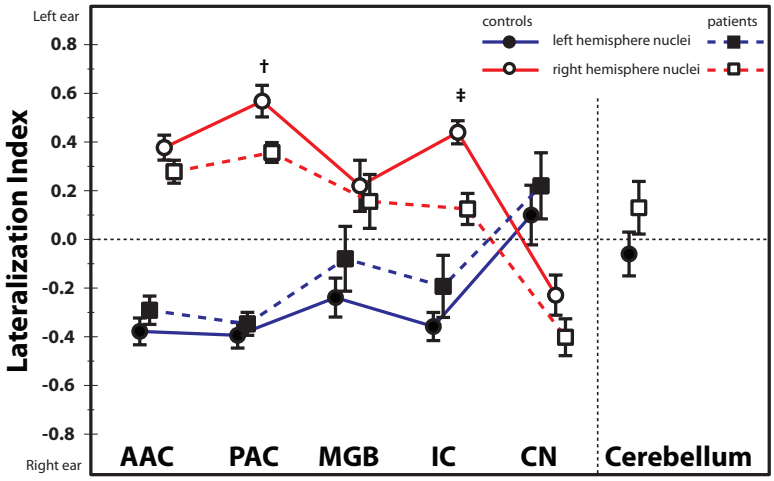

Figure 4.5 The lateralization indices for the left hemisphere nuclei (filled symbols) and the right hemisphere nuclei (open symbols) of the auditory pathway (AAC, PAC, MGB, IC and CN) and the cerebellum. A lateralization index of +1 indicates a response to left-ear stimuli only, whereas a value of -1 indicates a response to right ear stimuli only. The dashed lines correspond to the patient group and the solid lines represent the controls. The error bars indicate the standard error of the mean. The symbols indicate the two nuclei († : PAC and ‡ : IC) where the difference in lateralization index is significantly different between the subject groups.

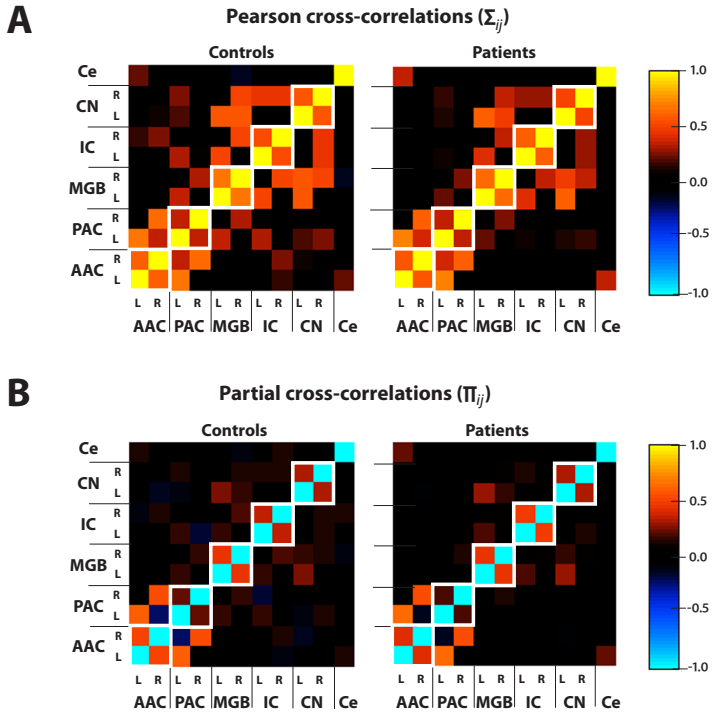

Figure 4.6 Observed functional and effective connectivity patterns in controls (left) and subjects with tinnitus (right). Pearson cross-correlation (**A**) and partial cross-correlation (**B**) coefficients were calculated and color-coded based on the value of the coefficient.

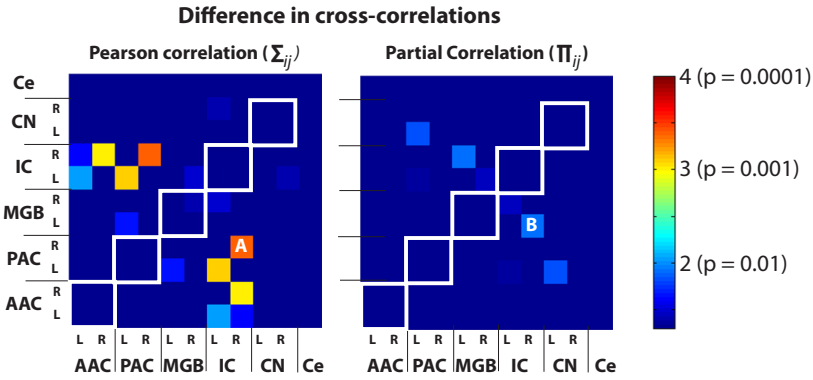

Figure 4.7 Significance maps associated with the observed difference between subject groups for the Pearson correlation coefficients (left) and partial correlation coefficients (right) for each connection. The scale is logarithmic, e.g., $p = 0.01 = 10^{-2}$ is associated to a log-significance of 2. The minimum (blue) is $-\log_{10}(0.05) = 1.30$. **A** and **B** indicate, respectively, the Pearson correlation coefficient between the right IC and the right PAC and the partial correlation coefficient between the right IC and the left MGB. Details of these connections are shown in figure 4.8.

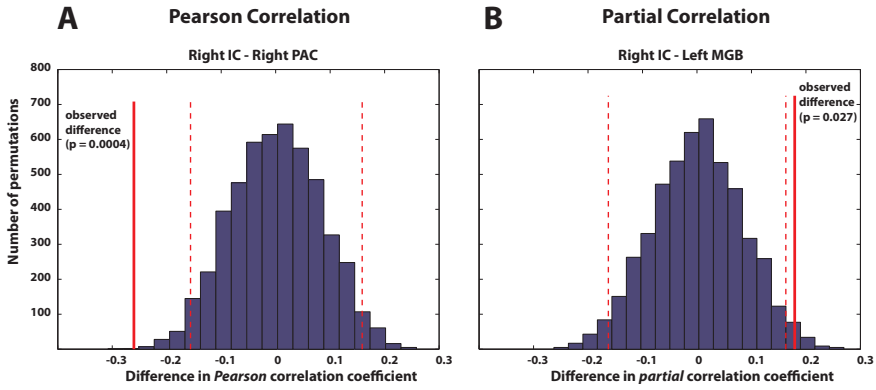

Figure 4.8 Inference of the significance levels of the difference in correlation between patients and controls in two connections. The distributions are based on the permutation of 5000 possible combinations. Panel **A** shows the distribution of possible differences in the Pearson correlation coefficient of the connection between the right IC and right PAC. Panel **B** shows the distribution of possible differences in the partial correlation coefficient between the right IC and left MGB. The bold red lines indicate the observed differences and dotted lines indicate the $p = 0.05$ significance levels. (See also A and B in figure 4.7)

The most prominent differences in Pearson correlation between the controls and patients related to the connections between the IC (left and right) and PAC (left and right) and between the IC and the AAC (see figure 7, left panel). The Pearson correlation was significantly higher in the controls than in the patient group, or equivalently, the difference between the correlation coefficients of patients and controls was negative. The most significant difference was -0.26 and corresponded to the connection between the right IC and right PAC (see figure 4.8A).

The differences as measured with the partial correlation coefficients, showed a different pattern. Three connections displayed a significant difference between subject groups: (1) the connection between the right IC and left MGB was stronger in the patients than in the controls (difference of + 0.18, p=0.027; see figure 7, right panel). (2) The opposite pattern was found for the connection between the left IC and right MGB, with a weaker connection in patients (difference of -0.16, p=0.05). (3) Finally, the connection between the left CN and left PAC showed also a significant difference, where it was stronger in the patient group (difference: + 0.11, p=0.03).

Figure 4.9 shows a summary of the partial correlation coefficients between the various nuclei. It shows for both the controls (left) and the patients (middle) the partial correlations coefficients between nuclei of the auditory pathway. Note that, for clarity reasons, not all existing connections are displayed. While figure 4.6B shows a measure of all existing partial correlations coefficients, figure 4.9 only shows significant partial correlations (that is, partial correlations larger than, or equal to 0.08). From the CN to the IC the connections decussate from ipsilateral to contralateral (see also figure 4.5) and continue contralaterally. The thickness of the lines indicates the strength of the partial correlation coefficients and show that especially the inter-hemispheric connections were strong. Note, however, that the ipsilateral connections between the PAC and the AAC were the strongest connections measured (varying between 0.53 and 0.62).

The differences between the two groups are indicated in panel C of figure 4.9. The contralateral connections between the IC and MGB showed a different strength of effective connectivity between controls and patients as well as the connection between the left CN and left PAC.

4.4 Discussion

In this paper, we investigated possible neural correlates of unilateral tinnitus using fMRI. First, we analyzed the sound-evoked responses and compared differences between subject groups. Based on previous functional imaging studies on unilateral tinnitus (Melcher *et al.*, 2000; Kovacs *et al.*, 2006; Smits *et al.*, 2007) we assumed that the lateralization of the tinnitus would be somehow represented in the brain. We thus performed a random-effects analysis with the two patient groups (a group that perceived tinnitus at their right ear and a group that perceived tinnitus at their left ear) and compared the measured responses to those of controls. This analysis revealed that the sound-evoked responses between both patient groups did not differ significantly, which is in line with previous work (Lanting *et al.*, 2008) where there was no dependency of the strength of the sound-evoked response on the side of the tinnitus. This was also confirmed by a recent paper of Melcher *et al.*

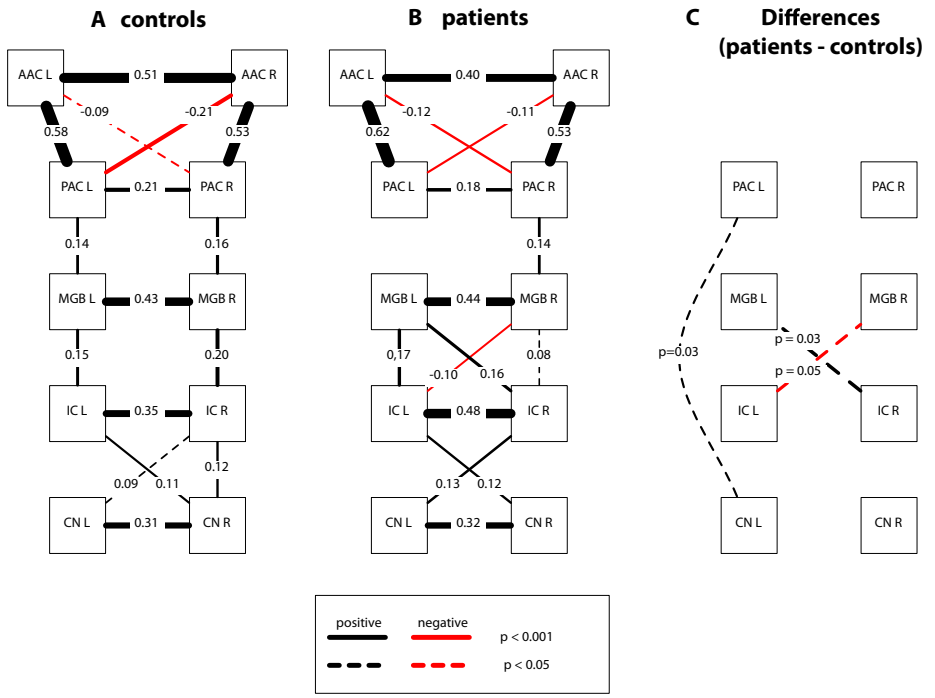

Figure 4.9 Partial correlation coefficients between nuclei in the auditory pathway for the controls (**A**), for the patients (**B**) and the differences between patients and controls (**C**). The graph shows significant partial correlation coefficients between nuclei as solid lines ($p < 0.001$) or as dashed lines ($0.001 < p < 0.05$). The thickness of the lines represents the strength of the partial correlation coefficient between nuclei.

(2009) showing that the lateralization of the tinnitus is not reflected in the strength of the evoked responses in the IC. The other studies that did show a relation between tinnitus lateralization and brain activity, either did not properly match their subject groups based on e.g. hearing loss (Kovacs *et al.*, 2006; Smits *et al.*, 2007), or had ongoing background noise that might have saturated neural responses (Melcher *et al.*, 2000). This presumably caused changes in the lateralization of the brain responses. Based on our findings, we further analyzed the results by pooling the two patient groups together. In summary, the laterality of the tinnitus did not correspond to a lateralized change in the neural response to sound.

The second level random-effects analysis revealed that the vermis of the cerebellum responded significantly stronger in the patient group compared to the controls. The role of the vermis of the cerebellum is not known, but several authors discussed its role. Animal studies reported anatomical connections between the CN and parts of the cerebellum (Huang *et al.*, 1982, 1990). Lesions in the vermis of the cerebellum in rats have been reported to block the long-term habituation of the acoustic startle response (Leaton and

Supple, 1991). Also, in humans, the medial part of the cerebellum is important in the long-term habituation of the acoustic startle response (Timmann *et al.*, 1998; Maschke *et al.*, 2000). A meta analysis, summarizing the findings of fifteen studies on the neural correlates of active and passive listening, reported a general role of the cerebellum in auditory processing (Petacchi *et al.*, 2005). Linking these findings to tinnitus, the vermis of the cerebellum was suggested to play a role in lateral gaze, which in particular subjects with tinnitus changed the perceived loudness of the tinnitus (Lockwood *et al.*, 2001). We can speculate about the possible relation of these results to ours: one could suggest that the habituation of the continuous percept of tinnitus might be impaired in these patients, leading to the prolonged complaints of tinnitus. The vermis of the cerebellum might thus not directly relate to the percept but might influence the habituation to perceived sounds–in this case tinnitus. Nevertheless, given our data, we cannot draw any firm conclusion about the cerebellum, except pointing out that it shows a larger response to sound in patients with tinnitus compared controls. A similar result (increased activity in the paraflocculus of the cerebellum in rats with behavioral evidence for tinnitus) was reported by (Brozoski *et al.*, 2007).

Further ROI analysis showed that, at many levels in the auditory pathway, there were no differences in the strength of the response between subject groups. In general, nuclei of the auditory pathway showed a stronger response to 70 dB (SPL) stimuli than to 40 dB stimuli. In addition, the auditory pathway showed stronger responses to contralateral stimuli—with the exception of the CN, which responded most strongly to ipsilateral stimuli. The pattern of responses to the sound stimuli was different between the subject groups in only two cases: the right PAC and the right IC. Here, in the patient group, there was a reduced difference between ipsilateral and contralateral stimuli. This could also be observed by looking solely at the lateralization index, which was significantly lower in these same nuclei (right IC and PAC). Interestingly, the patients lateralization was lower in almost all nuclei and was significantly lower when performing a repeated measures ANOVA on all right-hemisphere nuclei, except the CN. Unilateral tinnitus thus relates to a decreased lateralization of the auditory pathway. This decreased lateralization might relate to a diminished efficiency in the inhibitory ipsilateral input to the IC. Disinhibition could effectively lead to a more equal input from both ears (via contralateral excitatory input and a dysfunctional inhibition from the ipsilateral ear, see Ehret and Romand (1997)) and therefore decrease the lateralization index. Figure 4.10 shows schematically the normal situation (A) and the situation where the inhibitory pathway is absent (B), which may cause a decreased lateralization index.

In contrast to our earlier work (Lanting *et al.*, 2008) and a recent article by Melcher *et al.* (2009), current analyses indicate that the IC of the patients does not show increased sound-evoked responses. It did in the subjects that we studied earlier but we were not able to replicate this finding here, with a larger group of subjects. The fact that the tinnitus subjects were, on average, 10 years older than the controls might influence our findings, since there are reports that show that induced cortical fMRI activation declines with advancing age (D'Esposito *et al.*, 2003).

Another possible explanation lies in the voxel-selection method. In our previous work, for each condition, we selected the 10 % voxels that had the highest T-value according to

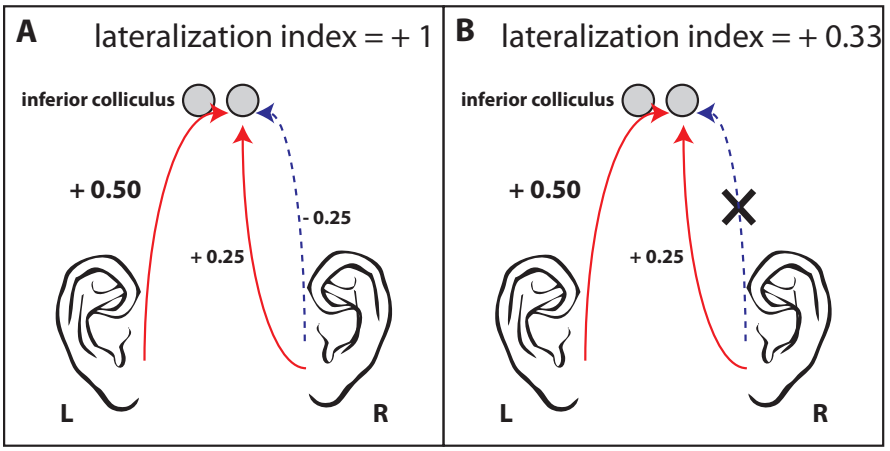

Figure 4.10 A schematic representation of excitatory (solid lines) and inhibitory (dashed lines) connections between both ears and the right IC. In the normal situation (**A**) there is a strong contralateral excitatory connection. In addition there are both excitatory and inhibitory ipsilateral connections. The right panel (**B**) shows a case where the ipsilateral inhibitory path is diminished (as hypothesized in tinnitus, causing hyperactivity), thereby reducing the lateralization index. Note that this is a simplified numerical example to demonstrate how a reduced efficiency of the inhibitory path may lead to a reduced lateralization index. Note that the connections do not necessarily reflect the actual connections between the ears and the IC but reflect the integral path up and until the level of the IC.

the single-subject analysis while in this work, we took the 10 % voxels that have the highest F-value (i.e., the voxels that are best described by the GLM). Melcher *et al.* (2009) used the single voxel that showed the maximum significance level (i.e., lowest p-value). When using the same voxel selection criterion as applied by Melcher, we still do not reproduce their results (see figure 4.11). Thus, the voxel selection criterium does not account for the difference between our result and that of Melcher *et al.* (2009).

An alternative explanation relates to the stimuli that were used. We used monaural stimuli (40 and 70 dB SPL) while Melcher *et al.* (2009) used binaural stimuli of approximately 75 dB (SPL), which might have some influence on the response strength. Another option that we cannot rule out is that there might be a hidden variable among the patients that correlates well with the level of sound-evoked activity like e.g. hyperacusis (Gu *et al.*, 2008) or tinnitus handicap.

So it seems that the effects of tinnitus on neural activity are subtle and might not translate to differences in levels of evoked BOLD activity, possibly since changes in evoked

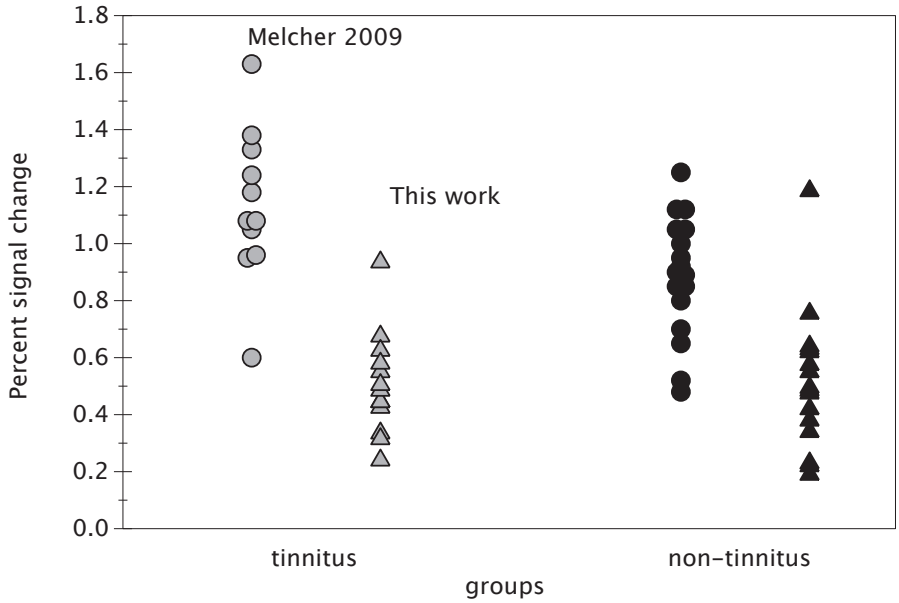

Figure 4.11 Percent signal change in the inferior colliculi of each subject for two separate and independent studies. Each circle indicates percentage signal change averaged between the voxel with the largest response in the left and right inferior colliculi, respectively, of a given subject in a recent study of Melcher *et al.* (2009) (data used with kind permission). Each triangle indicates percentage signal change averages between the left and right inferior colliculus and the four experimental conditions (i.e., the conditions as represented in figure 4.4 in this study). Whereas Melcher *et al.* (2009) find differences between the subject groups, we are not able to distinguish between the groups.

activity do not necessarily reflect changes in spontaneous firing rates (SFRs) or changes in neural synchrony.

