Neuroimaging correlates of hearing loss, tinnitus, and hyperacusis

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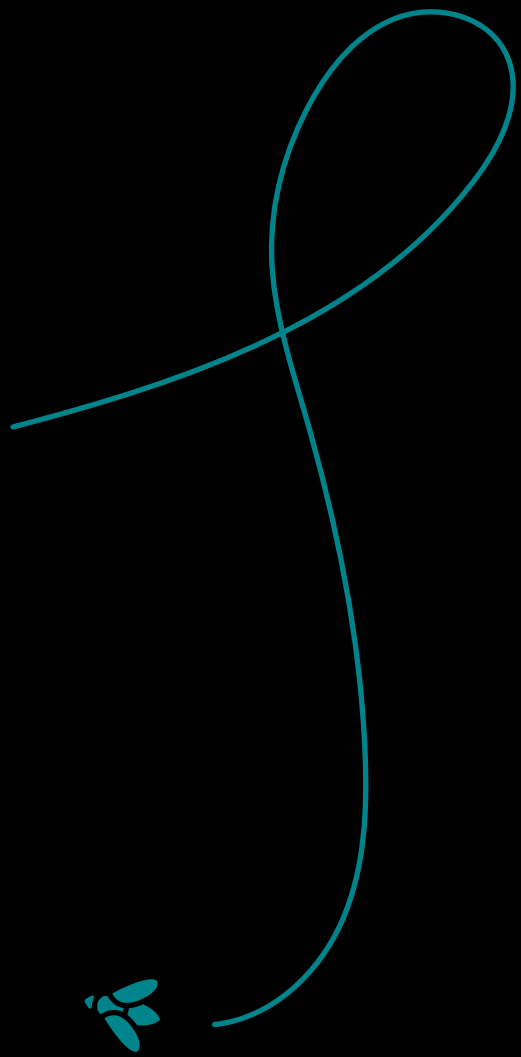

CHAPTER 01

INTRODUCTION

ELOUISE KOOPS

PREFACE

Worldwide, hearing loss is the most prevalent acquired sensory impairment, affecting a large proportion of the elderly population (Mathers et al., 2000). Both the accumulation of noise exposure and aging impact the quality of hearing. With a globally aging population, the current prevalence of 466 million people with hearing loss is expected to rise to 630 million by 2030 (OMS, 2018). Hearing loss is not only related to the deterioration of the quality of hearing, but it is also associated with a decrease in the quality of life (Ciorba et al., 2012), an increased likelihood of cognitive impairments, and even dementia (Jafari et al., 2019). The presence of hearing loss increases the chances of developing other auditory domain symptoms such as tinnitus and hyperacusis. Tinnitus is the perception of a phantom sound, a common and potentially devastating condition. Hyperacusis is characterized by the experience of uncomfortable loudness for sounds that are not uncomfortably loud to most people (Anari et al., 1999; Baguley, 2003). Both tinnitus and hyperacusis are debilitating symptoms, and even though several treatment options are available, there is currently no cure for either condition. Considering that the population prevalence of hearing loss is expected to rise from ~6% to ~7.4% in the coming ten years, and we are aware that hearing loss decreases the quality of life and puts people at risk of developing other conditions, it is essential to gain more insight in hearing loss and related symptoms such as tinnitus and hyperacusis.

The theoretical framework of the relationship between hearing loss, tinnitus, and hyperacusis is built around our brain's incredible capacity to adapt to environmental needs. In hearing loss, it is well established that adaptations to the reduction in sensory input occur throughout the auditory pathway. Presumably, the course of this plasticity process can underlie tinnitus and hyperacusis (Chen et al., 2015). The typically valuable trait of plasticity that allows the brain to rewire and recover from injury and sensory deprivation may give rise to the debilitating symptoms of tinnitus and hyperacusis. In this light, the sensory consequences of this plasticity are experienced as a maladaptive process. It has been theorized that tinnitus is explicitly related to maladaptive cortical reorganization (Shore et al., 2016). At present, it is unclear whether excessive reorganization or an incomplete form of reorganization in response to hearing loss may give rise to the tinnitus percept.