The last part of the results section described connectivity patterns between nuclei of the auditory pathway, with in addition the vermis of the cerebellum. A similar approach was performed by Langers *et al.* (2005b) studying connectivity patterns in subjects with unilateral hearing loss. In functional MR imaging, connectivity measures express the extent of similarity of the measured signals in time in various areas of the brain. Activities that covary together, suggest that the neural processes underlying this activity may be related. Two types of connectivity measures have been distinguished (Friston, 1994; Horwitz, 2003). The first type is functional connectivity and usually calculates the temporal correlation between pairs of time signals from two spatially remote areas. The second type of connectivity is effective connectivity which is intended to describe the influence of one area on another area (Friston, 1994).

We adopted two distinctive forms of connectivity analysis in this work (Horwitz,

2003). In addition to the simple (Pearson) cross-correlation as a measure for functional connectivity (Friston, 1994) we studied partial cross-correlation as measure for effective connectivity (Marrelec *et al.*, 2006, 2007, 2009). By using partial correlation, mutual characteristics like sound-evoked responses or other task related features are taken out leaving an inherent measure of effective connectivity.

We observed that for all connections between elements in the model, the Pearson correlation was higher than the partial correlation, indicating that much of the correlation could be driven by the experimental paradigm. We assessed the normal connectivity patterns and observed high partial correlation coefficients between the ipsilateral PAC and AAC. Also, in subjects with tinnitus, the partial correlation coefficient between the left AAC and the vermis of the cerebellum was increased; indicating that the cerebellum appears to show effective connectivity with the auditory association cortex. We also found differences in connectivity in patients with tinnitus based on permutation testing procedures. Specifically, the effective connectivity was disturbed between the IC and the contralateral MGB, as well as between the left CN and the left PAC.

4.5 Conclusion

In conclusion, we did not find tinnitus related differences in the strength of response to sound in the auditory pathway. Yet, we did find changes in lateralization and connectivity, especially from the IC to the contralateral MGB. Apparently, tinnitus is somehow related to changes in connectivity patterns, which may lead to a change in lateralization. The role of the cerebellum in tinnitus remains unknown, although it shows a stronger response to sound in patients with unilateral tinnitus, compared to subjects without tinnitus.

Acknowledgments

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5

Neural correlates of human somatosensory integration in tinnitus

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Abstract

Possible neural correlates of somatosensory modulation of tinnitus were assessed. Functional magnetic resonance imaging (fMRI) was used to investigate differences in neural activity between subjects that can modulate their tinnitus by jaw protrusion and normal hearing controls. We measured sound evoked responses, responses to jaw protrusion and the combined multimodal response. Additionally we studied multimodal integration of somatosensory jaw protrusion and sound. The auditory system responded to jaw protrusion. These responses occurred both in subjects with tinnitus and controls. The responses of the auditory brain areas to jaw protrusion presumably account for the modulation of tinnitus by jaw protrusion. Responses to jaw protrusion of the cochlear nuclei (CN) and the inferior colliculi (IC) were larger in subjects with tinnitus than in healthy controls, suggesting an abnormal strong auditory-somatic interaction in the patient group.

5.1 Introduction

Tinnitus is an auditory sensation without the presence of an external acoustic stimulus. Almost all adults have experienced some form of tinnitus, mostly transient in nature. The exact etiology of tinnitus remains unknown but may involve increased spontaneous neural activity, increased neural synchrony or reorganized tonotopic maps as a neural substrate of tinnitus in humans.

Tinnitus is affected in complex ways by somatosensory influences. Somatic maneuvers can elicit tinnitus or modulate the psychoacoustic attributes of tinnitus (e.g. the loudness or pitch). Examples of these somatosensory modulators are forceful head and neck contractions (Levine, 1999; Levine *et al.*, 2003; Abel and Levine, 2004; Levine *et al.*, 2008), oral facial movements like jaw clenching or jaw protrusion (Chole and Parker, 1992; Rubinstein, 1993; Lockwood *et al.*, 1998; Pinchoff *et al.*, 1998), electrical stimulation of the median nerve (Møller *et al.*, 1992; Møller and Rollins, 2002) and cutaneous stimulation (Cacace *et al.*, 1999a,b). In a remarkable case, finger movement evoked tinnitus (Cullington, 2001). A change of gaze is also known to modulate tinnitus in some patients with a vestibular schwannoma removed (Cacace *et al.*, 1994b,a; Giraud *et al.*, 1999; Coad *et al.*, 2001; Lockwood *et al.*, 2001; Herraiz *et al.*, 2003; Albuquerque and Bronstein, 2004; Baguley *et al.*, 2006).

Somatic modulation or induction of tinnitus may be considered a special case of multisensory integration–a phenomenon, in which one (sensory) modality influences another. Examples of this multisensory integration are visual stimuli that modulate activity measured in the auditory cortex (Pekkola *et al.*, 2005) and audio-visual speech or communication signals that modulate activity in the auditory cortex (Calvert *et al.*, 1999; Ruytjens *et al.*, 2006, 2007a). In addition to auditory-visual integration, there are studies that specifically assess multisensory integration between the auditory system and the somatosensory system. One illustrative example is a study of Jousmaki and Hari (1998) showing that auditory input can modulate touch sensation. Subjects were asked to rub their hands, and the evoked sounds were played back to them. When the high-frequency content of this

auditory signal was increased in loudness, subjects felt the skin under their palms becoming dry–the parchment-skin illusion.

Somatosensory stimulation can also influence auditory perception. The fact that subjects with somatosensory tinnitus can modulate their tinnitus is an example of this somatosensory auditory modulation, and might be explained by changes in normal multisensory integration. Noise-induced hearing-loss, for example, has been reported to alter the normal somatosensory input. The somatosensory input to the cochlear nucleus (CN) was increased after hearing loss (Shore *et al.*, 2008). This change in balance in somatosensory and auditory input at the level of the brainstem might thus be the neural correlate of somatosensory modulation of tinnitus.

Functional magnetic resonance imaging (fMRI) methods have been used to study multisensory integration of auditory and somatosensory input in the auditory cortex of the macaque monkey. Kayser *et al.* (2005) showed multisensory integration of tactile stimuli of the palm and the foot, and auditory stimuli in the belt areas (caudal medial and caudal lateral belt area) of the auditory cortex. Superadditive effects were demonstrated in the belt area, showing voxels with a response to the multisensory stimuli that was larger than the sum of the unisensory stimuli. Foxe *et al.* (2002) used an fMRI design to assess multisensory integration in humans. The unisensory response to sound and somatosensory stimulation was determined. There were voxels that showed overlap in activity between the two conditions. A cluster showing overlap was determined as the posterior part of the left superior temporal gyrus (Brodmann area (BA) 22 and 39) and the right auditory association cortex (BA22). Within the left-hemisphere a small area was found where the bimodal response exceeded the summed unimodal responses (e.g., a superadditive effect). Schurmann *et al.* (2006) later used vibrotactile, pulsed tactile and white noise auditory stimuli in an fMRI design to assess human multisensory integration. In the posterior auditory belt area, bilateral areas were found that showed overlap in activity between the unisensory conditions. These were the same area as found by Foxe *et al.* (2002), showing voxels with overlap between tactile and auditory stimuli.

Multisensory integration and spatial overlap of auditory and somatosensory input was also shown in several parts of the auditory system using anatomical labeling methods and electrophysiological measurements. Sites of neurophysiologic auditory-somatosensory integration were identified as the lower brainstem (the dorsal and ventral CN) and the inferior colliculus (IC) in guinea pigs (see review by Dehmel *et al.* (2008)). In addition, multisensory areas (i.e., areas that receive both auditory and somatosensory input, but not necessarily exhibit multisensory integration) were found in macaque monkeys using anatomical labeling methods. These areas were identified as the medial geniculate complex and the caudal medial belt area of the auditory cortex (Schroeder *et al.*, 2001; Smiley *et al.*, 2007; Hackett *et al.*, 2007a,b). These studies suggest that somatosensory auditory integration may already take place at the brainstem auditory nuclei. Specifically, these brainstem interactions may explain somatic modulation of tinnitus.

In this work we investigated the phenomenon of somatic modulation of tinnitus. For this purpose, we studied two groups of subjects: normal controls and subjects with tinni-

tus. The subjects in the tinnitus group were included based on their ability to change the psychoacoustical characteristics of their tinnitus by jaw protrusion. We hypothesize that this may be based on somatosensory auditory interaction already in the brainstem. We studied both the unisensory fMRI responses (to sound stimuli and jaw protrusion) as well as the multisensory response, obtained by combining both unisensory stimuli.

5.2 Materials and methods

Subjects

Thirteen subjects (12 males and one female, age 28–68 years, median 52 years) with tinnitus were recruited at the University Medical Center Groningen in the multidisciplinary tinnitus outpatient clinic, all with no known neurological and psychiatric history. The subjects with tinnitus were selected based on their ability to alter the loudness or pitch of their tinnitus by performing a protrusion of the jaw. Additionally, twenty control subjects (18 males and two females, age 20–59 years, median 31 years) without tinnitus were recruited. A selection criterion for all subjects comprised the hearing levels for both ears better than 30 dB hearing levels (HL) for frequencies 250, 500 and 1000 Hz, with the average difference between the left and right ear not exceeding 10 dB.

In the patient group, the perceived tinnitus frequency and loudness level were determined by a matching procedure. The frequency matching was performed with an external tone presented at the non-tinnitus ear or at the ear where the tinnitus was weakest, at a comfortable level. The loudness level was then determined by adjusting the level of this tone to match the tinnitus loudness.

Somatosensory modulation of tinnitus was assessed using a questionnaire as described in table 5.2. In this questionnaire–presented here as a translated version of the original Dutch version, the loudness of the tinnitus and loudness of the tinnitus during jaw protrusion was assessed using a visual analog scale. In addition to these loudness values, subjects were asked to rate the duration (in seconds) of the period that subjects could pertain the jaw protrusion that lead to a change of their tinnitus.

Subjects reported loudness values prior to the fMRI study (see figure 5.3). Subjects without tinnitus were also asked to report any perceptual change corresponding to jaw protrusion but no changes were reported.

The handedness of each subject was determined using a translated version of the Edinburgh inventory (Oldfield, 1971). Details of most subject characteristics are shown in table 5.1 and the assessment of the somatosensory modulation can be found in section 5.2. The study was approved by the local medical ethics committee and written informed consent was obtained from each participant.

MRI Protocol

All imaging experiments were performed on a 3T MRI system (Philips Intera, Philips Medical Systems, Best, The Netherlands) with an eight-channel phased-array head coil (SENSE head coil). First, a T1-weighted fast-field echo scan was acquired for anatomical

Table 5.1 Subject characteristics

Characteristics	Controls ($n = 20$)	Subjects with tinnitus ($n = 13$)
Age (years)		
average	32.8	51.8
standard deviation	10.5	10.4
range	20–59	28–68
Gender		
male	18 (90%)	12 (82%)
Tinnitus		
lateralization (left/right/non-lateralized)	–	1 / 1 / 11
average pitch (Hz)	–	6400
range (Hz)	–	750–12000
average loudness (dB SL)	–	16.7
range (dB SL)	–	10–25
Modulation of tinnitus		
changes in frequency	–	2 (15%)
changes in loudness	–	10 (77%)
changes in frequency and loudness	–	1 (8%)
Handedness		
right handed	19 (95%)	11 (85%)
left handed	1 (5%)	1 (8%)
ambidextrous	–	1 (8%)

orientation (TR 11.1 ms; TE 4.6 ms; flip-angle 15°; matrix 256 × 256 × 9; voxel-size 1.0 × 1.0 × 2.0 mm^3).

The functional scans consisted of 2179-ms single-shot T_2*-sensitive echo planar imaging (EPI) sequences with 41 2-mm thick slices (TR 10 s; TE 22 ms; flip-angle 30°; matrix 128 × 128, field of view 224 mm, SENSE reduction factor 2.7) and were acquired using a coronal orientation, aligned to the brainstem when viewed on a midsaggital cross-section. The influence of acoustic scanner noise was reduced using a sparse sampling strategy (Hall *et al.*, 1999) in which auditory stimuli were presented during a 7.8-s gap of scanner silence between two successive acquisitions. For each subject three runs of 61 acquisitions were performed. An additional 3D T_1-weighted fast-field echo scan (TR 25 ms; TE 4.6 ms; flip-angle 30°; matrix 256 × 256 × 160; voxel-size 0.94 × 0.94 × 1.0 mm^3) was acquired with the same orientation as the functional scans to serve as anatomical reference.

Experimental paradigm

Each functional run consisted of the acquisition of 61 volumes with 3 experimental conditions that were contrasted against a baseline condition: [1] a condition in which bilateral rippled broadband noise was presented, [2] a condition in which subjects protruded their jaw (protrusion of the mandible) and [3] a bimodal condition including both protrusion of the jaw and presentation of sound. Each condition was presented 15 times per functional run, the baseline condition was presented 16 times.

During the experiment, subjects were looking at the instruction as projected on a screen

mounted in the bore of the scanner. The instruction consisted of words describing the jaw protrusion task (either 'rest' or 'protrude jaw'). Subjects were instructed to attend to the sound stimuli, but were not required to perform any task related to the sound stimuli.

The auditory stimuli consisted of temporally and spectrally modulated noise (Langers *et al.*, 2003). The stimuli had a frequency-range of 125–8000 Hz with a spectral modulation density of one cycle per octave, a temporal modulation frequency of two cycles per second and a modulation amplitude of 80%. These stimuli were generated using Matlab 6.5 (The Mathworks Inc., Natick, MA) and were saved as wave files.

A PC setup equipped with a digital-analogue card (National Instruments 6052E, National Instruments Corporation, Austin, TX) in combination with Labview (National Instruments Corporation, Austin, TX) was used to present the auditory stimuli bilaterally at 70 dB (SPL) to the subjects through an MR compatible electrodynamic system (MR Confon GmbH, Baumgart *et al.* (1998)).

The experimental paradigm was not randomized and the jaw protrusion condition was performed alternating with either baseline or presentation of sound to prevent fatigue of jaw muscles as was reported by subjects in initial pilot measurements.

In order to minimize the within-scan movement, the protrusion started 4s after the beginning of the acquisition and ended 1.5s before the next acquisition (see fig. 5.1). Due to the long latency of the hemodynamic response of the blood oxygen level dependent (BOLD) effect (typically about 4-5 sec, although latencies vary between subjects and brain areas; see Neumann *et al.* (2003) and Langers *et al.* (2005a)), we were still able to measure the jaw-related neural activity. Since the jaw was always fully relaxed during the MRI acquisitions, the jaw protrusion task did not degrade the quality of the data. In addition, our timing paradigm ensured the reliable response of subjects to the visual instructions.

Data analysis

MR images were analyzed using Matlab 7.1 (R14) (The Mathworks Inc., Natick, MA) and SPM5 (Functional Imaging Laboratory, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/). The functional images were corrected for motion using realignment of all images to the first acquired volume of each subject and were spatially coregistered with the T1-weighted high-resolution anatomical image. The high-resolution anatomical image was segmented in gray matter, white matter and cerebral spinal fluid (CSF) segments. The gray-matter segment of the anatomical image was normalized to a custom normalization template (for more details, see Lanting *et al.* (2008)) and the resulting transformation parameters were also applied to the functional data. Functional data were spatially smoothed using an isotropic Gaussian kernel with a full width at half maximum of 4 mm, to improve signal-to-noise ratio characteristics while retaining the ability to discern small auditory structures (e.g. the cochlear nuclei). Functional images were interpolated to voxel dimensions of $2.0 \times 2.0 \times 2.0$ mm^3.

Statistical Parameter mapping

For each subject, a general linear model was set up to analyze the relative contribution of each condition to the measured response. The multiple regression model included three covariates of interest (one for each condition with the exception of the baseline condition), and for each run one constant factor to model the mean and a linear term to correct for

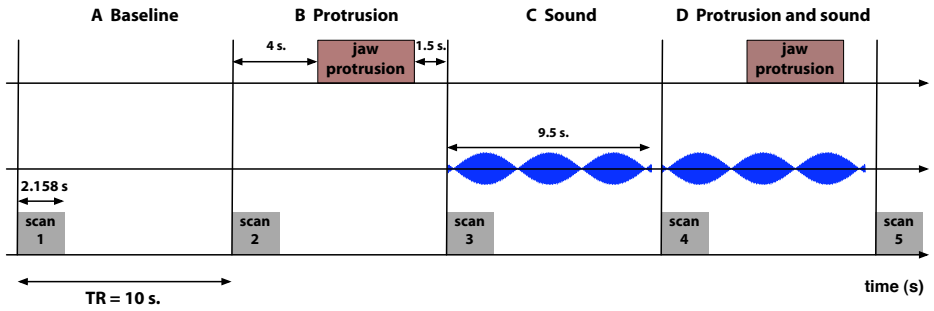

Figure 5.1 The experimental design. The design consisted of 4 conditions: a baseline condition (panel **A**: baseline) to which all other conditions were contrasted, two unisensory conditions (panel **B**: jaw protrusion and panel **C**: sound) and one multisensory condition (panel **D**: jaw protrusion combined with sound). Presentation of the sound stimulus started with scanning and ended 0.5 s prior to the next scan. The instruction for jaw protrusion was projected on a screen 4 s after the beginning of a scan until 1.5 s prior to the next. Note that the data acquisition of each condition started after the stimulus presentation or instruction was finished (e.g., the unisensory jaw protrusion condition (**B**) corresponds to hemodynamic fMRI responses that are measured during scan 3 and the multisensory condition (**D**) corresponds to the fMRI responses measured during scan 5). The sequence of the four conditions was repeated 15x3 times.

drift in the scanner signal. The model was applied to the data of all individual voxels and contrast images were created, one for each condition (i.e. sound vs. baseline, jaw protrusion vs. baseline and sound combined with jaw protrusion vs. baseline).

The three contrast images obtained per subject based on the general linear model were further analyzed in a random effects analysis using a repeated measures ANOVA implemented as a factorial design in SPM5. Three factors were defined to model the responses. One factor was a subject factor; one factor was defined as group factor (i.e., subjects with tinnitus or controls) and one factor was a within-subjects stimulus factor (i.e., the experimental conditions). Using such a design, several main effects could be tested. First we assessed which voxels responded significantly to sound only, to jaw protrusion only and to the bimodal condition that combined both modalities. This was achieved by pooling the data of all subjects. In addition, a contrast was defined showing voxels that have a significantly larger response to the bimodal condition than to the sum of the unimodal conditions. This last contrast can be thought of as a measure of multisensory integration.

Probability maps

A drawback of a random effects analysis is that, when the between-subject variability is high and the mean response is weak, it can prove rather unreliable and insensitive (Thirion *et al.*, 2007). An alternative method to represent a response that is common within a particular subject group is to make use of a map containing a descriptive statistic like an

incidence or probability (Hall and Plack, 2009). This method depicts a percentage of subjects that exhibit (significant) activity at a certain voxel.

In the present study we used a probability threshold of $p < 0.001$, uncorrected for multiple comparisons, for the unisensory conditions (i.e. sound vs. baseline and jaw protrusion vs. baseline). Now, if in a single subject, the response measured at a certain voxel exceeded threshold in both unimodal conditions, it was given a value of one. If it did not exceed threshold, it was given a value of zero. Next, the values were summed over all subjects and divided by the number of subjects to create a probability map. This map now shows the percentage of subjects that exhibit overlap in activation between the unisensory conditions at a certain voxel (Schurmann *et al.*, 2006).

Region of interest analysis

In addition to the second level analysis and the analysis of the probability maps we performed a region of interest (ROI) analysis, assessing sound-evoked responses in 13 anatomical areas comprising parts of the auditory pathway and parts of the somatosensory pathway. The ROIs were defined based on anatomical atlasses. The left and right primary auditory cortices (PAC) were defined as the combination of the TE1.0, TE 1.1 and TE 1.2 areas using the SPM5 Anatomy toolbox (Morosan *et al.*, 2001; Rademacher *et al.*, 2001; Eickhoff *et al.*, 2005, 2007). For the left and right auditory association cortices (AAC) we used the left and right superior temporal gyrus as defined by Brodmann (BA 22) based on the AAL template in MRIcron (`http://www.sph.sc.edu/comd/rorden/mricron/`).

Both the ROIs of the primary and auditory association cortices were first normalized to match our anatomical template in order to have a corresponding image space. The left and right part of the medial geniculate body of the thalamus (MGB), the left and right inferior colliculi (IC) and the left and right cochlear nuclei (CN) were manually drawn based on an anatomical atlas (Woolsey, 2003; Martin, 2003). In addition to these auditory areas, the left and right somatosensory cortices were manually drawn approximately coinciding with BA 6 and SII. Also, the left and right ventrolateral (VL) nucleus of the thalamus were manually drawn based on an anatomical atlas (Woolsey, 2003; Martin, 2003). Finally, the vermis of the cerebellum was taken as a region of interest since it showed aberrant responses in subjects with tinnitus in a previous study (chapter 4). Figure 5.4 shows the location of the ROIs of the primary auditory cortex, the auditory association cortex, the medial geniculate body and the somatosensory cortex.

Based on a t-test comparing the responses to the bimodal condition to a baseline condition, the 10% of the voxels that responded most strongly (i.e., with the highest T-value) within each ROI were selected. A percent signal change compared to baseline was calculated for each condition based on the (averaged) regression coefficients within each ROI. For each subject we calculated a percent signal change for all 13 ROIs. Box plots were used to visualize the data and show for each ROI the distribution of the percent signal change values for each condition within each group.

Next, we determined if there were significant differences between the percent signal change in the left hemisphere ROI compared to the right hemisphere ROI using a two-

sample t-test where the data was pooled over all subjects. This excluded the vermis of the cerebellum since we made no distinction between the left and right hemisphere.

The responses of both unimodal conditions, each compared to a baseline signal level were tested for statistical significant deviation from zero using a one-sample t-test. The bimodal condition was not tested, since the voxels used in the ROI analysis were determined based on the strength of the responses in the bimodal condition and is therefore biased (Kriegeskorte *et al.*, 2009). Next, a two-sample t-test was used for each condition and each ROI to assess potential differences between subject groups.

Finally, the bimodal condition (jaw protrusion combined with the presentation of sound) was compared to the unimodal conditions. This comparison was based on the difference between the multisensory condition and the sum of the unisensory conditions (Calvert *et al.*, 2001; Kayser *et al.*, 2005). A difference that was larger than zero was considered evidence for multisensory integration.

5.3 Results

Audiometry

Pure tone audiometry (250-8000 Hz) was performed prior to the functional session. The mean audiogram and the standard deviation around the mean are displayed per group in figure 5.2. For the control group, hearing threshold was 13 ± 8 dB hearing level (HL), averaged over both ears and over frequencies of 250-8000 Hz (mean ± standard deviation across subjects). In subjects with tinnitus, this average was 25 ± 20 dB HL. For the frequency-range of 250-2000 Hz, average hearing thresholds were determined at 11 ± 2 and 15 ± 8 dB HL for, respectively, the controls and subjects with tinnitus. The frequency-range of 4000–8000 Hz showed larger (and significant) differences between the two subject groups with an average of 17 ± 11 and 39 ± 23 dB for, respectively, the controls and subjects with tinnitus (see figure 5.2).

Table 5.2 shows the questions that subjects with tinnitus were asked. The last column shows the incidence (in % of all subjects with tinnitus) that a maneuver changed the perceptual characteristics of the tinnitus. All tinnitus subjects were able to alter their tinnitus by protrusion of the jaw. Upon protrusion of the jaw, two subjects (15%) reported only a change in pitch of their tinnitus or the appearance of another sound and ten (77%) subjects reported only a loudness change of their tinnitus. One subject reported both a change in frequency and loudness. The change in loudness varied from -9 to +4 units on a visual analog scale, where a negative number indicates a loudness decrease (two subjects) and a positive number an increase of the loudness (eight subjects). Figure 5.3 shows the loudness of the tinnitus during jaw protrusion as function of the loudness as measured during rest. In all tinnitus subjects, the modulation effect of jaw protrusion was stable and persisting throughout the maneuver. Question 7 and 8 of the questionnaire were asked specifically since subjects had to perform the maneuver without excessive head movement while in a supine position (i.e., lying in the MR scanner).

Table 5.2 Somatosensory modulation of tinnitus questionnaire

	Question		percentage
1.	Does the tinnitus changes by performing the following manipulations?		
a.	protrusion of the jaw (mandible)	y/n	100%
b.	movement of the mandible to the right	y/n	77%
c.	movement of the mandible to the left	y/n	62%
d.	retraction of the mandible	y/n	46%
e.	touching of / pressure on the skin of the face	y/n	62%
f.	touching of / pressure on the neck area	y/n	31%
g.	touch of / pressure on the left hand / making fist	y/n	23%
h.	touch of / pressure on the right hand / making fist	y/n	8%
i.	turn head left	y/n	54%
j.	turn head right	y/n	69%
k.	gaze to the left (hold head fixed in normal position)	y/n	0%
l.	gaze to the right (hold head fixed in normal position)	y/n	8%
m.	gaze upwards (hold head fixed in normal position)	y/n	8%
n.	gaze downwards (hold head fixed in normal position)	y/n	0%
2.	Describe the effect of the manipulations in 1.)		
a.	change in pitch		15%
b.	change in loudness		77%
c.	another sound		8%
d.	any other effect		8%
3.	Is this modulation persistent if you hold the manipulation in a fixed position	y/n	
4.	does the loudness of the tinnitus change?	y/n	
5.	what is the loudness of your tinnitus (visual analog score 0–10)?		average: 6.0
6.	what is the loudness of your tinnitus if you perform any of the manipulations in 1.)		average: 6.6
7.	can you manipulate your tinnitus without movement of the head	y/n	
8.	can you manipulate your tinnitus while in a supine position	y/n	

Statistical parameter mapping and probability maps

Figure 5.5 shows a statistical parameter map (SPM), obtained through the random-effects analysis, across all (33) subjects for the contrast sound vs. baseline and jaw-protrusion vs. baseline. The contrast sound vs. baseline (indicated as 'sound') showed part of the auditory pathway consisting of the CN, IC, MGB, PAC and AAC. The contrast jaw-protrusion vs. baseline (indicated as 'jaw') showed the following structures: the ventrolateral nucleus of the thalamus (VL), the putamen (Put) and the secondary somatosensory cortex (SII). Activation patterns obtained exceeded the voxel-wise threshold, showing significant responses. A small area in the primary auditory cortex responded to both sound and jaw protrusion.

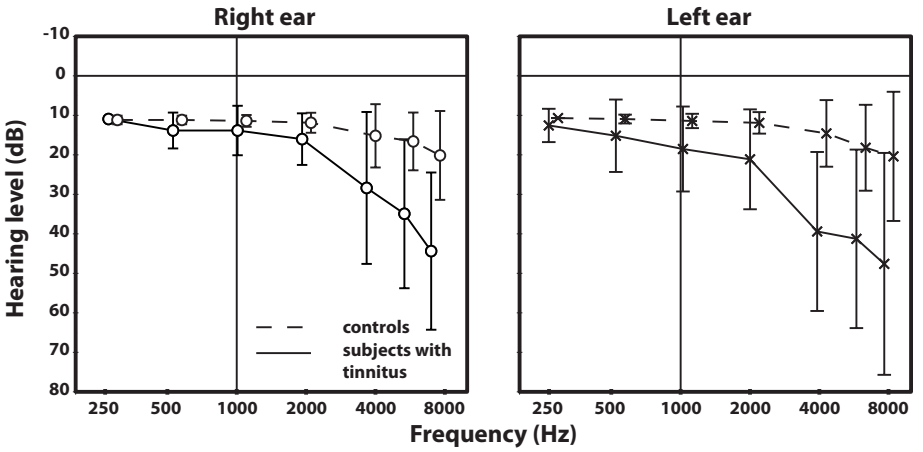

Figure 5.2 Average hearing thresholds for the right and left ear of the two subject groups. The solid lines represent hearing thresholds of subjects with tinnitus and the dashed lines represent hearing thresholds of the controls. The thresholds were normal in the control group (i.e., better than 20 dB hearing level). For the high frequency range (4.0-8.0 kHz), thresholds in subjects with tinnitus were elevated as compared to controls. The error bars indicate the group standard deviation around the mean.

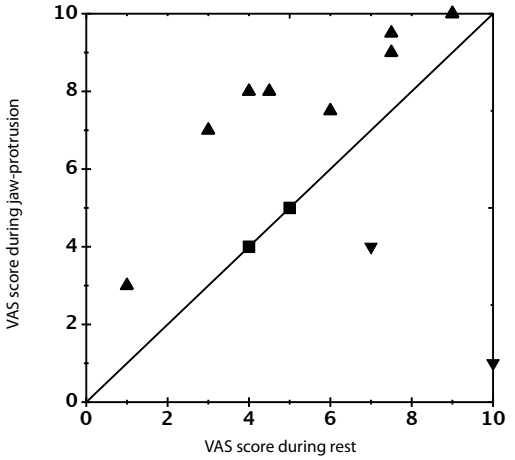

Figure 5.3 Tinnitus loudness during rest and jaw protrusion. Visual analogue scores (VAS) show for each subject the loudness of their tinnitus during jaw protrusion vs. the loudness during rest. Eight subjects reported an increase, two subjects reported a decrease and two subjects reported no change in the loudness as a result of jaw protrusion. The latter two subjects described a change in the pitch of their tinnitus.

Figure 5.6 shows a probability map indicating overlap between the two unisensory conditions and additionally shows a measure of multisensory integration. In blue/red colors it shows the probability-map indicating the incidence that a voxel shows a significant response to both jaw-protrusion and sound at the level of single subjects (thresholded at $p < 0.001$, uncorrected for multiple comparisons). Voxels with more than 5% overlap across subjects are shown. These areas include the bilateral BA 41 and 22. Similarly, it shows in orange/yellow colors, the voxels for which the sum of the jaw and sound responses was smaller that the response to the combined jaw plus sound stimulation. These areas are considered to exhibit multisensory integration. They include the bilateral middle temporal gyrus (including BA 21) and the inferior temporal gyrus. The coronal cross-section shows voxels that exhibit multisensory integration in the cingulate gyrus, which is part of the limbic lobe.

Region of interest analysis

A region of interest analysis was performed in all subjects using ROIs comprising parts of the auditory pathway (the CN, the IC, the MGB, the PAC and the AAC) and the somatosensory areas including the somatosensory cortex, the ventrolateral nucleus of the thalamus and the vermis of the cerebellum. Within each ROI, the 10% best responding voxels to the bimodal condition were selected and the mean signal change compared to baseline was calculated. Since the responses of each left and right ROI were not statistically significantly different from each other, the average value of the left and right ROI was taken.

The box plots in figure 5.7 show the distribution of ROI-responses for controls (white) and subjects with tinnitus (gray) for the different ROIs. For each ROI, it shows the distribution of measured percent signal changes for the three experimental conditions, sound, jaw protrusion and the combined (bimodal) condition–all compared to baseline. In addition, a measure for multisensory integration is shown in each rightmost box plot, where the distribution of values of the multisensory response minus the sum of the two unisensory responses for each single subject is represented. Summary statistics on the experimental conditions are presented in table 5.3 and summary statistics on the group differences are presented in table 5.4.

Response to sound

All nuclei of the auditory pathway (figure 5.7A–E) showed a significant ($p < 0.001$) response to sound in both subject groups. The somatosensory cortex (figure 5.7F) did not show a significant response to sound stimuli ($p = 0.06$ and $p = 0.93$ for, respectively, the controls and subjects with tinnitus). The ventrolateral nucleus of the thalamus (figure 5.7G), in contrast, showed a small, but significant response to sound ($p = 0.004$ and $p = 0.008$ for, respectively, the controls and subjects with tinnitus). The vermis responded significantly to sound in controls ($p = 0.001$). In tinnitus subjects the response of the vermis to sound was not significant ($p = 0.06$). When comparing groups (controls vs. subjects with tinnitus) no significant differences in sound-evoked responses were observed (see table 5.4).

Response to jaw protrusion

While the somatosensory cortex (figure 5.7F) did not show a response to sound, it did show a significant ($p < 0.001$) response to jaw-protrusion as can also be observed at the voxel-wise group analysis in figure 5.5. The ventrolateral nucleus of the thalamus and the cerebellum also showed a significant response to jaw-protrusion in both subject groups (figure 5.7G–H). Interestingly, almost all ROIs in the auditory pathway responded significantly to jaw-protrusion, except for the IC in the control group ($p = 0.48$). Evidently, somatosensory input enhances activity in the auditory pathway, even in the absence of sound stimuli.

Multisensory integration

For each ROI we determined whether the multisensory response exceeded the sum of both unisensory responses. A positive difference is considered strong evidence of multisensory integration (Calvert, 2001). The difference was positive in the MGB and the IC of controls (figure 5.7CD and table 5.4). The patient group did not show evidence of multisensory integration at this level. Also the somatosensory cortex (both groups) and the ventrolateral nucleus of the thalamus in the control group showed some integration. Note, however, that the effects of this integration were small (figure 5.7F-H).

Group differences

The responses measured in several ROIs allow for a comparison between both subject groups. At the level of the CN, the subjects with tinnitus showed a larger response to jaw protrusion than the controls (tinnitus - controls : 0.35 ± 0.24; mean difference ± 95% CI). Also, the IC showed larger jaw responses in tinnitus subjects than in controls (p = 0.014; tinnitus – controls: 0.28 ± 0.22; mean difference ± 95% CI). The other ROIs did not show significant differences between the subject groups.

In summary, jaw protrusion generated extensive responses in the auditory pathway. The only differences between tinnitus subjects and controls was found in the CN and the IC, for which the responses to jaw protrusion were larger in tinnitus.

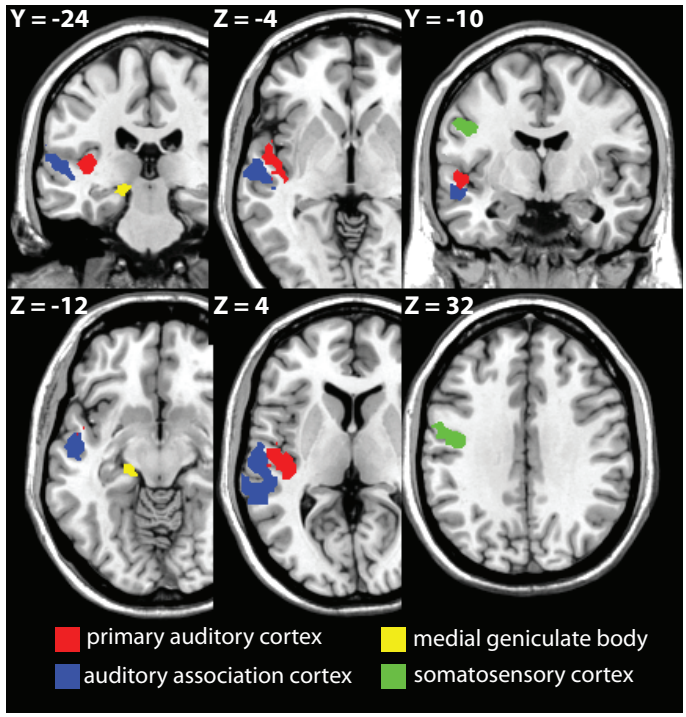

Figure 5.4 Regions of interest (ROIs). Four ROIs are displayed as colored overlays over a gray-scale anatomical image. Shown in red is the ROI of the primary auditory cortex, in blue the auditory association cortex, in yellow the medial geniculate body, and in green the ROI corresponding to the somatosensory cortex (see text for definition of ROIs). Note that, for visualization purposes, only the left hemisphere ROIs are shown. The ROIs of the inferior colliculus (IC), cochlear nucleus (CN), ventrolateral (VL) nucleus of the thalamus and cerebellum (vermis) are not shown in this figure.

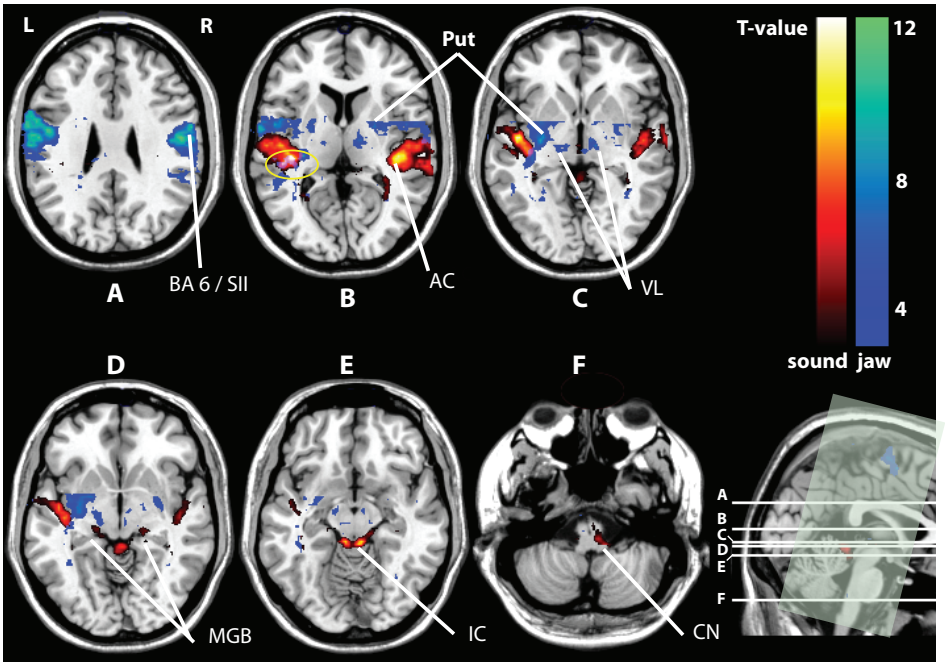

Figure 5.5 Group responses to sound stimuli and jaw protrusion. The group analysis (random effects analysis) shows the spatial distribution of voxels that have a significantly large ($p < 0.01$ FDR ; T>3.43) response to sound (red/yellow colored overlay) and jaw protrusion (blue/green colored overlay). The responding voxels are displayed over a gray-scale anatomical image. The bottom-right midsaggittal cross-section shows (semi-transparent) the imaging volume and the slices of interest (A–F). These slices include the following structures: (A) BA6 / SII, Brodmann area 6 / somatosensory cortex; (B) AC, auditory cortex; Put, Putamen; (C) VL, ventral lateral nucleus of the thalamus; (D) MGB, medial geniculate body; (E) IC, inferior colliculus and (F) CN, cochlear nucleus. The yellow circle in panel B indicates an area in the primary auditory cortex that shows overlap of activity between the two conditions (sound and jaw protrusion). This overlap is visible by the purple color; mixing red (sound) and blue (jaw protrusion).