Moreover, the characteristic changes that occur at the central level of the auditory system, i.e., the brain, in tinnitus and hyperacusis are still unclear. Even though it is well established that tinnitus is maintained at the level of the central auditory system, the exact brain areas and networks involved remain to be discovered. Due to the high comorbidity of hearing loss, tinnitus, and hyperacusis, it has proven difficult to tease apart the effects of these different yet

possibly related conditions on the brain. To that end, it is crucial to gain insight into the characteristic differences and adaptation of the central auditory system that accompany hearing loss, tinnitus, and hyperacusis.

This first chapter gives a short overview of the auditory system and the ramifications if the system is compromised due to hearing loss and associated symptoms such as tinnitus and hyperacusis. The pathway from sensation to auditory perception is described, starting at the peripheral auditory system and progressing to the auditory cortex. Furthermore, I present the current state of knowledge on subcortical and cortical substrates of hearing loss, tinnitus, and hyperacusis. Then, I will give a short introduction to magnetic resonance imaging (MRI), the method used to investigate the auditory system's structural and functional characteristics in this work. Finally, I will provide an overview of the subsequent chapters within this thesis.

SOUND PERCEPTION

Sound perception arises from a complex system that translates the variations in pressure, which form propagating sound waves, into an audible sound. The human ear has the incredible capacity to detect pressure variations as small as 2×10^{-5} Newtons per square meter. This detection threshold has led to the convention of expressing the level of a sound that travels through the air in sound pressure level relative to this reference intensity (dB SPL) (Fletcher and Munson, 1933). Decibels (dB) are commonly used to describe the intensity of a sound on a logarithmic scale. The practical use of this becomes evident when you consider that the human auditory system can code for a $10^{\wedge 13}$-fold increase in sound intensity from a just noticeable sound to a sound that is painfully loud: an impressive dynamic range.

The sensitivity of hearing is dependent on the frequency of sound. The frequency spectrum that the human auditory system can encode ranges from 20 Hz to 20 kHz. The dB Hearing Level scale (dB HL) reflects this frequency-dependent difference in sensitivity and is expressed relative to standardized or average thresholds. The dB HL scale is used to describe hearing thresholds that are depicted with an audiogram. The perceptual correlate of sound intensity is the loudness of a sound, which is additionally affected by the bandwidth, frequency, and temporal characteristics of a sound (Fletcher and Munson, 1933; Behler and Uppenkamp, 2016). The subjective loudness of pure tones can be expressed with a phon scale, as is illustrated in figure 1.1. This scale equates the loudness of pure tones of a particular frequency to a reference tone of 1000 Hz at set intensities and was originally devised in 1933 by Fletcher and Munson. Therefore, they are often called Fletcher-Munson curves. These equal loudness contours have since been revised and incorporated into an international standard (ISO).

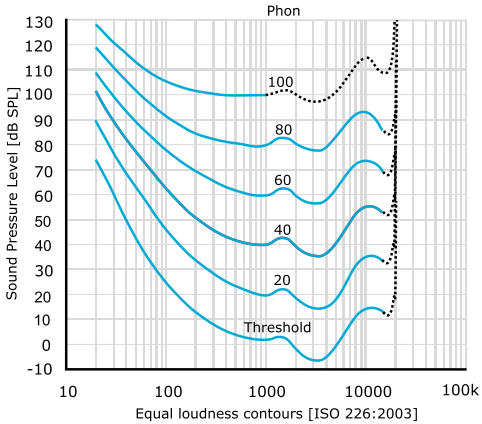

Figure 1.1. The equal loudness contours as described by the international standard (ISO 226: 2003 revised). These loudness contours, or phon curves, reflect that human hearing is most sensitive to frequencies between 1 and 5 kHz. From these contours, it can be extracted that for a pure tone at 7 kHz to sound equally loud as a reference tone of 40 dB SPL at 1 kHz, an intensity of 50 dB SPL is needed.