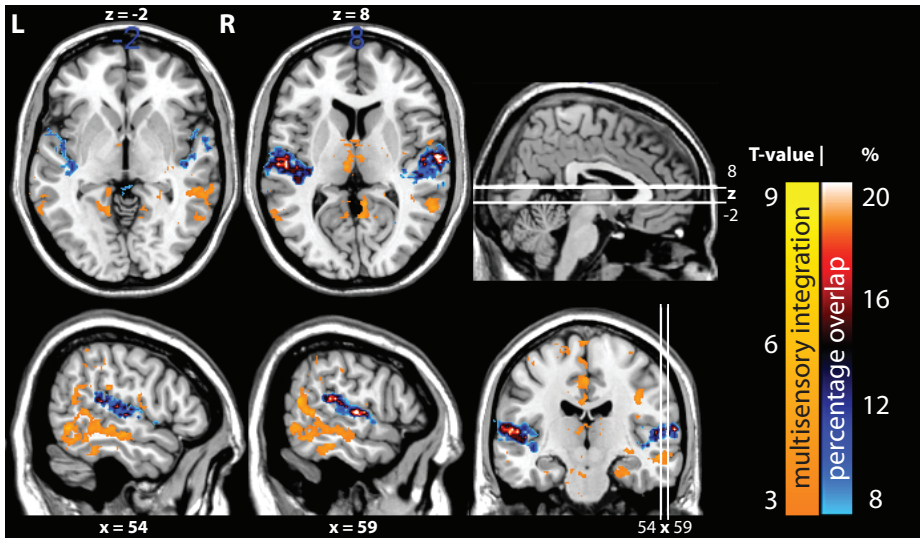

Figure 5.6 Probability map showing the incidence, in percent overlap between all subjects (thresholded at 5% and coded in blue-black-red colors), that a voxel showed significant responses to both jaw protrusion and sound (each thresholded at the single subject level of $p < 0.001$, F-test, uncorrected for multiple comparisons). Areas that showed overlap include the primary auditory cortex (PAC, BA 41) and the auditory association cortex (AAC, BA 22). In addition, orange/yellow colors show voxels that exhibited multisensory integration (i.e., when the bimodal condition showed a larger response than the sum of the unimodal stimuli; $p < 0.05$ FDR, $T > 2.58$). Areas that showed multisensory integration included the bilateral middle temporal gyrus (including BA 21) and the inferior temporal gyrus. The coronal cross-section (bottom-right) shows also multisensory integration in the cingulate gyrus, which is part of the limbic lobe.

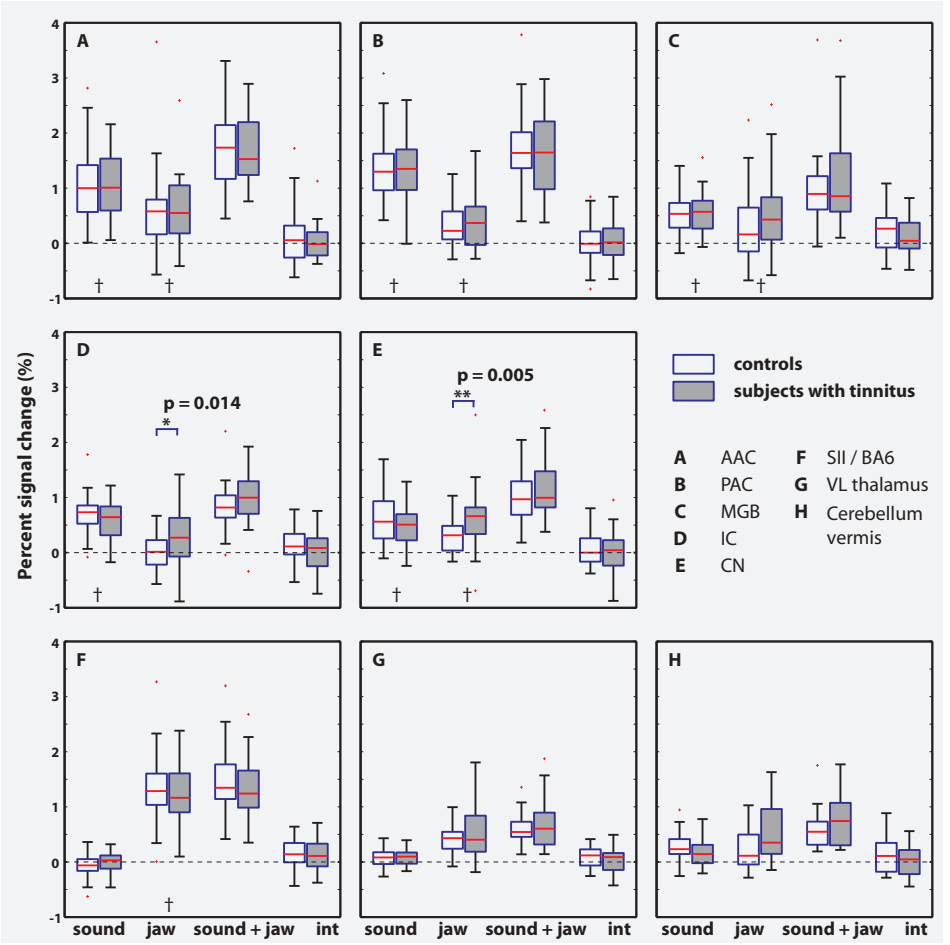

Figure 5.7 Region of interest (ROI) responses in (A) the auditory association cortex (BA 22), (B) the primary auditory cortex (BA41), (C) the medial geniculate body, (D) inferior colliculus and (E) the cochlear nucleus. In addition, the responses in (F) the somatosensory cortex, (G) the ventral lateral nucleus of the thalamus and the (H) cerebellum (vermis) are shown. Responses are shown for controls (white) and subjects with tinnitus (gray) as box plots (showing smallest observation, 25^{th}, 50^{th} and 75^{th} percentile, and largest observation). The responses are given in percent signal changed compared to baseline. For each ROI, the responses for three conditions are displayed: the response to sound ("Sound"), the response to jaw-protrusion ("Jaw"), and the response to both conditions together ("Sound + Jaw"). In addition a measure for multisensory integration is shown ("Int", defined as the difference between the multisensory condition and the sum of the unisensory condition).

Table 5.3 Significance values (p-values, t-test) of the region-of-interest (ROI) responses to sound, jaw and integration in both subject groups; significant values ($p < 0.05$) are typeset in bold.

	Controls			Subjects with tinnitus		
Region of interest	sound	jaw-protrusion	integration	sound	jaw-protrusion	integration
Auditory association cortex (AAC)	**< 0.001**	**< 0.001**	0.08	**< 0.001**	**< 0.001**	0.41
Primary auditory cortex (PAC)	**< 0.001**	**< 0.001**	0.66	**< 0.001**	**< 0.001**	0.80
Medial geniculate body (MGB)	**< 0.001**	**0.002**	**0.01**	**< 0.001**	**0.001**	0.22
Inferior colliculus (IC)	**< 0.001**	0.48	**0.005**	**< 0.001**	**0.012**	0.47
Cochlear nucleus (CN)	**< 0.001**	**< 0.001**	0.27	**< 0.001**	**< 0.001**	0.83
Somatosensory cortex (SII)	0.06	**< 0.001**	**< 0.001**	0.93	**< 0.001**	**0.049**
Ventrolateral nucleus of the thalamus	**0.004**	**< 0.001**	**0.006**	**0.008**	**< 0.001**	0.22
Cerebellum vermis	**0.001**	**0.022**	0.10	0.06	**0.003**	0.80

Table 5.4 Differences between subject groups; significant differences (two-sided t-test; p < 0.05) are typeset in bold. For each ROI, the table shows the mean difference (a positive difference indicates that the responses in patients are bigger than those in controls subjects), the 95% confidence interval (CI) and the corresponding significance level (p-value).

	Sound			Protrusion			Integration		
Region of interest	mean	±CI	p-value	mean	±CI	p-value	mean	±CI	p-value
AAC	0.02	0.31	0.89	-0.04	0.36	0.81	-0.09	0.24	0.44
PAC	-0.02	0.22	0.90	0.08	0.22	0.44	0.00	0.19	0.93
MGB	0.04	0.18	0.66	0.20	0.37	0.30	-0.09	0.20	0.35
IC	-0.09	0.17	0.28	**0.28**	**0.22**	**0.014**	-0.08	0.16	0.30
CN	-0.13	0.22	0.23	**0.35**	**0.24**	**0.005**	-0.07	0.18	0.46
SII	0.07	0.10	0.13	-0.12	0.33	0.47	-0.05	0.13	0.45
VL thalamus	0.09	0.08	0.75	0.09	0.18	0.29	-0.03	0.10	0.57
Cerebellum vermis	-0.10	0.21	0.34	0.33	0.35	0.06	-0.12	0.24	0.33

5.4 Discussion

Somatic tinnitus is a phenomenon, which refers to tinnitus that is elicited or modulated by somatosensory input. This may be considered to be a specific form of multimodal integration. The present study demonstrated that overlap of somatosensory and auditory responses could be measured throughout the auditory pathway (i.e., non-zero responses to both auditory and somatosensory input), which may explain the influence of jaw protrusion on tinnitus. In addition to overlap in activity between the sensory modalities, multisensory integration between them was measured.

Multimodal integration refers to the responsiveness of a single neuron to stimulation of different sensory modalities, or the modulation of the response to one sensory modality by another sensory modality. Typically, this influence has been described in terms of changes in firing rates of the neuron, being either enhancing or suppressive. Bimodal enhancement is based on the magnitude of the bimodal response compared to the larger of the unimodal responses (Stein and Meredith, 1993). Bimodal suppression on the other hand is based on a bimodal response that is smaller than the larger of the unimodal responses.

Auditory and somatosensory integration: neurophysiologic and histological evidence

Somatic sensation of the head, including the oral cavity is conveyed by four cranial nerves, of which the trigeminal nerve is most important. The trigeminal nerve consists of three branches: the ophthalmic branch (nV/I), which innervates the forehead, upper eyelid, and extraocular muscles; the maxillar branch (nV/II), which innervates the upper lip and jaw, the roof of the mouth and the lower eyelid; and the mandibular branch (nV/III), which innervates the lower lip, the floor of the mouth, and the anterior two thirds of the tongue and the mucous membranes of the lower jaw. These three branches converge to the trigeminal ganglion (TG). Neurons of the trigeminal ganglion project to the brainstem trigeminal sensory complex, which receives proprioceptive information from the jaw and the vocal tract and intraoral structures like the temporomandibular joint.

Extensive evidence of multisensory integration of auditory and somatosensory input in the brainstem comes from labeling and electrophysiological measurements in animals. Nuclei of the dorsal column project to the ventral cochlear nucleus (VCN) and dorsal cochlear nucleus (DCN) (Shore *et al.*, 2000; Shore and Zhou, 2006). In addition, the dorsal column nuclei and the spinal trigeminal nucleus (Sp5) also project to the ventrolateral border region of the IC (Shore and Zhou, 2006).

The somatosensory integration extends up to the cortex. Smiley and Hackett (Smiley *et al.*, 2007; Hackett *et al.*, 2007a) showed that in macaque monkeys there are somatosensory connections to the caudal medial auditory area (CM) (presumably secondary auditory cortex or association cortex in humans) and indicate this area as a site of multisensory integration. They also found input from the ventral, anterodorsal and magnocellular divisions of the medial geniculate complex. This indicates that auditory input may reach CM through the anterodorsal division of the medial geniculate complex while somatosensory inputs may reach the CM area through the magnocellular division of the medial geniculate complex (Hackett *et al.*, 2007a). So, in addition to the CN and the IC, which receive (and

integrate) both somatosensory and auditory signals, there are cortical areas that exhibit multisensory processing.

Multisensory integration: fMRI findings

Not only is there evidence from animal studies that use electrophysiological measures of multisensory integration, but there are also studies that use functional imaging methods. Calvert (2001) reviewed the use of PET and fMRI to study cross-modal matching, integration and learning in 18 studies. Also, the translation of criteria describing multisensory integration from the electrophysiological cellular level to functional imaging methods are described and discussed. In essence, since in fMRI each voxel reflects the response of a large population of neurons, an additive response (i.e., when the bimodal response equals the sum of the unimodal responses) could simply reflect linear summation of the responses of two sets of sensory-specific neurons that happen to fall within the same voxel.

Several articles have described a form of multisensory integration and overlap of activation patterns to different unisensory stimuli. Foxe *et al.* (2002) and Schurmann *et al.* (2006) both show overlap of responses to somatosensory (touch) and auditory stimuli in the auditory cortex. Foxe *et al.* (2002) argued that this coincides with the CM belt area that was found earlier to show bimodal responses in cellular recordings (Foxe *et al.*, 2000). In addition to these studies, Beauchamp *et al.* (2008) located an area on the human superior temporal sulcus that showed overlap to auditory input, somatosensory input and visual input.

A study that used the superadditivity as marker for bimodal integration was performed by Kayser *et al.* (2005) and shows integration of somatosensory and auditory input in the superior temporal gyrus, which coincides with the (CM) belt area. The same group recently combined electrophysiology and fMRI in primates to study multisensory integration and pointed out that although the measured field potentials as well as the fMRI activity in the primary areas are strongly influenced by somatosensory (and also visual) stimulation, at the level of a single unit, there is only a minority of neurons that actually integrate the bimodal stimuli (Kayser *et al.*, 2009). It is thus of importance to know the relation between the strength of neural signals and fMRI activity to infer multimodal integration.

Multisensory processing: overlap in activation and multisensory integration

This work considers several levels of evidence for multisensory processing (i.e., a response to both somatosensory stimulation and auditory signals). The first level is the spatial overlap in activity patterns between the unisensory modalities and can be visualized using probability maps (figure 5.6). We found overlap between the auditory modality and the somatosensory modality in the primary auditory cortex (BA 41) and the auditory association cortex (BA 22) (see e.g. figure 5.6). Note however, that the overlap only occurs in maximally 30 % of all subjects, when applying a threshold of p = 0.001 (uncorrected for multiple comparisons) at single subject level.

A second level of evidence for multisensory processing comes from the ROI analyses. All auditory ROIs showed responses to both sound and jaw movement (Fig. 7.) Interestingly, the only differences between subjects with tinnitus and controls were found in the

CN and IC. In both ROIs, jaw protrusion evoked a larger response in subjects with tinnitus compared to controls. This may indicate an abnormal somatic input to the auditory system in the tinnitus subjects.

Additionally, we determined for each region of interest, the difference between the sum of the jaw and sound responses and the combined response (figure 5.7), and specifically looked for superadditivity of the combined response. We found superadditivity in the MGB and the IC (at least, in the control group; this was not detected in the patient group). Also the somatosensory cortex and the ventrolateral nucleus of the thalamus showed integration, although the effects of this integration seem small; presumably since in all ROIs at least one of the unisensory modalities gives a large response (and can be detected without the use of another modality).

Finally, multisensory integration was tested for all individual voxels separately. Several areas besides regions that were selected for the ROI analyses were identified exhibiting multisensory integration. Figure 5.6 shows the middle temporal gyrus, the inferior temporal gyrus and the cingulate gyrus as areas that have a significant higher response to multisensory stimuli than the sum of the unisensory stimuli. These areas only showed significant responses to the multimodal stimulus compared to the sum of the unimodal responses, which is suggestive for multisensory integration.

Integrating evidence

Although we found voxels in the brain that show overlap between auditory and somatosensory input, it does not necessarily reflect multisensory integration. Stein and Meredith (1993) pointed out three principles of multisensory integration: temporal coincidence, spatial coincidence and inverse effectiveness. This last point indicates that if the unisensory responses are weak (and may not be detected individually) they may be detected if an area in the brain integrates neural signals from two modalities and enhances the output such, that the multimodal response is actually exceeding a perceptual threshold. Yet, given our findings that all auditory nuclei show a response to somatosensory stimulation, in addition to the multisensory integration in other areas (especially the middle and inferior temporal gyrus), it underlines the importance of somatosensory interaction in the (extralemniscal) auditory system (Møller *et al.*, 1992) in defining possible mechanisms underlying tinnitus.

One hypothesis, relating tinnitus to changes in normal somatosensory integration, is that a change in input from the auditory system (due to e.g. noise-induced hearing loss) might influence the somatosensory input to the brainstem (Shore *et al.*, 2008). This might thus form a neurophysiological basis for modulating perceptual characteristics of tinnitus. Our finding that jaw protrusion shows enhanced responses in the IC and CN of subjects with tinnitus compared to controls is consistent with this hypothesis.

5.5 Conclusion

In conclusion, we showed responses to jaw protrusion throughout the auditory pathway. These responses occurred in both tinnitus patients and control subjects. The somatosensory responses of the auditory brain areas to jaw protrusion presumably account for the modulation of tinnitus by jaw protrusion. The response to jaw protrusion of the CN and the IC was larger in subjects with tinnitus than in healthy controls, suggesting an abnormal auditory-somatic interaction in the patient group.

6

A diffusion tensor imaging study on the auditory system and tinnitus

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Abstract

Tinnitus is an auditory percept in the absence of an external sound source. Mechanisms in the central nervous system are believed to be key in the pathophysiology of tinnitus. Diffusion tensor imaging (DTI) is an MR imaging technique that allows in vivo exploration of white matter tissue in the human brain. Using a probabilistic DTI approach, we determined the characteristics of fiber tracts from the inferior colliculus to the medial geniculate body up to the primary auditory cortex. We also investigated the connections between the auditory system and the amygdala, which may be involved in some forms of tinnitus. White matter tracts were characterized by three quantities: the mean fractional anisotropy, the weighted mean fractional anisotropy and the path strength. All these quantities are measures of the patency of white matter tracts. The most important finding is an increased patency of the white matter tracts between the auditory cortex and the amygdala in tinnitus patients as compared to healthy controls.

6.1 Introduction

Auditory System and Tinnitus

The central auditory system starts at the auditory nerve (AN) which conveys action potentials in response to neurotransmitters released by the hair cells in the cochlea. The cochlear nucleus (CN) is the first nucleus of the auditory system receiving information from the ipsilateral cochlea. A next step in the pathway is the superior olivary complex (SOC), in which input from the two cochlear nuclei converge. Neurons from the CN and SOC project to the inferior colliculus (IC) through axons that form the lateral lemniscus (LL). The next step is formed by connections between the inferior colliculus and the medial geniculate body (MGB) of the thalamus, a relay station of several types of information of which the auditory pathway is only one. Fibers leaving the MGB project to the primary auditory cortex (AC). For more detailed information we refer to Ehret and Romand (1997). In addition to this classical auditory pathway there are connections between the auditory system and the limbic system (Møller *et al.*, 1992). The limbic system is involved in motivation, mood and emotion (Dalgleish, 2004) and consists of many subsystems, including the hippocampus, the amygloid complex, the cingulate gyrus and the prefrontal cortex (Morgane and Mokler, 2006). Typical complaints attached to tinnitus such as anxiety, depression, and emotions such as fear indicate the association of the limbic system with tinnitus (Jastreboff, 1990). Cognitive therapies focus on the reduction of alteration of the emotional content of the percept of tinnitus by habituation (Jastreboff and Jastreboff, 2003; Jastreboff, 2007). Changes in regional cerebral blood flow (rCBF) and blood oxygenation (BOLD) signal have been reported in the limbic system by several studies (Lanting *et al.*, 2009). The connection between the auditory system and the limbic system may thus be of importance in the pathology of tinnitus.

In this paper we study the characteristics of white matter fiber tracts defining the (classical) auditory pathway, especially the pathways from the IC to the MGB up to the primary auditory cortex. We also investigate the connections between the auditory system and the limbic system, especially the amygdala (AM). Both pathways were studied using diffusion tensor imaging (DTI) methods. Additionally, we investigated possible differ-

ences in structural brain connectivity between subjects affected by tinnitus and healthy subjects. Since hemispheric differences have been reported in patients (Smits *et al.*, 2007; Melcher *et al.*, 2000; Devlin *et al.*, 2003), also lateralization of DTI findings was investigated.

Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a recently developed MR acquisition modality that enables the measurement of structural organization of tissues (Basser *et al.*, 1994; Pierpaoli *et al.*, 1996). As a powerful and non-invasive technique for *in vivo* exploration of human tissues, DTI is widely used in various medical fields, especially in brain imaging (Werring *et al.*, 1999, 2000).

DTI is based on the diffusion properties of water molecules in white matter of the brain. The diffusion is limited by the fibrous nature of white matter: the well-organized axon structure, axon membranes, neurofilaments and overall the myelin coating surrounding the neurons induce the displacement of water molecules to occur preferentially along the axon fibers rather than perpendicularly to them (Beaulieu, 2002). This anisotropic diffusion of water molecules can be measured by an MR scanner, allowing us to infer information on white matter connectivity. Two interesting features of DTI techniques are the ability to derive local information, such as the amount of anisotropy and the principal water diffusion direction in a single brain voxel (Kindlmann, 2004), and the possibility to track fiber bundles from a selected brain area (Basser *et al.*, 2000).

Although several imaging studies have examined the auditory system and tinnitus (Lockwood *et al.*, 1998; Melcher *et al.*, 2000; Lanting *et al.*, 2008), only a few of them applied DTI techniques (Yoo *et al.*, 2006; Lutz *et al.*, 2007; Lee *et al.*, 2007). The main reason for the infrequent application of DTI in studies on the auditory system is the poor spatial resolution of DTI images. Partial volume effects lead to underestimation of diffusion anisotropy, and multiple nerve fiber tracts crossing a single brain voxel may disturb the fiber tracking algorithm. According to tracing studies on the macaque (Schmahmann and Pandya, 2006) using radioactive tracers, we know that the fibers of the auditory pathway intersect motor bundles (in the CN-SOC-IC path) and are close (within millimeter range) to the corticospinal tract (in the IC-MGB path) and do cross the internal capsule (Martin, 2003) when connecting the MGB with the AC.

Standard deterministic fiber tracking techniques only consider the main diffusion direction in each voxel, which is provided by the eigenvector corresponding to the largest eigenvalue of the diffusion tensor in the voxel. This technique is incapable of resolving voxels with multiple fiber directions that occur when two fibers cross each other within a voxel, but also has problems in the presence of gray matter voxels. For this reason, we used probabilistic tractography with multiple fiber orientations (Behrens *et al.*, 2007); this technique has been shown to provide significant advantages in sensitivity when tracking non-dominant fiber populations and allowed us to track the auditory paths, which are impossible to detect with deterministic tractography. Although probabilistic tractography does not allow the visualization of the actual fiber bundles, it outputs a whole-brain probabilistic connectivity map for localizing white matter tracts. Using this method, we investigated the connections of the auditory pathways and the connections between the auditory system and the limbic system in a focused per-subject analysis. We also studied

differences in tractography results of these paths in subjects with and without subjective tinnitus.

Related Work on the Auditory System

The auditory system and tinnitus have been studied using functional imaging techniques (Lockwood *et al.*, 1998; Melcher *et al.*, 2000; Lanting *et al.*, 2008). DTI methods have been used in a few studies on the auditory system, but in most cases only scalar values derived from DTI analysis, such as the fractional anisotropy (FA) index, describing the amount of anisotropy per voxel, were considered. For instance, Lee *et al.* (2007) performed a study where the FA index in several areas of the brain was determined. Reduced FA values in tinnitus patients were found in the left frontal Arcuate Fasciculus and the right parietal Arcuate Fasciculus. However, the classical auditory pathway was not studied. Lutz *et al.* (2007) were able to detect changes in FA maps in cortical and subcortical auditory regions, in relation with the age of the subjects (although the study did not concern tinnitus): elder subjects showed bigger FA values in IC and lower FA values in the auditory radiation and in the temporal gyri. Moreover, Lee *et al.* (2003) showed differences in FA values between subjects with conductive hearing loss and subjects with profound sensorineural hearing loss, reporting that DTI findings of subjects with profound sensorineural hearing loss revealed neural damages in the auditory pathway. De Groof *et al.* (2006) used DTI to study the auditory and vocal system in birds. They discerned a number of song control and auditory nuclei, and discriminated the tracts running from and to these nuclei. Using DTI fiber tracking techniques, Upadhyay *et al.* (2007) studied the connectivity patterns within the primary auditory cortex.

Different methods are available for exploring differences in brain structure between two groups. Using voxel-based morphometry (VBM), brain data from different subjects are aligned to a reference volume so that voxel-wise statistics can be computed (Ashburner and Friston, 2000). This method has been used for comparing diffusion tensor data (Jones *et al.*, 2005; Park *et al.*, 2003) and provides a whole-brain picture of structural differences; the major drawback of this technique is that the results could be strongly influenced by the quality of the alignment among the brain data and by the spatial smoothing usually applied to the data (Smith *et al.*, 2006). Tract-based spatial statistics (Smith *et al.*, 2006) is another tool for comparing DTI data among subjects. This technique seems to be very robust to misalignment of brain data; however, it only allows comparisons of high-FA white matter voxels and is not able to analyze a specific fiber bundle *per se*.

6.2 Materials

Subjects

DTI data were acquired from 25 subjects: 15 healthy subjects and 10 subjects with tinnitus. The group of healthy volunteers consisted of 12 men and 3 women; the age of these varied from 26 to 75, with a mean of 46 and a standard deviation of 16. The subjects affected by tinnitus (9 men and 1 woman) had ages varying from 30 to 70, with an average of 49 and a standard deviation of 12. All the subjects were right-handed. Six of the patients suffered from bilateral tinnitus. Four suffered from tinnitus in the left ear only. Figure 6.1A shows the hearing levels of the patients at different octave frequencies; Fig. 6.1B shows the severity of tinnitus as perceived by the patients during rest, according to a visual analogue scale (VAS) ranging from 1 to 10. The frequency and loudness level of tinnitus were determined by a matching procedure. Frequency matching was performed with an external tone presented at the non-tinnitus ear (unilateral tinnitus) or at both ears (bilateral tinnitus) at a comfortable level. The loudness level was then determined by adjusting the level of this tone to match the tinnitus loudness. All subjects gave written informed consent for their participation in the study.

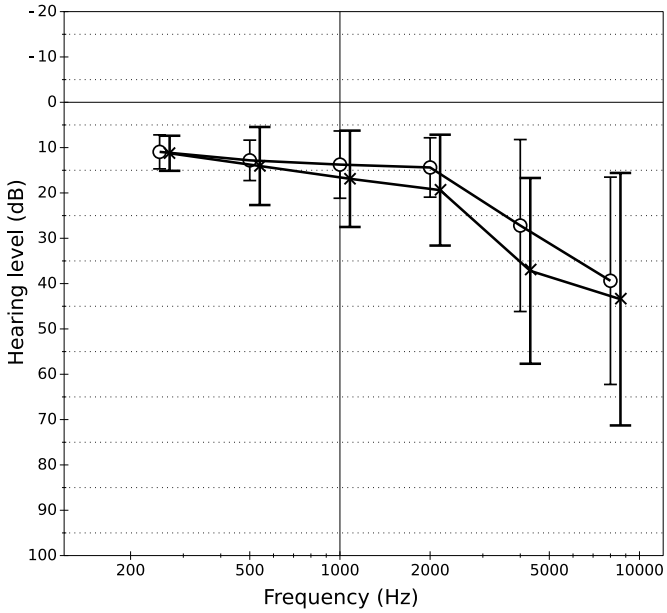
Magnetic Resonance Scanner

All the imaging experiments were performed on a 3T Philips Intera MRI scanner. DTI was performed using a diffusion weighted spin-echo, echo-planar imaging technique. The DTI parameters were as follows: field of view = 240 × 240 mm; matrix size = 128×128; no. of slices = 50; imaging resolution = 1.85×1.85×2 mm^3; TR = 5485 ms; TE = 74 ms. In total, 61 volumes were acquired per subject, one without diffusion weighting (b = 0 s/mm^2) and 60 volumes with diffusion weighting (b = 800 s/mm^2) along 60 non-collinear directions. To correct for susceptibility artifacts, two acquisitions were used: one with fat-shift direction in the posterior direction (APP) and one in the anterior direction (APA).

An anatomical scan was acquired to serve as reference (T1-weighed fast-field echo scan, TR= 25 ms, TE = 4.5 ms, flip angle = 30°, imaging resolution = 0.94×0.94×1 mm^3, matrix = 256×256×160 slices).

For the region of interest (ROI) selection, functional MRI data were acquired. These consisted of 2179 ms single shot $T2^*$-sensitive echo planar imaging (EPI) sequences, 41 slices, 2 mm thickness, TR = 10 s, TE = 22 ms, flip angle 30°, matrix 128×128, field of view 224 mm, SENSE reduction factor 2.7, 51 acquisition per subject. The influence of acoustic scanner noise on fMRI data was reduced by using a sparse sampling strategy (Hall *et al.*, 1999). A TE of 22 ms was chosen for optimal SNR at areas close to air-tissue boundaries, like the temporal lobes, at the cost of a small SNR decrease in the BOLD signal (see e.g., the introduction of Deichmann *et al.* (2002)).

Auditory stimuli were delivered by a MR-compatible electrodynamic system (MR Confon GmbH (Baumgart *et al.*, 1998)). This system was driven by a PC equipped with a digital-analogue card (National Instruments 6052E) controlled by Labview 6.1 (National Instruments Corporation, Austin, TX). The auditory stimuli were generated off-line using Matlab© and consisted of temporally and spectrally modulated broadband "rippled" noise (Langers *et al.*, 2003). The stimuli had a frequency-range of 125 − −8000 Hz with



patient	1	2	3	4	5	6	7	8	9	10
VAS	X	X	1	5	4	4	7	10	3	7.5
freq (kHz)	0.75	8	9	4	4	9	6	0.75	9	11
loudness (dB)	12	13	10	10	20	15	15	15	15	X

Figure 6.1 *A*: Hearing levels (in dB HL) of the tinnitus patients at several frequencies. Circles represent the average levels for the right ear, crosses represent average levels for the left ear. The whiskers show standard deviations. *B*: Severity (VAS), frequency and loudness level (in dB SL) of tinnitus perceived by the patients. An "X" is used when data were not available.

a spectral modulation density of 1 cycle per octave, a temporal modulation frequency of 2 cycles per second and a modulation-amplitude of 80%.

Each of the three functional runs consisted of the acquisition of 61 volumes under 3 experimental conditions: (i) a condition in which bilateral rippled noise noise was presented at a level of 70 dB (SPL), (ii) a condition in which subjects were visually instructed to protrude their jaw, and (iii) a combination of these two conditions (jaw protrusion + sound stimuli). Each condition was presented 15 times per functional run.

6.3 Methods

First we investigated the auditory connections and the connections between the auditory and the limbic system using standard deterministic DTI tracking provided by Track-

Vis (http://www.trackvis.org). This method was inadequate and failed to reveal any connections. Therefore we reverted to probabilistic tracking (Smith *et al.*, 2004). While standard DTI tracking computes a single main diffusion direction per voxel, probabilistic DTI tries to determine the most probable diffusion direction in each voxel by computing voxel-wise probability distributions of the main diffusion direction. The technique consists of sampling these distributions in each voxel to find the probabilities to reach the neighboring voxels, and repeating the process in the voxels reached. In this way it is possible to compute the probability to reach any voxel, starting from a certain voxel (or group of voxels).

Preprocessing

Before DTI analysis, preprocessing of raw data is necessary. This preprocessing step includes susceptibility correction, as well as eddy current and gradient table correction, depending on the scanner used for the acquisition. Susceptibility correction, which requires two DTI acquisitions with different fat-shift directions, was performed using MatLab, combined with the Statistical Parametric Mapping toolbox (http://www.fil.ion.ucl.ac.uk/spm/), the FieldMap toolbox (Jezzard and Balaban, 2005), and the MatLab routines created by Andersson et al. (Andersson *et al.*, 2003). Correction for eddy currents was performed with the software package FSL (Smith *et al.*, 2004), a library of analysis tools for fMRI, MRI and DTI data. The gradient table modification was achieved using a MatLab toolbox created by Farrell et al. (http://godzilla.kennedykrieger.org/~jfarrell/software_web.htm). The last step before tracking is skull stripping (i.e., disregarding all non-brain data) and it was performed using BET (Smith, 2002).

Region of Interest selection

We studied a number of brain areas involved in auditory processing and investigated how they are connected to each other. In particular, we determined the connectivity profiles between auditory cortex and inferior colliculus (AC-IC), auditory cortex and amygdala (AC-AM), inferior colliculus and amygdala (IC-AM) and all reverse connections. We disregarded the connections between inferior colliculus and cochlear nucleus (IC-CN), because a preliminary analysis showed that the motor fibers in the brain stem, running next to the acoustic fibers, have a strong influence on the results due to the poor spatial resolution of DTI. The selection of these ROIs (AC, IC, and AM) was done manually and for each individual patient, using the anatomical T1 scan as a primary reference. When possible (in 6 datasets), the location of the AC was checked by a comparison with BOLD activation maps (contrast: sound - baseline) provided by fMRI analysis. Location and size of the ROIs were checked by overlapping the drawn ROIs, normalized to the Montreal Neurological Institute (MNI-152) template brain (Brett *et al.*, 2002), to the correspondent areas defined by the Juelich Histological Atlas, thresholded at 5% (Morosan *et al.*, 2001; Amunts *et al.*, 2005); ACs showed an average overlap of 70%, AMs showed an average overlap of 77%.

Following Anwander *et al.* (2007), each ROI selection included both the gray matter and a part of the white matter directly beneath it. This was done to avoid dispersion of samples already at the early stages of the tracking procedure, due to the low anisotropy of

gray matter areas. In this way the first part of the axon bundles leaving from the concerned gray matter area was included in the ROI.

To prevent the tractography from finding connections passing through cortical regions or the cerebrospinal fluid (CSF), masks were defined where tracts could not enter. These were positioned in the CSF above the AC and along Reil's Insula, and served as barriers to avoid tracts passing through these regions. Nevertheless, the usage of these masks was not sufficient and after the probabilistic tracking an additional filtering was necessary, i.e., fiber tracts leading to the cerebellum or to the motor cortex were manually removed.

Probabilistic Tractography

The BEDPOST tool (Behrens *et al.*, 2003), which runs a Markov Chain Monte Carlo sampler, was used for building distributions of parameters describing the diffusion direction in each voxel. Probabilistic tractography was accomplished using the FMRIB Diffusion Toolkit (Behrens *et al.*, 2003). For each voxel in a ROI, 5000 samples were taken from the distribution.

The probabilistic tractography outputs a connectivity map depicting the voxel-wise probability to reach any given voxel starting from a user-defined ROI, and it is defined as the percentage of samples leaving from the starting ROI that pass through that voxel. The connectivity map was then filtered so that only those paths connecting two different ROIs, a seed and a target ROI, were considered. An intrinsic problem of the sampling is that regions nearby the starting ROI are reached by a large number of samples, whereas the further a region is from the starting ROI, the fewer samples are able to reach it. This is a well-known and still unsolved problem. We addressed it by computing the connections between two ROIs in both directions: first starting from a region and reaching the other one, and then the other way around. Statistics on the paths, as described in the next section, were computed independently on each single path.

Statistical Analysis

Although the voxel-wise FA value is the conventional statistical measure used in DTI analysis, we considered various novel statistics to compare the two groups. For each path between a seed ROI and a target ROI (and vice versa) the following three statistical values were computed. First, the *mean FA value* of a path was determined as the mean of the FA values of all voxels in the path. Second, the *weighted mean FA value* (wFA) of a path was determined, where the weighting was provided by the probability value of each voxel (i.e., the chance that a voxel is reached as determined by the probabilistic tracking). Voxels with a larger weight are more likely to represent the actual anatomical pathway. The third statistical value was the *strength* (S) of a path, defined as the percentage of the samples leaving from the starting ROI that were able to reach the target ROI. We consider the strength of a path as a key feature that describes the relevance of that path with respect to any other path connecting the starting ROI to other brain areas. Additionally, hemispheric differences were determined for all three statistical values (features) for each subject and path, using a lateralization index L defined as:

$$L_{feature} = 2 \cdot \frac{feature_{right} - feature_{left}}{feature_{right} + feature_{left}}. \tag{6.1}$$

These statistical values were used to compare the two groups, resulting in a distribution of statistical values for each group and path. Since a chi-square test revealed that the distributions were not normally distributed, we used the non-parametric Kruskal-Wallis test in which the equality of the medians of the distributions is assessed to test for differences between control subjects and tinnitus patients.

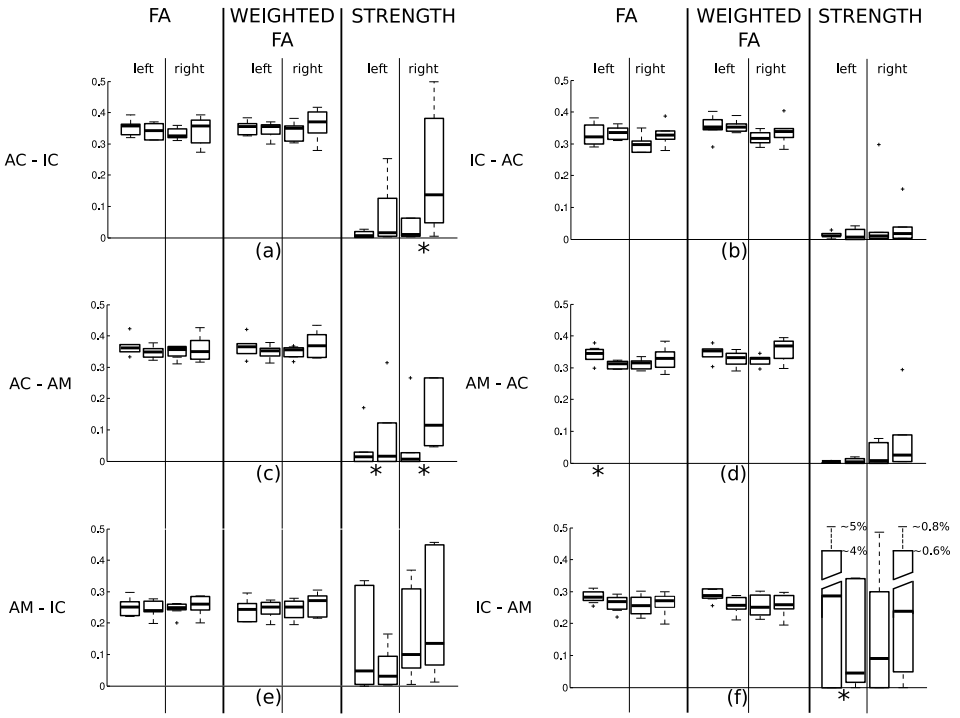


Figure 6.2 Box & whisker plots of the distributions of FA, weighted FA and strength in the paths of tinnitus patients and controls. For each pair of box and whiskers, the left data set corresponds to control subjects and the right one to tinnitus patients. Panel (a) shows the statistical results computed on the tract AC-IC; panel (b) of the tract IC-AC, panel (c) of the tract AC-AM, panel (d) of the track AM-AC, panel (e) of the tract AM-IC, panel (f) of the tract IC-AM. Paths where a statistically significant differences between tinnitus patients and controls were found (see Table 6.1a)) are marked with an asterisk below the plot.

6.4 Results

The fiber tracts of the classical auditory system and of the connections between the auditory nuclei and the limbic system could be identified by the probabilistic tracking technique. These connections were consistent between the two directions of tracking. We also ascertained that standard deterministic DTI tracking is not able to reveal these connections. However, probabilistic tractography was not able to detect every path in a statistically significant way for every subject. The easiest path to track was the auditory path, which was detected in 50% (from AC to IC) and 35% (from IC to AC) of the subjects, respectively. The connection between IC and AM was found in 40% of the cases (50% from AM to IC), and the path AC-AM (and vice versa) was found in 40% of the subjects. We did not find any relation between the cases where it was impossible to find a path and the type of subject (control or tinnitus patient). It appears that the difficulties in tracking the paths are due only to the low imaging resolution or to the low signal-to-noise ratio. No significant differences were found between the positions of the paths in the control group and the tinnitus group: all subjects showed the same connectivity pattern. This was true both for the connections between the auditory nuclei (AC and IC) and for the connections between the auditory nuclei and the amygdala.