In contrast to the perceived loudness of pure tones expressed on the phon scale, the sounds we perceive and decode in daily life are often composed of multiple frequencies that all interact to form environmental sounds and speech. Our auditory system is well up to this task of decomposing a sound into its component frequencies to facilitate sound processing at the brain level. This sound decoding relies on a high temporal resolution of processing and adequate frequency decomposition, necessary ingredients for sound and speech perception, which provides us with the required information about our auditory environment. The detection, forwarding, and processing of these environmental sounds and, ultimately, the interaction with our auditory environment all occur due to our auditory system's intricate structure and function.

THE ASCENDING AUDITORY PATHWAY

The auditory system consists of the peripheral auditory system of the ear and the central auditory pathway located in the brain. The auditor periphery can be partitioned into the outer, middle, and inner ear. The central auditory pathway consists of the auditory brainstem nuclei: the cochlear nuclei, the olivary complex, and the lateral lemniscal pathway, and the inferior colliculus in the auditory midbrain, and the medial geniculate body of the thalamus. From there, the signal reaches the auditory cortical areas via the acoustic radiation tract.

THE AUDITORY PERIPHERY

The outer ear receives sound waves, which are transmitted by the middle ear to the inner ear, see figure 1.2 for a schematic representation of the auditory periphery. The outer ear consists of the visible parts of the auditory system: the auricle, or pinna, and the external auditory canal. The auricle directs sound into the external auditory canal, where these directed sound waves hit the tympanic

membrane causing it to vibrate. These vibrations are then relayed to the middle ear, consisting of the three auditory ossicles: the malleus, incus, and stapes. These ossicles aid the signal transfer from the outer ear to the inner ear by providing leverage that offsets the pressure difference between the air-filled ear canal and the fluid-filled cochlear duct. When the human ear is exposed to a very loud sound, the stapedius muscle contracts to dampen the energy transmitted to the inner ear. This contraction, or acoustic reflex, is influenced via complex routes and various neural systems (Lauer et al., 2017). The acoustic startle reflex is thought to provide some minimal protection against noise damage (Møller, 1974).

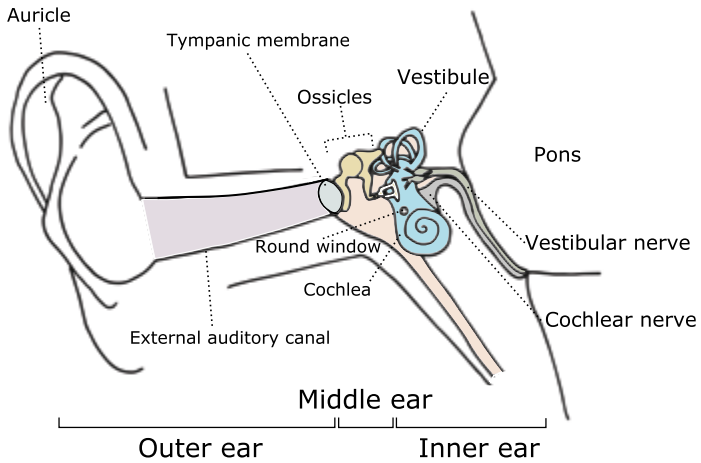

Figure 1.2 Schematic representation of the peripheral auditory system. The peripheral auditory system consists of the outer ear, middle ear, and inner ear. Sound waves hit the tympanic membrane, which causes it to vibrate. These vibrations are transmitted via the ossicles of the middle ear to the cochlea in the inner ear. In the inner ear, the mechanically transduced signal is transformed into an electrical signal. This electrical signal is forwarded to the central auditory system via the vestibulocochlear nerve.

The stapes, the smallest bone in the human body, is responsible for transferring energy from the middle ear to the inner ear. This tiny bone is mobilized by the smallest human skeletal muscle, just over 1 mm long: the stapedius muscle. The stapes' footplate connects to the oval window: the membrane that marks the transition to the inner ear structures. With a piston-like movement, the stapes transduces the acoustic vibrations to the fluid-filled inner ear. The round window moves outward upon the pressure of the stapes on the oval window to allow for movement of this fluid.