Table 6.1 Significance values (p-values) determined by the Kruskal-Wallis test of differences between tinnitus group and control group. *(a)*: Differences in path indices between the groups for each hemisphere. *(b)*: Differences in path lateralization between the groups. Significant p-values (0.05 threshold) are indicated in bold.

left hem.	AC-IC	AC-AM	IC-AC	IC-AM	AM-AC	AM-IC
FA	0.943	0.148	0.630	0.108	**0.033**	0.512
wFA	0.936	0.624	0.261	0.935	0.732	0.262
S	0.127	**0.020**	0.377	**0.016**	0.739	0.261

right hem.	AC-IC	AC-AM	IC-AC	IC-AM	AM-AC	AM-IC
FA	0.423	0.333	0.149	0.192	0.422	0.631
wFA	0.521	0.106	0.423	0.744	0.077	0.149
S	**0.047**	**0.006**	0.199	0.109	0.135	0.631

(a)

	FA	wFA	S
AC-IC	0.377	0.262	0.631
AC-AM	**0.010**	**0.037**	0.261
IC-AC	0.053	**0.024**	0.108
IC-AM	0.628	0.628	0.333
AM-AC	0.739	0.618	0.998
AM-IC	0.423	0.994	0.631

(b)

The results of the statistical analysis of differences between tinnitus patients and control subjects are summarized in Table 6.1 and in Figure 6.2. Statistics were computed on each single path connecting two different ROIs. Note that in this phase of the analysis, we consider the path from a ROI "A" to a ROI "B" different from the path from "B" to "A".

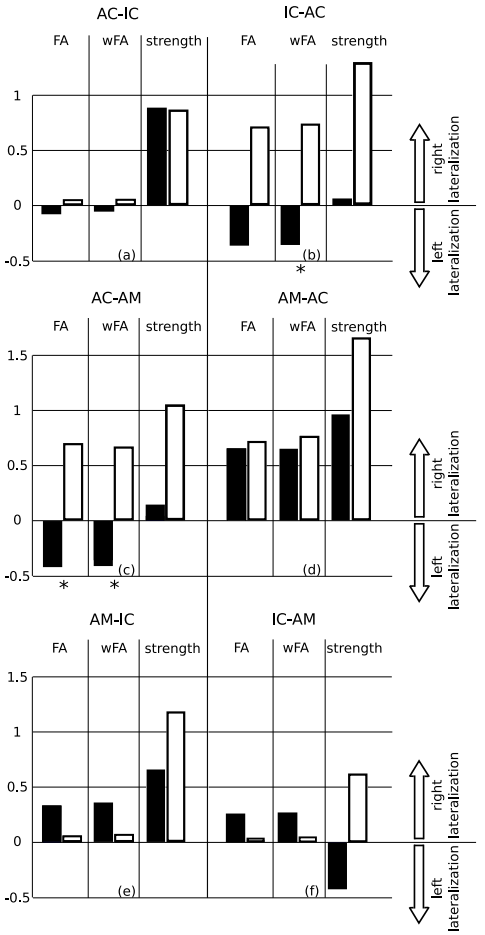


Figure 6.3 Lateralization of the paths in the two groups. The black bars represent lateralization in the control group, the white bars represent lateralization in the tinnitus group. Positive values mean right lateralization, and negative values mean left lateralization (the range of the lateralization is [-2,2]). The Kruskal-Wallis test (see Table 6.1b) found significant differences (marked with an asterisk in the picture) in lateralization of fractional anisotropy (FA) and weighted fractional anisotropy (wFA) of the path leaving from the auditory cortex (AC) to the amygdala (AM), and in lateralization of wFA in the path leaving from the inferior colliculus (IC) to the auditory cortex.

Table 6.1a) shows the significance values (p-values) determined by the Kruskal-Wallis test of differences between tinnitus patients and control subjects in fractional anisotropy (of the detected paths), weighted fractional anisotropy (of the detected paths) and strength of all the paths. The test detects a number of significant differences between the paths of the two groups. Statistically significant differences (threshold 0.05) can be noted in the strength of the AC-AM connection: it is higher in the tinnitus group, as seen from Fig. 6.2, both

for the right and the left hemisphere. Other significant differences are present in the FA values of the path AM-AC in the left hemisphere and in the strengths of the path AC-IC in the right hemisphere (stronger in the tinnitus group) and the path IC-AM in the left hemisphere (stronger in the control group).

Lateralization of FA, weighted FA and strength of the paths in the two groups is shown in Fig. 6.3. Table 6.1b) shows the significance values (p-values determined using the Kruskal-Wallis test) of the differences in these indices between the two groups. Statistically relevant differences (threshold 0.05) can be noted in the lateralization of FA and of weighted FA in the path AC-AM, and in the lateralization of weighted FA in the path IC-AC. In both cases the tinnitus group shows a right lateralization whereas the control group shows a left lateralization, as can be seen from Fig. 6.3.

So far, statistics on the paths were computed *independently* on each single path between ROIs. For the *sole* purpose of visualizing the auditory paths as found by the probabilistic tractography, we used the intersection of the two paths (A→B, B→A) between any pair (A,B) of ROIs to define the location of a connection. For this purpose we normalized all the paths to the MNI template brain (Brett *et al.*, 2002) using FLIRT (Jenkinson and Smith, 2001), and averaged the per-subject results to obtain a group-wise connectivity map. The result is shown in Fig. 6.4. The paths in Fig. 6.4(A) show connections between AC and IC. These paths also involve the MGB through which they pass. Note that the MGB was not used as a ROI. In Fig. 6.4(B) we show the connections between the amygdala and the inferior colliculus, overlapped with the connections between the amygdala and the auditory cortex. The two paths follow the same route from the AM to the MGB, where they split into two separate paths, one to the IC and one to the AC.

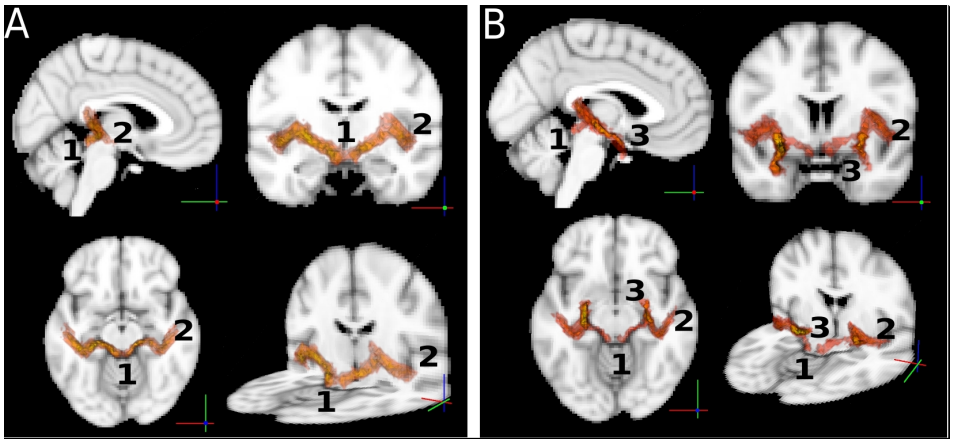


Figure 6.4 *A*: Connections of the auditory pathways. Each path shown is the group average of the normalized (to the MNI standard template) paths of all the subjects. The intersection of two paths (A→B, B→A) between any pair (A,B) of ROIs was used to define the location of a connection. The image shows iso-probability surfaces, coded with color (the color scale ranges from yellow to red, where yellow indicates the highest probability to find the path in that brain region, and red indicates lower probabilities). Track endpoints are identified by numbers. 1: inferior colliculus, 2: auditory cortex, 3: amygdala. The iso-probability surfaces are semitransparent so that an exploration of all voxels is possible. *B*: the connections between the amygdala and the inferior colliculus, overlapped with the connections between the amygdala and the auditory cortex.

6.5 Discussion

In this study we extended the application of DTI of the brain to the study of auditory pathways in tinnitus patients and controls. We considered DTI tracks that connect the inferior colliculus, the auditory cortex and the amygdala and vice versa. In other words, we only considered tracks that connected these pre-selected seed and target ROIs. Obviously, such an approach will not identify new connections. Rather, it allows for the quantification of known connections in the brain.

The first interesting result is the ability to track the classical auditory pathway. The tracks that connect the AC and the IC all pass through the MGB. Thus, although the MGB was not preselected as a region of interest, these tracks follow the expected pathway of the classical auditory system. Hence, as in a recent validation study performed in the macaque (Dauguet *et al.*, 2007), DTI identifies known neuronal tracks in the brain.

Based on earlier hypotheses (Møller *et al.*, 1992; Jastreboff, 1990) we expected that an anatomical connection between the auditory system and the limbic system would exist and indeed, we found such a connection between the auditory cortex and the amygdala which also connected to the MGB. This suggests that DTI is able to detect an anatomical pathway which is part of the non-classical auditory pathway, e.g., the connection from the dorsal MGB to the limbic system (amygdala).

In order to summarize the track properties, we computed three quantities for each connection in each subject: the fractional anisotropy (FA), the weighted fractional anisotropy (wFA), and the connection strength (S). The anisotropy is a property of each voxel in the brain. It is a measure of the directionality of water diffusion in the voxel. If the water diffusion is primarily in a particular direction, the voxel is assumed to contain neural fibers that are oriented in that direction. A fiber track consists of a large number of neighboring voxels. The average FA of a track is thus assumed to be a measure of the patency of the track. We assumed that the wFA is an improved measure of this patency, as it takes the probability that a voxel is actually part of the track into account. Obviously, the wFA can only be computed when using probabilistic fiber tracking. Finally, the connection strength S is the fraction of samples in a seed region that actually reaches the target region. A high strength S is again a measure of the patency of the track. Conversely, a low strength may indicate that the seed region is connected primarily to other end points. Although these three measures (FA, wFA and S) are the result of considerable data reduction, they provide measures that allow for straightforward comparisons between subjects and subject groups.

By quantifying the tracks that pairwise connect the IC, AC and AM, we were able to make comparisons between control subjects and tinnitus patients. These three ROIs were selected because they may play an important role in the mechanisms that lead to tinnitus. Tinnitus is an auditory percept that occurs in the absence of a known acoustical source outside the body. In many cases, tinnitus is presumably related to abnormal spontaneous neural activity in the brain. Such patterns may occur in cases of peripheral hearing loss, as reviewed in Eggermont and Roberts (2004), apparently as a consequence of altered (often reduced) peripheral input to the central auditory system.

Despite the fact that a relation between tinnitus and peripheral hearing loss is present, it is not straightforward. For example, tinnitus may be present in the absence of any substantial hearing loss. Also, the presence of hearing loss is associated with tinnitus in only about 20% of the cases (Lockwood *et al.*, 2002). The mechanisms underlying the diffuse relation between hearing loss and tinnitus are unclear. It is possible that subtle characteristics of the functional or anatomical (structural) connectivity of the central auditory systems determine whether a subject develops tinnitus. In addition, non-auditory brain areas are believed to be involved in tinnitus. Specifically, the interaction between the limbic and the auditory system has been proposed in models that explain tinnitus (Lockwood *et al.*, 1998; Jastreboff, 1990; Møller, 2006a).

Abnormal spontaneous brain rhythms in tinnitus patients are indicative of abnormal functional connectivity in such patients. These brain rhythms reflect the activity of forward and backward loops connecting brain areas, specifically of the cortical-thalamical connections (Llinas *et al.*, 2005). In tinnitus patients, the alpha brain rhythm is reduced, while the delta rhythm is substantially enhanced (Weisz *et al.*, 2005a). These abnormal brain rhythms, which differentiate tinnitus patients from control subjects, could in part be due to differences in the anatomical connections.

Our study is an attempt to show possible anatomical differences between subject groups using DTI. The computation of FA, wFA and S allowed us to compute such differences. We found differences and similarities between tinnitus patients and healthy controls. For example, the variability across subjects for FA and wFA of the paths was remarkably small within each group, and was also very similar between both groups.

Significant differences in path strength between tinnitus patients and healthy controls were found for the left IC-AM connection, the right AC-IC connection, and the AC-AM connection for both hemispheres (see Table 6.1a) and Fig. 6.2c), which also resulted in a significant difference for the lateralization (see Table 6.1b) and Fig. 6.3c). Tinnitus patients also showed a higher FA in the AM-AC connection.

Regarding lateralization, differences between tinnitus patients and controls were found for the FA of the AC-AM connection and the weighted FA of the AC-AM and IC-AC connections, cf. Fig. 6.3b). This result may correspond to the abnormal lateralization in brain function observed in tinnitus patients in a PET study (Langguth *et al.*, 2006).

The difference in strength of the connection between auditory cortex and amygdala in subjects with tinnitus compared to controls indicates that the limbic system may indeed play a major role in tinnitus, especially concerning the emotional content of the percept of tinnitus. Although cognitive therapies, focused on treating tinnitus by habitation, have been used for many years (Jastreboff and Jastreboff, 2003; Jastreboff, 2007), no imaging study prior to the present one has shown a potential anatomical pathway that might function differently between tinnitus patients and normal hearing controls.

6.6 Acknowledgements

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7

General conclusions

7.1 Introduction

In this thesis, the phenomenon of subjective tinnitus was studied with functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI). First, experimental paradigms suitable for imaging methods were discussed as well as the results that were generated using these paradigms. Second, two distinct forms of tinnitus were studied. The first was lateralized tinnitus where subjects perceive tinnitus at one side of the head, predominantly at one side of the head or centrally located 'in the head'. The second 'type' of tinnitus was somatic tinnitus, a phenomenon that refers to somatosensory maneuvers that elicit or modulate the psycho-acoustical attributes of tinnitus (e.g., the loudness or pitch of the tinnitus). In the following paragraphs the main findings will be discussed and further speculated on in relation to hypotheses of tinnitus generation.

7.2 Experimental paradigms on functional imaging methods of subjective tinnitus

Neuroimaging methods like functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) measure signals that presumably reflect the firing rates of multiple neurons and are assumed to be sensitive to changes in the level of neural activity. Both imaging modalities depend on the hemodynamic or vascular response to neural activity. They may identify changes in local neural activity that result from induced modulation of tinnitus and, in some cases, may identify abnormal steady-state activity associated with tinnitus.

The spatial en temporal resolution limit the use of these methods to the investigation of the rather slow hemodynamic responses. These can be identified in brain areas, summarizing responses of a large number of neurons. In addition, these methods only measure the strength of activity. Changes in, for example, neural synchrony that have also been suggested to relate to tinnitus (see e.g. Eggermont (2007a)) presumably remain unnoticed when the brain is studied with PET or fMRI.

In addition, changes in spontaneous activity–another marker of tinnitus, is not measured with blood oxygen level dependent (BOLD) fMRI since it only measures differences between conditions and cannot be used to assess baseline levels of activity. Some PET methods however have been used to study steady state neural activity in subjects with tinnitus and in subjects without tinnitus.

Nevertheless, there are two basic paradigms that have been applied in functional neuroimaging of tinnitus. Firstly, sound-evoked responses as well as steady state neural activity have been measured to compare patients with tinnitus to healthy controls. Secondly, paradigms that involve modulation of tinnitus by a controlled stimulus allow for a within-subject comparison that identifies neural activity that may be correlated to the tinnitus percept.

Even though there are many differences across studies, the general trend emerging from the neuroimaging studies, is that tinnitus in humans may correspond to enhanced neural activity across several centers of the central auditory system. Also, neural activity in non-auditory areas including frontal areas, the limbic system and the cerebellum seems to be associated with the perception of tinnitus. These results indicate that in addition

to the auditory system, non-auditory systems may represent a neural correlate of tinnitus. The studies reviewed in chapter 2 suggest abnormal neural activity in tinnitus patients at several levels in the brain. Specifically, cortical and sub-cortical auditory brain areas show a correlation between blood flow and tinnitus loudness. However, in many cases, it is unclear to what extent the abnormalities truly relate to tinnitus. Some aspects may also be related to hearing loss or hyperacusis, rather than tinnitus. Also, the presented differences between subject groups may have been confounded to differences in matching criteria between groups (e.g., hearing levels and age).

Although the currently published neuroimaging studies typically show a correspondence between tinnitus and enhanced neural activity, it will be important to perform future studies on subject groups that are closely matched for characteristics such as age, gender and especially hearing loss in order to rule out the contribution of these factors to the abnormalities specifically ascribed to tinnitus.

The observation that tinnitus corresponds to abnormal neural activity in auditory brain areas is not very surprising. After all, tinnitus is the abnormal percept of sound. The question remains as to how the abnormalities emerge. To what extent does the abnormal activity in the auditory cortex, which presumably has a close correspondence to the tinnitus percept, reflect an inherent abnormality of the cortex? In other words, does it reflect pathology of the cortex or is it a consequence of an abnormal interaction with subcortical brain areas and possibly limbic or frontal regions? And how does the abnormality simply reflect the consequence of peripheral hearing loss? These questions remain to be answered and the answers are key in understanding the pathology of tinnitus.

7.3 Increased sound evoked responses in subjects with unilateral tinnitus

Chapter 3 shows the results of a study on sound-evoked responses as a marker of tinnitus. Based on the lateralization of the tinnitus in a subgroup of patients we expected to measure a lateralization in the responses in the central auditory system that would reflect the lateralization of the tinnitus.

The results show an increased sound-evoked response of the inferior colliculi (IC) of subjects with lateralized tinnitus when compared to those in subjects without tinnitus (see figure 3.4), which is in close agreement with Salvi *et al.* (1990) who measured enhanced evoked response amplitudes in the inferior colliculus of the chinchilla following acoustic trauma. The responses in the auditory cortex (combining primary and secondary auditory cortices) however where not different between the subject groups.

As can be observed in the auditory cortex (see figure 3.3), contralateral stimuli gave a larger response than ipsilateral stimuli. We also found an intensity dependency, i.e., stimuli of 70 dB (SPL) gave a larger response than stimuli of 40 dB (SPL). In the control group we found a functional asymmetry as described earlier (Devlin *et al.*, 2003; Krumbholz *et al.*, 2005). In the inferior colliculi of subjects with tinnitus we observed a change in this asymmetry; subjects with tinnitus showed no clear contralateral dominance in the strength of the responses. So, in addition to the change in the level of the sound-evoked responses

we also observed a difference in response lateralization.

7.4 Changes in lateralization and connectivity patterns in subjects with unilateral tinnitus

Based on the main results described in chapter 3 (increased sound-evoked responses and changes in the response lateralization in the inferior colliculi of subjects with tinnitus), more subjects were included in the study. The strength and lateralization of sound-evoked responses and the connectivity patterns between nuclei in the auditory pathway were assessed and the results were described in chapter 4. However, there was no dependency of the strength of the sound-evoked response on the side of the tinnitus. This is in line with previous work (Lanting *et al.*, 2008) and was also confirmed by a recent paper of Melcher *et al.* (2009) showing that the lateralization of the tinnitus is not reflected in the strength of the evoked responses in the IC. The other studies that did show a relation between tinnitus lateralization and brain activity, either did not match their subject groups based on e.g. hearing loss (Kovacs *et al.*, 2006; Smits *et al.*, 2007), or had ongoing background noise that might have saturated neural responses (Melcher *et al.*, 2000). This presumably caused changes in the lateralization of the brain responses. In summary, the laterality of the tinnitus did not correspond to a lateralized change in the neural response to sound.

The vermis of the cerebellum responded significantly stronger in the patient group compared to the controls. The role of the vermis of the cerebellum is not known, but several authors discussed its role. Lesions in the vermis of the cerebellum in rats have been reported to block the long-term habituation of the acoustic startle response (Leaton and Supple, 1991). Also, in humans, the medial part of the cerebellum is important in the long-term habituation of the acoustic startle response (Timmann *et al.*, 1998; Maschke *et al.*, 2000). A meta analysis, summarizing the findings of fifteen studies on the neural correlates of active and passive listening, reported a general role of the cerebellum in auditory processing (Petacchi *et al.*, 2005).