The inner ear contains the semicircular canals, the cochlea, and the peripheral part of the vestibulocochlear nerve. The semicircular canals form the vestibular system. Their function is to retain balance and assess motion, a task that it accomplishes due to the three fluid-filled canals positioned in 90° orthogonal angles. The cochlea is the inner ear structure that is essential for mammalian hearing, and it consists of three fluid-filled ducts: the scala tympani, scala media, and scala vestibuli. These structures are illustrated in figure 1.3. The stapes transfers the sound vibrations to the perilymph-filled scala vestibuli and the scala tympani. The scala media, or cochlear duct, is filled with endolymph and is situated between the scala vestibuli and the scala tympani and contains the sensory hair cells.

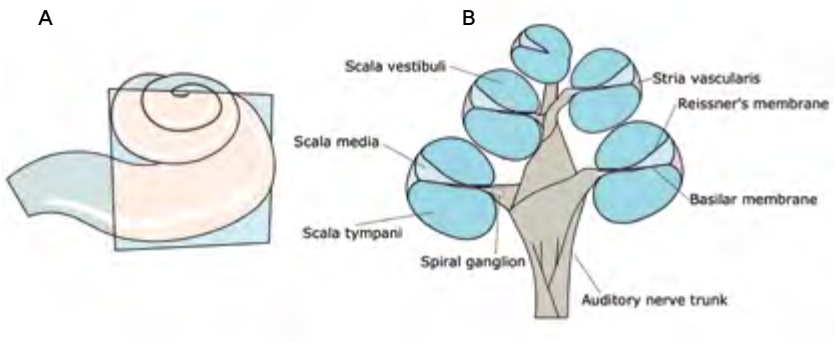

Figure 1.3 The cochlea consists of three fluid-filled ducts. (A) The coiled human cochlea, with the plane along which a virtual cut is made to obtain the image in panel B. (B) The scala tympani and scala vestibuli are filled with endolymph, a fluid very similar to cerebral spinal fluid with high Na^+ content. The scala media is filled with endolymph, which is high in K^+. The stria vascularis maintains the ionic composition of the endolymph.

Whereas sodium (Na^+) is the predominant positively charged ion in the perilymph, the endolymph of the scala media has a high potassium (K^+) ion concentration, crucial for the generation of the hair cell receptor potentials. The stria vascularis is the lateral wall structure within the cochlea maintaining the ion composition of the endolymph (Hudspeth, 2014). The endolymph-filled cochlear duct is separated from the scala vestibuli by Reissner's membrane and from the scala tympani by the basilar membrane. The basilar membrane is a structural element organized tonotopically, allowing frequency decomposition of an incoming signal.

An acoustic signal evokes a traveling wave that progresses over the length of the basilar membrane, as depicted in figure 1.4. Low frequencies within this wave cause the basilar membrane to vibrate stronger on the apical end, whereas higher frequencies cause vibrations near the basal end of the basilar membrane. The

sensory epithelium, or Organ of Corti, is situated on top of the basilar membrane, illustrated in figure 1.5. The elements of this structure convert the mechanical movement of the basilar membrane into an electrical signal. Three rows of outer hair cells and one row of inner hair cells create these nerve impulses.