The vermis of the cerebellum was suggested to play a role in lateral gaze, which in particular subjects with tinnitus changed the perceived loudness of the tinnitus (Lockwood *et al.*, 2001). We can speculate about the possible relation of these results to ours: one could suggest that the habituation of the continuous percept of tinnitus might be impaired in these patients, leading to the prolonged complaints of tinnitus. The vermis of the cerebellum might thus not directly relate to the percept but might influence the habituation to perceived sounds–in this case tinnitus. Nevertheless, given our data, we cannot draw any firm conclusion about the cerebellum, except pointing out that it shows a larger response to sound in patients with tinnitus as compared to controls.

Further region of interest (ROI) analysis showed that, at many levels in the auditory pathway, there were no differences in the strength of the response between subject groups. In general, nuclei of the auditory pathway showed a stronger response to 70 dB (SPL) stimuli than to 40 dB stimuli. In addition, the auditory pathway showed stronger responses to contralateral stimuli—with the exception of the cochlear nucleus (CN), which responded most strongly to ipsilateral stimuli. The pattern of responses to the sound stim-

uli was deviating between the subject groups in only two cases: the right primary auditory cortex (PAC) and the right IC. Here, in the patient group, there was a reduced difference between ipsilateral and contralateral stimuli. This could also be observed by looking solely to the lateralization index, which was significantly lower in these same nuclei (right IC and PAC). Interestingly, the patients lateralization was lower in almost all nuclei and was significant lower when performing a repeated measures ANOVA on all right-hemisphere nuclei, except the CN. Unilateral tinnitus thus relates to a decreased lateralization of the auditory pathway. This decreased lateralization might relate to a diminished efficiency in the inhibitory ipsilateral input to the IC (see figure 4.10). Disinhibition could effectively lead to a more equal input from both ears (via contralateral excitatory input and a dysfunctional inhibition from the ipsilateral ear, see Ehret and Romand (1997)) and therefore decrease the lateralization index.

In contrast to our earlier work (chapter 3) and a recent article by Melcher *et al.* (2009), the results in chapter 3 indicate that the IC of the patients does not show increased sound-evoked responses. It did in the subjects that we studied earlier but we were not able to replicate this finding here, with a larger group of subjects. The fact that the tinnitus subjects were, on average, 10 years older than the controls and the difference in auditory stimuli (binaural stimuli vs. monaural stimuli) might influence our findings. Also the methodological difference in the selection of voxels might have an influence, although there is little evidence for that.

The last part of chapter 4 described connectivity patterns between nuclei of the auditory pathway, with in addition the vermis of the cerebellum. We adopted two distinctive forms of connectivity analysis in this work (Horwitz, 2003). In addition to the simple (Pearson) cross-correlation as a measure for functional connectivity (Friston, 1994) we studied partial cross-correlation as measure for effective connectivity (Marrelec *et al.*, 2006, 2007, 2009). By using partial correlation, mutual characteristics like sound-evoked responses or other task-related features are taken out leaving an inherent measure of effective connectivity.

We observed that for all connections between elements in the model, the Pearson correlation was higher than the partial correlation, indicating that much of the correlation could be driven by the experimental paradigm. We assessed the normal connectivity patterns and observed high partial correlation coefficients between the ipsilateral PAC and auditory association cortex (AAC). Also, in subjects with tinnitus, the partial correlation coefficient between the left AAC and the vermis of the cerebellum was increased; indicating that the cerebellum appears to show effective connectivity with the auditory association cortex. We also found differences in connectivity in patients with tinnitus based on permutation testing procedures. Specifically, the effective connectivity was disturbed between the IC and the contralateral medial geniculate body (MGB), as well as between the left CN and the left PAC.

In conclusion, we did not find tinnitus related differences in the strength of response to sound in the auditory pathway. Yet, we did find changes in lateralization and connectivity, especially from the IC to the contralateral MGB. Apparently, tinnitus is somehow related to changes in connectivity patterns, which may lead to a change in lateralization. The role of the cerebellum in tinnitus remains unknown, although it shows a stronger response to

sound in patients with unilateral tinnitus, compared to subjects without tinnitus.

7.5 Neural correlates of somatosensory modulation of tinnitus

Somatic tinnitus is a phenomenon which refers to tinnitus that is elicited or modulated by somatosensory input. This may be a specific form of multimodal integration. The study in chapter 5 demonstrates that overlap of somatosensory and auditory input can be measured in the auditory pathway; jaw protrusion caused a response in the auditory system and may explain the influence of jaw protrusion on the perceived loudness of tinnitus. Probability maps can be used to indicate voxels that show functional overlap between unimodal conditions. We found overlap between the auditory modality and the somatosensory modality in the primary auditory cortex (BA 41) and the auditory association cortex (BA 22) (see figure 5.6). Interestingly, the only differences between subject with tinnitus and controls were found in the cochlear nuclei and IC. Jaw protrusion evoked in both ROIs a larger response in subjects with tinnitus compared to those in controls.

At the level of the brainstem there is already integration of somatosensory input and auditory input. The dorsal column-medial lemniscal system and the trigeminal sensory complex are key structures showing modulation of neural activity in the auditory system at the level of the cochlear nucleus and the inferior colliculus. Nuclei of the dorsal column are involved in relaying proprioceptive information from the trunk, shoulders, head (pinna) and posterior neck muscles to the ventral cochlear nucleus and dorsal cochlear nucleus (Shore *et al.*, 2000; Shore and Zhou, 2006). In addition to multimodal integration at the level of the cochlear nuclei and inferior colliculi, there is some evidence for cortical integration of somatosensory input and auditory input. The caudal medial belt area gets indirect and direct somatosensory input and receives auditory input through the anterodorsal division of the medial geniculate complex (Smiley *et al.*, 2007; Hackett *et al.*, 2007a,b).

Our findings, in combination with existing literature stress the importance of somatosensory interaction in the extralemniscal or non-classical auditory system (Møller *et al.*, 1992) in defining possible mechanisms underlying tinnitus. One hypothesis, relating tinnitus to changes in normal somatosensory integration, is that a change in input from the auditory system (due to e.g. noise-induced hearing loss) might influence the somatosensory input to the brainstem and might thus form a neurophysiological basis for modulating perceptual characteristics of tinnitus (Shore *et al.*, 2008). Especially our finding that jaw protrusion shows enhanced responses in the inferior colliculi and cochlear nuclei of subjects with tinnitus compared to controls underlines this hypothesis. It however remains a question what the exact neurophysiologic mechanisms are that may underlie tinnitus itself.

In conclusion, we showed responses to jaw protrusion throughout the auditory pathway. These responses occurred in both tinnitus and control subjects. The somatic responses of the auditory brain areas to jaw protrusion presumably account for the modulation of tinnitus by jaw protrusion. The response to jaw protrusion of the cochlear nuclei and the inferior colliculi was larger in subjects with tinnitus than in healthy controls, suggesting an unusually auditory-somatic interaction in the patient group.

7.6 The auditory pathway – is the limbic system involved?

Chapters 3–5 show the results of fMRI studies on subjective tinnitus. On the other hand, an imaging technique sensitive to diffusion of water in tissue was used in chapter 6: diffusion tensor imaging (DTI). This technique allows us to track anatomical pathways between predefined regions of interest and we specifically assessed part of the auditory pathway. The first interesting result is the ability to track the classical auditory pathway. The tracks that connect the auditory cortex (AC) and the IC all pass through the MGB. Thus, although the MGB was not preselected as a region of interest, these tracks follow the expected pathway of the classical auditory system. Hence, as in a recent validation study performed in the macaque (Dauguet *et al.*, 2007), DTI identifies known neuronal tracks in the brain.

Based on earlier hypotheses (Møller *et al.*, 1992; Jastreboff, 1990) we expected that an anatomical connection between the auditory system and the limbic system would exist and indeed, we found such a connection between the auditory cortex and the amygdala which also connected to the MGB. This suggests that DTI is able to detect an anatomical pathway which is part of the non-classical auditory pathway, e.g., the connection from the dorsal MGB to the limbic system (amygdala).

In order to summarize the track properties, we computed three quantities for each connection in each subject: the fractional anisotropy (FA), the weighted fractional anisotropy (wFA), and the connection strength (S). The anisotropy is a property of each voxel in the brain. It is a measure of the directionality of water diffusion in the voxel. If the water diffusion is primarily in a particular direction, the voxel is assumed to contain neural fibers that are oriented in that direction. A fiber track consists of a large number of neighboring voxels. The average FA of a track is thus assumed to be a measure of the patency of the track.

We assumed that the wFA is an improved measure of this patency, as it takes the probability that a voxel is actually part of the track into account. Obviously, the wFA can only be computed when using probabilistic fiber tracking.

Finally, the connection strength S is the fraction of samples in a seed region that actually reaches the target region. A high strength S is again a measure of the patency of the track. Conversely, a low strength may indicate that the seed region is connected primarily to other end points. Although these three measures (FA, wFA and S) are the result of considerable data reduction, they provide measures that allow for straightforward comparisons between subjects and subject groups.

By quantifying the tracks that pairwise connect the IC, AC and amygdala (AM), we were able to make comparisons between control subjects and tinnitus patients. These three ROIs were selected because they may play an important role in the mechanisms that lead to tinnitus.

Despite the fact that a relation between tinnitus and peripheral hearing loss is present, it is not straightforward. The mechanisms underlying the diffuse relation between hearing loss and tinnitus are unclear. It is possible that subtle characteristics of the functional or anatomical (structural) connectivity of the central auditory systems determine whether a subject develops tinnitus. In addition, non-auditory brain areas are believed to be involved in tinnitus. Specifically, the interaction between the limbic and the auditory system has been proposed in models that explain tinnitus (Lockwood *et al.*, 1998; Jastreboff, 1990;

Møller, 2006a).

Abnormal spontaneous brain rhythms in tinnitus patients are indicative of abnormal functional connectivity in such patients. These brain rhythms reflect the activity of forward and backward loops connecting brain areas, specifically of the cortical-thalamical connections (Llinas *et al.*, 2005). In tinnitus patients, the alpha brain rhythm is reduced, while the delta rhythm is substantially enhanced (Weisz *et al.*, 2005a). These abnormal brain rhythms, which differentiate tinnitus patients from control subjects, could in part be due to differences in the anatomical connections.

Our study is an attempt to show possible anatomical differences between subject groups using DTI. The computation of FA, wFA and S allowed us to compute such differences. We found differences and similarities between tinnitus patients and healthy controls. For example, the variability across subjects for FA and wFA of the paths was remarkably small within each group, and was also very similar between both groups.

Significant differences in path strength between tinnitus patients and healthy controls were found for the left IC-AM connection, the right AC-IC connection, and the AC-AM connection for both hemispheres, which also resulted in a significant difference for the lateralization. Tinnitus patients also showed a higher FA in the AM-AC connection.

Regarding lateralization, differences between tinnitus patients and controls were found for the FA of the AC-AM connection and the weighted FA of the AC-AM and IC-AC connections. This result may correspond to the abnormal lateralization in brain function observed in tinnitus patients in a PET study (see chapter 4).

The difference in strength of the connection between auditory cortex and amygdala in subjects with tinnitus compared to controls indicates that the limbic system may indeed play a major role in tinnitus, especially concerning the emotional content of the percept of tinnitus. Although cognitive therapies, focused on treating tinnitus by habituation, have been used for many years (Jastreboff and Jastreboff, 2003; Jastreboff, 2007), no imaging study prior to the present one has shown a potential anatomical pathway that might function differently between tinnitus patients and normal hearing controls.

7.7 Conclusions and outlook

In this work we found evidence linking tinnitus to the central auditory system. We found increased sound-evoked responses in a subset of subjects with lateralized tinnitus (chapter 3) while it was absent in another subset (chapter 4). This could reflect a 'hidden variable' like hyperacusis that may be present in some patients but not all. The increased sound-evoked activity might thus not necessarily be a marker of tinnitus but could as well be a marker for a phenomenon that in many cases accompanies tinnitus (i.e., hyperacusis).

Additionally we found evidence for a change in response lateralization and connectivity at the level of the midbrain (i.e., the inferior colliculus) in subjects with tinnitus. As suggested earlier (Møller, 2006c), a change in the balance between excitation and inhibition could not only lead to a change in the 'gain-setting' of the auditory pathway (Salvi *et al.*, 2000), but also lead to a change in the response lateralization (chapter 4).

A change in the balance between excitation and inhibition not only affects the auditory pathway but may also affect normal somatosensory-auditory integration. A recent study

has shown that reduced input to the auditory system (due to e.g. hearing loss) affected the level of somatosensory input at the level of brainstem (Shore *et al.*, 2008). In chapter 5 we investigated somatic tinnitus and found increased levels of response to jaw protrusion at the level of the cochlear nucleus in subjects with tinnitus compared to controls. A disturbance in the normal integration might be the basis for the somatosensory modulation of the loudness of tinnitus while the integration is not disturbed in subjects without tinnitus.

Chapter 6 explores the use of DTI to study tinnitus. The difference in strength of the connection between auditory cortex and amygdala in subjects with tinnitus compared to controls, indicates that the limbic system may indeed play a major role in tinnitus, especially concerning the emotional content of the percept of tinnitus.

Yet, there are many ways in which tinnitus can be studied. Functional MR imaging techniques have, for example, a limited temporal resolution (especially when performing auditory experiments using a sparse design). One of the hypothesized basis aspects of tinnitus–in addition to the increased spontaneous neural activity–corresponds to an increase in neural synchrony (Seki and Eggermont, 2003). One might think of studies that specifically try to assess this synchrony by using EEG or MEG techniques. Also, specific fMRI analysis methods like blind source separation techniques (Langers, 2009) may offer valuable information about brain dynamics for which standard GLM approaches like the ones used in this thesis are not suitable.

So, although this PhD thesis reports evidence for involvement of the central auditory system in tinnitus, it is by far conclusive. Therefore I would like to end this thesis by quoting Frank Herbert

> "The beginning of knowledge is the discovery of something we do not understand." — Frank Herbert (1920–1986)

and conclude that there is a lot we do not understand about tinnitus and the brain.

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Summary

Summary

Tinnitus resides between the ears

Tinnitus, a pathology between the ears

Besides tissues like bone, fluid and brain tissue, there is more between the ears, at least that is what the google-results show us after a search. Obesity, feeling cold and even an orgasm can all be found between the ears. This is not really strange if you think about it. The brain processes sensory signals in such a way that we are able to perceive the world around us. They also co-ordinate the muscles which make us either move or stop us from moving. If everything goes according to plan these processes may not be noticeable. Yet, if there is interruption of some kind we will notice it, either directly or somewhat later.

Problems in communication may be caused by hearing loss–a problem with the auditory senses that has its roots outside the central nervous system (and therefore peripheral). Parkinson's disease, on the contrary, is an example of a degenerative affection of the central nervous system and is characterized by motor dysfunction (among others) .

There is also a category of affections where a change in the peripheral function may lead to a change at a central level. One example is phantom-limb pain which may start after amputation of a limb. The sensory input to the central nervous system is decreased which may cause the complaints of pain in the phantom limb. This thesis describes a number of studies to a possible analogous problem in the auditory system: tinnitus.

Tinnitus

Tinnitus may be the term for a number of possible different pathologies that all lead to the same percept: a sound that is only heard by the patients. A common theory describes the etiology of tinnitus as the disruption in the balance between excitatory and inhibitory input to the auditory cortex. If, for example, the auditory input is diminished through a peripheral hearing loss, it may cause the activity in the central auditory system to increase. Since the loudness of sound is also coded by the level of activity, an increase in the (spontaneous) activity may effectively lead to the percept of a sound: tinnitus.

Imaging techniques and tinnitus

In an attempt to objectify this abnormal levels of activity *functional magnetic resonance imaging* (fMRI) was used. This technique measures the change in the vascular oxygen level in the brain, which is caused by (task-related) changes in the levels of neural activity. Chapter 2

In a number of studies on tinnitus, both fMRI as *positron emission tomography* (PET) have been used to show differences between subjects with tinnitus and subjects without. A number of experimental paradigms have been used. A common method measures sound-evoked responses in patients and compares it to those in subjects without tinnitus. From work performed in animals with tinnitus they found–in addition to the increased levels of spontaneous activity–an increased sound-evoked response. This might also hold for patients with tinnitus.

Another methods studies a specific group of subjects that have the ability to modulate the loudness of their tinnitus by eye-movements or jaw-movements. By measuring the activity during this modulation and during 'rest' it may possible to localize the activity that corresponds to the modulation of the loudness.

The results of these studies show a trend, although differences between individual studies, that tinnitus corresponds to an increased level of activity in number of auditory areas in the brain. They also found differences in activity in non-auditory areas (such as the frontal lobe, the limbic system and the cerebellum) that seem associated with tinnitus. None-auditory areas seem associated with tinnitus although the role of these non-auditory brain is not yet clear.

Nevertheless, it remains unclear whether the differences in neural activity between the subject groups are related to tinnitus or that they may have been caused by differences in the hearing levels or age between the groups. Hyperacusis, a reduced tolerance to loud sounds–often coinciding with tinnitus–may also responsible for the aberrant levels of activity.

Increased sound-evoked responses

The first study (as described in chapter 3) studies a specific group of subject with lateralized tinnitus. Based on this lateralization of tinnitus, a corresponding lateralization at cortical levels was expected. This functional lateralization, on the contrary, was not apparent. The results, however, did show an increased sound-evoked response in the inferior colliculus (IC) of subjects with lateralized tinnitus as compared to those in a control group. Chapter 3

The results also showed that contra-lateral stimuli gave larger responses than ipsilateral stimuli. Moreover, 70 dB (SPL) stimuli gave larger responses than those of 40 dB (SPL).

In the IC of subjects with tinnitus, the pattern of responses was different; patients with tinnitus do not show the contra-lateral dominance of the responses. In addition to the

increase sound-evoked responses, we measured a difference in the response lateralization. A striking conclusion is that the lateralization of the response did not correspond to the lateralization of the tinnitus

Lateralization and connectivity

Chapter 4 The response lateralization to sound may give a measure of the efficiency of the input from the periphery to the central nervous system. From the auditory peripheral system to the central auditory system there are generally speaking two pathways; contra-lateral excitatory pathways and ipsilateral inhibitory pathways. A reduction in the efficiency of this inhibitory pathway may (by a reduction of inhibition) theoretically lead to increased activity in ipsilateral auditory nuclei. This in turn leads to a reduction in the so-called lateralization index.

The lateralization index was determined for each nucleus in the auditory pathway. This index was systematically lower in the patient-group, but only significant at two levels: the right primary auditory cortex (PAC) and the right IC. This suggest that there is a reduced efficiency of the ipsilateral inhibitory afferent input to the IC. In contrast to the findings in chapter 3, there were no increase sound-evoked responses in the IC of patients.

In addition to this analysis, we studied the connectivity patterns between nuclei of the auditory pathway. The degree of influence of one nucleus onto another can be described in terms of correlation of the time-courses of these nuclei. This *functional connectivity* suggested that if nuclei are active at the same time, they may be functionally connected. Yet, correlation does not imply causality and cannot distinguish the direction of the stream of information between nuclei.

The partial correlation was used as a measure of *effective connectivity* to assess the influence that one nucleus has on another nucleus. The partial correlation is the correlation that remains between two nuclei after subtracting the influence of the other nuclei.

The functional connectivity in the auditory pathway was larger than the effective connectivity and suggests that a great deal of the correlation can be explained by the experimental paradigm that was used; presenting sound stimuli. The results showed a disturbed pattern of effective connectivity between the IC and the contralateral *medial geniculate body* (MGB) as well as between the left *cochlear nucleus* (CN) and the left primary auditory cortex. In particular, the disturbed effective connection between the IC and MGB is in agreement with previous findings; a reduced lateralization in subjects with tinnitus.

Disturbed balance between somatosensory and auditory input

Chapter 5 Somatic tinnitus is a phenomenon which refers to tinnitus that is elicited or modulated by somatosensory modulation like e.g. jaw protrusion. The results demonstrate that over-

lap of somatosensory and auditory input can be measured in the auditory pathway; jaw protrusion caused a response in the auditory system and may explain the influence of jaw protrusion on the perceived loudness of tinnitus.

Overlap between the auditory modality and the somatosensory modality were found in the primary auditory cortex and the auditory association cortex. Interestingly, the only differences between subjects with tinnitus and controls were found in the CN and the IC. Jaw protrusion evoked in both ROIs a larger response in subjects with tinnitus compared to those in controls.

Our findings stress the importance of somatosensory interaction in the (extralemniscal or non-classical) auditory system in defining possible mechanisms underlying tinnitus. One hypothesis, relating tinnitus to changes in normal somatosensory integration, is that a change in input from the auditory system (due to e.g. noise-induced hearing loss) might influence the somatosensory input to the central nervous system. This thus form a neurophysiological basis for modulating perceptual characteristics of tinnitus. Especially our finding that jaw protrusion shows enhanced responses in the inferior colliculi and cochlear nuclei of subjects with tinnitus compared to controls underlines this hypothesis.

The auditory pathway

Where the results of our fMRI findings were summarized in chapter 3–5 summarizes chapter 6 the results from a study that used *Diffusion tensor imaging* (DTI) to gain insight in the anatomical pathway formed by white matter fiber bundles. DTI is a technique sensitive to the diffusion of water in tissue and can be used to track the anatomical pathway between two predefined areas. The first results shows that this method can be used to track part of the auditory pathway. The paths that the auditory cortex connect to the IC all pass the MGB of the thalamus. The structural properties of these paths were determined and compared between subject groups.

Chapter 6

By quantifying the tracks that pair-wise connect the IC, AC and amygdala (AM), we were able to make comparisons between control subjects and tinnitus patients. These three ROIs were selected because they may play an important role in the mechanisms that lead to tinnitus. We found differences and similarities between tinnitus patients and healthy controls.

Significant differences in path strength between tinnitus patients and healthy controls were found for the left IC-AM connection, the right AC-IC connection, and the AC-AM connection for both hemispheres.

The difference in strength of the connection between auditory cortex and amygdala in subjects with tinnitus compared to controls indicates that the limbic system may indeed play a major role in tinnitus, especially concerning the emotional content of the percept of

tinnitus. Although cognitive therapies, focused on treating tinnitus by habituation, have been used for many years, no imaging study prior to the present one has shown a potential anatomical pathway that might function differently between tinnitus patients and normal hearing controls.

Between the ears?

The experiments as described in this thesis show that there are subtle functional and structural differences between subjects with and without tinnitus. TInnitus seems thus a pathology between the ears. Note that, the difference may occur as a response of the central nervous system to a peripheral hearing loss. This may cause a disruption–like with the amputation of a limb–of the normal input to the brains. Tinnitus seems to be consequence of the changes in the central nervous system that follow peripheral hearing loss.

Samenvatting

Nederlandse samenvatting
Oorsuizen zit tussen de oren

Tussen de oren

Behalve fysiek tastbaar weefsel zoals bot, vloeistof en hersenen zit er veel meer tussen de oren, althans zo leert een rondje googlen ons. Overgewicht, kou en zelfs het orgasme zitten kennelijk allemaal tussen de oren. En eigenlijk is dat niet zo vreemd, want de hersenen zorgen er immers voor dat we de wereld om ons heen waarnemen; ze ontvangen signalen van onze sensoren. Ook coördineren ze de aansturing van spieren die ons uiteindelijk in beweging zetten of juist doen afremmen. Normaal gesproken merken we van deze processen niet zo veel maar als er een hapering of storing optreed merken we de gevolgen meestal vrij snel.

Problemen in communicatie kunnen optreden door bijvoorbeeld gehoorverlies–een probleem waarvan de oorzaak buiten het centrale zenuwstelsel ligt (ofwel perifeer). De ziekte van Parkinson daarentegen is een voorbeeld van een degeneratieve afwijking in het centraal zenuwstelsel en kenmerkt zich door (onder andere) afwijkingen in de motoriek.

Er is echter ook een categorie afwijkingen waarin een verandering in de periferie kan leiden tot een verandering op centraal niveau. Een voorbeeld hiervan is fantoompijn die kan ontstaan na amputatie van een ledemaat. Hierdoor is de sensorische invoer naar het centrale zenuwstelsel verminderd wat kan leiden tot een pijnsensatie. Dit proefschrift beschrijft studies naar een mogelijk analoog probleem in het auditieve systeem: oorsuizen of tinnitus.

Tinnitus

Oorsuizen is een verzamelterm voor waarschijnlijk een aantal verschillende afwijkingen die uiteindelijk tot hetzelfde percept leiden: een geluid dat alleen door de patiënt zelf wordt gehoord. De gangbare theorie beschrijft het ontstaan van tinnitus als een verstoring in de balans van exciterende en inhiberende invoer van auditieve hersengebieden. Als bijvoorbeeld de auditieve invoer wegvalt door een perifeer gehoorverlies dan kan de activiteit in het centrale zenuwstelsel toenemen. En aangezien de activiteit in het auditieve systeem samenhangt met de luidheid van geluid kan een toename in de (spontane) activiteit leiden tot de perceptie (de waarneming) van geluid: tinnitus.

Beeldvormende technieken en tinnitus

In een poging deze veronderstelde abnormale activiteit te objectiveren is gebruik gemaakt van functionele *magnetic resonance imaging* (fMRI). Deze techniek meet de verandering van het vasculaire zuurstofniveau in de hersenen die het gevolg is van een (taakgerelateerde) verandering in neurale activiteit. Hoofdstuk 2

In andere studies naar tinnitus zijn zowel fMRI als *positron emission tomography* (PET) gebruikt om verschillen aan te tonen tussen patiënten met tinnitus en proefpersonen zonder tinnitus. In deze studies zijn een aantal verschillende experimentele paradigma's gebruikt. Een veel gebruikte methode is het meten van de respons op geluid in een groep patiënten met tinnitus en deze te vergelijken met de respons gemeten in een groep proefpersonen zonder tinnitus. Uit onderzoek is gebleken dat in proefdieren met tinnitus -naast de toename in spontane activiteit- ook de respons op geluid is toegenomen. Dit is mogelijkerwijs ook het geval in patiënten met tinnitus.

Een andere methode is het bestuderen van een specifieke groep patiënten die de luidheid van hun tinnitus kan moduleren door oogbewegingen of kaakbewegingen. Door de activiteit te meten tijdens deze modulatie en dit te vergelijken met de activiteit tijdens 'rust' is het mogelijk de activiteit die samenhangt met verandering in luidheid te lokaliseren.

De resultaten van deze studies laten een trend zien waarbij, ondanks verschillen tussen individuele studies, tinnitus correspondeert met verhoogde activiteit in een aantal specifieke gebieden in het auditieve systeem. Ook verschillen in activiteit in niet-auditieve gebieden (zoals frontale hersengebieden, het limbische systeem (betrokken bij emotie) en het cerebellum) lijken geassocieerd met tinnitus. Niet-auditieve hersengebieden zijn dus kennelijk ook betrokken bij het fenomeen tinnitus, ook al is de precieze rol van deze gebieden hierbij niet geheel duidelijk.

Het is overigens niet helemaal duidelijk of de verschillen in neurale activiteit tussen de groepen alleen het gevolg zijn van tinnitus, of dat ze mogelijkerwijs te maken hebben met verschillen in bijvoorbeeld gehoorverlies of verschillen in leeftijd tussen de groepen. Ook hyperacusis, een verminderde tolerantie voor luid geluid, veel voorkomend in combinatie met tinnitus, zou deze afwijkende activiteit kunnen verklaren.

Verhoogde respons op geluid

In de eerste studie (zoals beschreven in hoofdstuk 3) is specifiek gekeken naar een groep patiënten met gelateraliseerde tinnitus. Op basis van de lateralisatie van de tinnitus werd een overeenstemmende lateralisatie op centraal niveau verwacht. Deze functionele lateralisatie bleek echter niet aanwezig. Wel lieten de resultaten een verhoogde respons op geluid zien in de inferior colliculus (IC) van patiënten met gelateraliseerde tinnitus in ver- Hoofdstuk 3

gelijking met de respons gemeten in een controlegroep.

De resultaten toonden ook dat contra-laterale stimuli een grotere respons lieten zien dan ipsi-laterale geluidsstimuli. Bovendien gaven stimuli van 70 dB (SPL) een grotere respons dan die van 40 dB (SPL).

In de IC van proefpersonen met tinnitus was het patroon van deze respons anders; patiënten met tinnitus laten geen duidelijke contralaterale dominantie zien. Dus, in aanvulling op de verhoogde respons op geluid, was er een verschil in de lateralisatie van de respons meetbaar. Opmerkelijk was dat de lateralisatie van de respons niet gecorreleerd was met de lateralisatie van de tinnitus.

Lateralisatie en connectiviteit

Hoofdstuk 4 De lateralisatie van de respons op geluid geeft mogelijk een indicatie van de efficiëntie van de aanvoer van de periferie naar het centrale zenuwstelsel. Van de auditieve periferie naar het centrale zenuwstelsel zijn er globaal gesproken twee paden: contra-laterale excitatoire banen en ipsi-laterale inhibitoire banen. Een verminderde efficiëntie van deze inhibitoire banen zou (door een vermindering van de remmende werking) theoretisch tot een verhoogde activiteit van de ipsi-laterale auditieve kernen kunnen leiden. Dit vertaalt zich uiteindelijk in een verlaging van de zogenaamde lateralisatie-index.

Voor elke kern in het auditieve pad werd de lateralisatie-index bepaald. De lateralisatie-index was systematisch lager in de groep patiënten, maar op slechts twee niveaus significant: de rechter primaire auditieve cortex (PAC) en de rechter IC. Dit suggereert dat efficiëntie van de ipsi-laterale inhibitoire afferente aanvoer naar de IC verminderd is. De resultaten toonden nu echter geen verhoogde respons op geluid op het niveau van de IC, zoals hoofdstuk 3 liet zien.

In aanvulling op deze analyse werden de verbindingspatronen tussen de kernen in het auditieve pad bestudeerd. De mate van invloed van één kern op een andere kern kan worden beschreven als een correlatie tussen tijdsreponsen van deze kernen. Deze *functionele connectiviteit* toonde dat kernen die tegelijkertijd actief zijn mogelijk functioneel verbonden zijn. Correlatie geeft echter geen informatie over causaliteit en geeft dus geen informatie over de richting van de informatiestroom tussen de kernen.

Om toch meer te kunnen zeggen over de invloed die kernen op elkaar hebben is de partiële correlatie gebruikt als maat voor *effectieve connectiviteit*. De partiële correlatie is de correlatie tussen kernen die overblijft als de invloed van de overige kernen van de simpele correlatie is afgetrokken.

De functionele connectiviteit in het auditieve pad was groter dan de effectieve connectiviteit en geeft daarmee een indicatie dat een deel van de correlatie kan worden verklaard

door het experimentele paradigma: het aanbieden van geluid. Resultaten lieten zien dat de effectieve connectiviteit tussen de IC en de contralaterale *medial geniculate body* (MGB) verstoord was, evenals de connectiviteit tussen de linker cochleaire nucleus (CN) en de linker primaire auditieve cortex. Met name de verstoorde verbinding tussen de IC en MGB is in overeenstemming met de eerdere bevindingen van de verminderde lateralisatie in de proefpersonen met tinnitus.

Verstoorde balans tussen somatosensorische en auditieve invoer

Somatische tinnitus ontstaat of wordt gemoduleerd door een somatosensorische manipulatie zoals kaakbeweging. Resultaten lieten zien dat overlap van somatosensorische en auditieve activiteit gemeten kon worden in het auditieve pad. Protrusie van de onderkaak veroorzaakte een meetbare respons in het auditieve pad en geeft mogelijkerwijs de invloed van deze beweging op tinnitus weer. Hoofdstuk 5

Overlap in respons tussen de auditieve signalen en de somatosensorische signalen werd gevonden in de primaire auditieve cortex en de associatieve auditieve cortex. Het enige verschil tussen de proefpersonen met en zonder tinnitus was te vinden in de CN en de IC. Protrusie van de kaak leidde in deze beide gebieden tot een grotere respons in de groep patiënten met tinnitus in vergelijking met de controles.

Deze bevindingen onderstrepen het belang van de somatosensorische interacties in het (niet-klassieke) auditieve pad als mogelijke verklaring voor deze specifieke vorm van tinnitus. Eén hypothese relateert tinnitus aan een verandering in de normale somatosensorische integratie en veronderstelt dat een verandering in invoer van auditieve informatie (door bijvoorbeeld gehoorverlies) tot een verandering van de somatosensorische invoer naar het centrale zenuwstelsel kan leiden. Dit kan een neurofysiologische basis vormen die de invloed van kaakbeweging op de tinnitus mogelijkerwijs kan verklaren. Vooral de verhoogde respons op kaakbeweging in de IC en de CN van proefpersonen met tinnitus draagt bij aan deze hypothese.

Het auditieve pad

Waar hoofdstuk 3–5 vooral de resultaten van de fMRI-studies naar tinnitus laten zien geeft hoofdstuk 6 meer inzicht in de anatomische banen die gevormd worden door banen van witte stof in de hersenen. *Diffusion tensor imaging* (DTI) is een beeldvormende techniek die gevoelig is voor diffusie van water in weefsel en kan worden gebruikt om anatomische paden tussen twee vooraf gedefinieerde gebieden in beeld te brengen. Het eerste resultaat liet zien dat deze methode kan worden gebruikt om een deel van het klassieke auditieve pad in beeld te brengen. De paden die de auditieve cortex verbinden met de Hoofdstuk 6

IC lopen allen via het mediale geniculate lichaam van de thalamus (MGB). Verder werden de structurele eigenschappen van deze paden bepaald en vergeleken tussen de groepen proefpersonen.

Een vergelijking tussen groepen proefpersonen met en zonder tinnitus werd gemaakt voor de paden die paarsgewijs de inferior colliculus (IC), de auditieve cortex (AC) en de amygdala (AM) met elkaar verbinden. Deze kernen waren geselecteerd omdat wordt verondersteld dat deze een belangrijke rol spelen in de mechanismen die betrokken zijn bij het ontstaan van tinnitus. Er zijn zowel overeenkomsten als verschillen gevonden.

Er werden significante verschillen gevonden in de sterkte van de paden tussen de groepen in de verbinding IC-AM in de linker hemisfeer, de verbinding AC-IC aan de rechterzijde en de AC-AM verbinding aan beide kanten.

De verschillen in sterkte van de verbinding tussen de auditieve cortex en de amygdala tussen de groepen proefpersonen geven een indicatie dat het limbische systeem een belangrijke rol speelt in tinnitus, met name met betrekking tot de emotionele associaties die tinnitus kan induceren. Alhoewel cognitieve therapiën -veelal gericht op gewenning aan de tinnitus- al jaren bekend zijn, is deze studie de eerste die de (potentiële) anatomische paden in beeld brengt en de toestand van deze paden vergelijkt tussen proefpersonen met en zonder tinnitus.

Tussen de oren?

De experimenten in dit proefschrift tonen aan dat er subtiele functionele en structurele verschillen zijn tussen mensen met en zonder tinnitus. Tinnitus lijkt dus echt clichématig 'tussen de oren' te zitten. Daarbij kan opgemerkt worden dat deze verschillen mogelijk ontstaan als gevolg van een perifeer gehoorverlies. Dit gehoorverlies zorgt ervoor dat -net als met de amputatie van een ledemaat- de invoer naar de hersenen verstoord is. De tinnitus lijkt te ontstaan als reactie van het centrale zenuwstelsel op het perifere gehoorverlies.

Nawoord

Nawoord

De resultaten van ruim 4 jaar onderzoek: een proefschrift die de resultaten beschrijft van (o.a.) fMRI studies naar tinnitus. Daarnaast een heleboel niet-gedocumenteerde ervaringen met collega's en vrienden en een persoonlijke ontwikkeling van 'student' naar 'young (urban) professional'. Het is maar goed dat een proefschrift zich beperkt tot de wetenschappelijke aspecten van het werk, omdat het op schrift zetten van deze ervaringen een veelvoud van de tijd zou hebben gekost. Evengoed zijn het juist díe ervaringen en contacten met collega's en vrienden die me hebben gevormd en een belangrijk deel van de herinneringen vormen aan een mooie periode – een periode die slechts enkele bewijsstukken heeft opgeleverd: een paar artikelen en een proefschrift.

Een proefschrift die de studies beschrijft naar een fenomeen dat relatief eenvoudig uit te leggen is aan de leek: Oorsuizen (tinnitus) en de mogelijke veranderingen in het centraal zenuwstelsel die er aan ten grondslag kunnen liggen of er het gevolg van zijn. Ingrediënten voor dit onderzoek: een proefpersoon mét oorsuizen, een proefpersoon zonder oorsuizen, een MRI scanner, veel data-analyse en nog meer geduld.

Terugkijkend op de belangrijkste resultaten moet ik bekennen dat, ondanks dat de resultaten zeker wel in het kader van hypotheses vallen, het toch wel indirecte metingen zijn en de resultaten bijvoorbeeld ook het gevolg kunnen zijn van andere (misschien veel voorkomend naast oorsuizen) pathologieën of oorzaken. Misschien is de belangrijkste les van dit onderzoek *juist* de bevinding dat er veel verschillende vormen van tinnitus zijn die elk potentieel een andere oorzaak en gevolg hebben. Dit maakt het onderzoek lastig interpreteerbaar en moeilijk te generaliseren. Eenvoudig was het in ieder geval niet.

Een promotieonderzoek is als een reis waarin het einddoel globaal bekend was, maar waarin de wegen die bewandeld werden soms dood liepen of een bocht beschreven die een nieuw perspectief op het onderzoek gaven, of –het tegenovergestelde– juist een cirkel vormden, terug naar het begin. Juist de collegae, vrienden en familie spelen een belangrijke rol als reisgenoot, klankbord en uitlaatklep.

Dit relatief korte nawoord is een poging om deze menselijke aspecten van het werk te benadrukken. Het gaat hier met name om de mensen die elk op eigen wijze een bijdrage hebben geleverd aan het werk. Zonder deze bijdrage was het een eenzame reis en misschien zelfs een onmogelijke. Ik wil iedereen die de afgelopen jaren heeft gezorgd voor een prettige werkomgeving, een luisterend oor, voor de kritische vragen en behulpzame antwoorden, suggesties en de broodnodige afleiding dan ook van harte bedanken hiervoor.

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Buiten deze selecte groep zijn er nog mensen binnen het NiC die een rol hebben gespeeld: **Remco**-ondanks dat je rol nooit formeel is geweest was je altijd oprecht geïnteresseerd in het onderzoek. Als ik een vraag had was jij veelal de persoon bij uitstek die het antwoord wist. Methodologie-sparring-partner–Dank daarvoor! **Diek**, tijdens het afstudeerproject als mentor op gepaste afstand in combinatie met **Hans** als directe begeleider. Tijdens het promoveren als geïnteresseerde toehoorder met een gezonde dosis scepsis.

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Curriculum Vitae

Cris Lanting werd geboren op 22 december 1980 te Bedum. Van 1993 tot 1999 doorliep hij zijn middelbare schooltijd op het Wessel Gansfort College te Groningen. Hierna begon hij met zijn studie *Technische Natuurkunde* aan de Rijksuniversiteit Groningen.

Tijdens deze studie koos hij voor de richting *Biomedische Technologie* en bestudeerde de doorbloeding van het brein met behulp van MRI labeling technieken (Arterial Spin Labeling, ASL). Dit afstudeerproject werd uitgevoerd in het Neuro Imaging Center (NIC) van de graduate school 'Behavioral and Cognitive Neurosciences' van de RUG.

Na het behalen van zijn ingenieurstitel in 2005 begon hij een promotieonderzoek op het gebied van auditieve fMRI en tinnitus vanuit de afdeling KNO van het Universitair Medisch Centrum Groningen (UMCG). Verscheidene studies werden uitgevoerd naar de neurale representatie van tinnitus in het auditieve systeem van de mens. Het project resulteerde in de openbare verdediging van een proefschrift en de verkrijging van de doctorsgraad in 2010.

Aansluitend heeft hij een positie als postdoc geaccepteerd aan het MRC Institute of Hearing Research te Nottingham (Verenigd Koninkrijk) waar hij onderzoek doet naar de representatie van geluid in de auditieve cortex met behulp van fMRI en EEG.

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