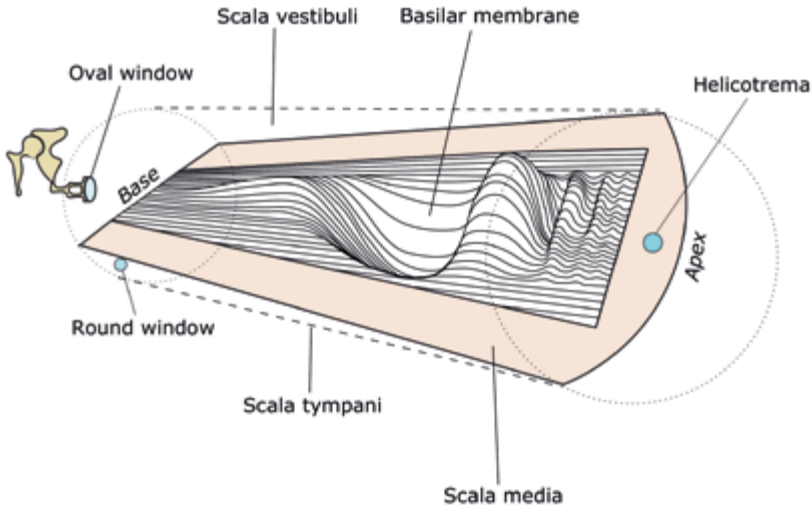

Figure 1.4 The tonotopic organization of the auditory system originates at the basilar membrane. Displacement of the fluid within the scala vestibuli causes the basilar membrane to fluctuate in response to sound. Different frequencies incite peak fluctuations at different areas along its length. The motion of the basilar membrane mimics a traveling wave that oscillates at the frequency of stimulation. The basilar membrane has been uncoiled here to depict the displacement over the length of the structure.

The shearing motion of the tectorial membrane relative to the basilar membrane causes the tips of the inner and outer hair cell stereocilia to be bent. The tallest stereocilia of the outer hair cells are embedded in the tectorial membrane, and are connected to the other stereocilia of the same hair cell via tip-links (Pickles et al., 1984; Verpy et al., 2011). The inner hair cell bundles do not directly contact the tectorial membrane, but are functionally coupled to it via movement of the fluid surrounding the inner hair cells (Ghaffari et al., 2007). The bending or deflecting of the stereocilia mechanically opens the hair cell potassium channels, allowing ions to enter and depolarise the cell. This event causes the presynaptic voltage-gated calcium channels at the base of the hair cells to open, allowing calcium to rush into the cell. The influx of calcium leads to the release of the neurotransmitter glutamate, which binds to receptors on the postsynaptic cell, the spiral ganglion cells, which can lead to the firing of an action potential. The central leading processes, or axons, of the spiral ganglion cells form the cochlear partition of the vestibulocochlear nerve, which is also called the auditory nerve.

At the acoustic meatus, where the auditory nerve enters the cranium alongside the vestibular and the facial nerve, the peripheral pathway ends, and the central auditory pathway begins.

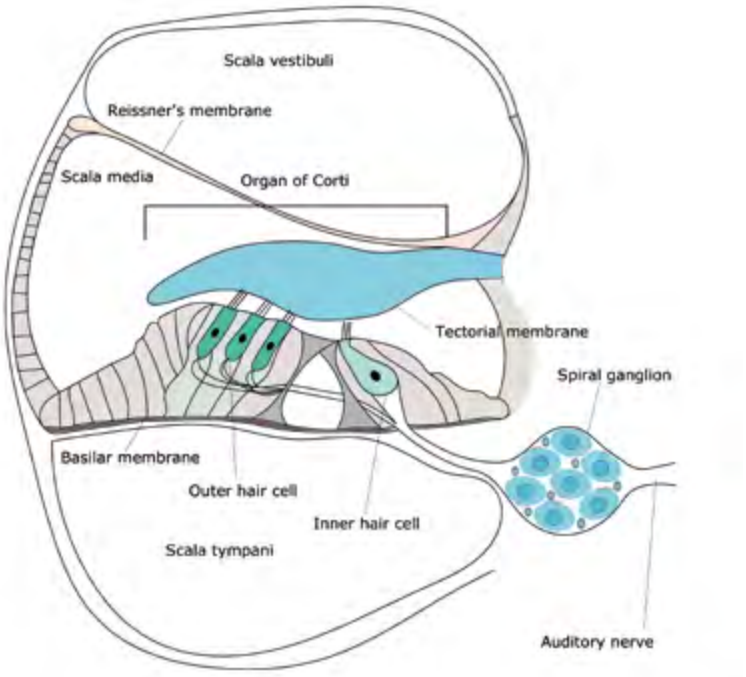

Figure 1.5 The organ of Corti resides within the scala media of the cochlea and contains the hair cells. The hair cells are the sensory receptors that can detect sound via the mechanical shearing of the stereocilia tip links of the hair cells relative to the tectorial membrane. This shearing occurs due to the movement of the basilar membrane in response to sound, which causes a displacement of the stereocilia relative to the tectorial membrane. Three rows of outer hair cells amplify the signal received by the cochlea, and one row of inner hair cells provide the transduction of the sound signal; via the spiral ganglion neurons and the cochlear nerve to the brain. Thus, the hair cells convert the mechanical motility of the peripheral auditory system into an electrical signal that the brain can process.

THE CENTRAL AUDITORY PATHWAY

The action potentials resulting from auditory stimulation are generated in the auditory periphery by the first-order auditory neurons, the spiral ganglion neurons. These action potentials are forwarded via the axons of the spiral ganglion neurons, that form the auditory nerve, to the brainstem. The auditory nerve fibers target the ventral and dorsal cochlear nuclei in the medulla at the brainstem level, as depicted in figure 1.6 A. From the cochlear nuclei, the signal

is forwarded to the superior olivary complex in the mid-pons. Part of the auditory pathway's fibers cross over to the contralateral side at this point, passing from the cochlear nucleus to the contralateral olivary nucleus. The superior olivary complex is essential for sound localization as it receives input from both ears. The medial superior olive is essential for detecting interaural time differences, whereas the lateral part encodes interaural intensity differences. The signal is then transmitted from the olivary complex to the inferior colliculus via the white matter tracts of the lateral lemniscus. Some of the fibers terminate in the nucleus of the lemniscus, whereas the majority of the fibers travel directly to the inferior colliculus. The inferior colliculus fibers synapse in the medial geniculate nucleus of the thalamus. From the thalamus, the signal is relayed to the auditory cortex in the temporal lobe via the acoustic radiation, as illustrated in figure 1.6 B. The auditory cortex connects to several cortical areas, and it projects back to the auditory pathway.

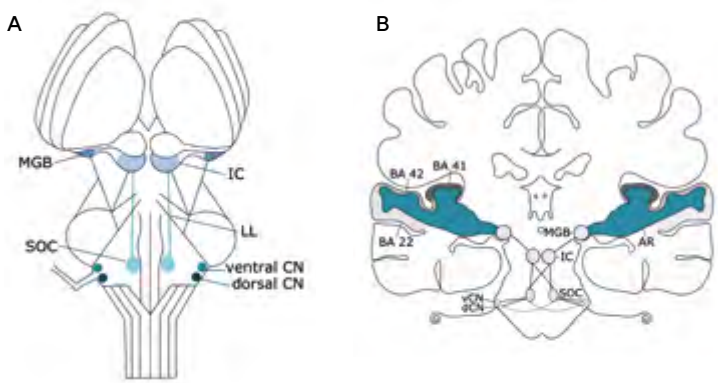

Figure 1.6 (A) Dorsal view of the brainstem and thalamus with a schematic representation of the central auditory pathway structures. The ascending auditory signal relayed via the auditory nerve enters the central auditory pathway at the cochlear nucleus (CN) level. The signal is then transferred to the superior olivary complex (SOC), and via the white matter of the lateral lemniscal tract (LL), arrives at the inferior colliculus (IC). From the IC, the signal is forwarded to the medial geniculate body in the thalamus (MGB), which is the last subcortical auditory area before the signal reaches the auditory cortex. (B) The auditory pathway is illustrated on a coronal slice of the brain. The acoustic radiation, depicted in color, is the white matter fiber bundle that connects the medial geniculate body of the thalamus to the auditory cortex. The auditory cortex consists of the primary auditory cortex (BA 41), the secondary auditory cortex (BA 42), and the associate auditory cortex (BA 22).

TONOTOPIC ORGANIZATION

A very striking characteristic of the auditory system is the extent to which it is organized in a frequency-wise manner. This organization of sound frequencies is called tonotopy, and it reflects the mechanical properties of the basilar

membrane in the cochlea, depicted in figure 1.7 A. The basilar membrane is narrower and stiffer near the base, where it resonates with higher frequencies. It gets progressively broader and more flexible near the apex, where it resonates with low frequencies. Thus, the tonotopic organization of the auditory system primarily reflects the mechanical properties of the basilar membrane. These properties make it selectively sensitive to different frequencies depending on the location along its length, as illustrated in figure 1.7 B. In this manner, the basilar membrane can decompose a complex sound into its component frequencies.

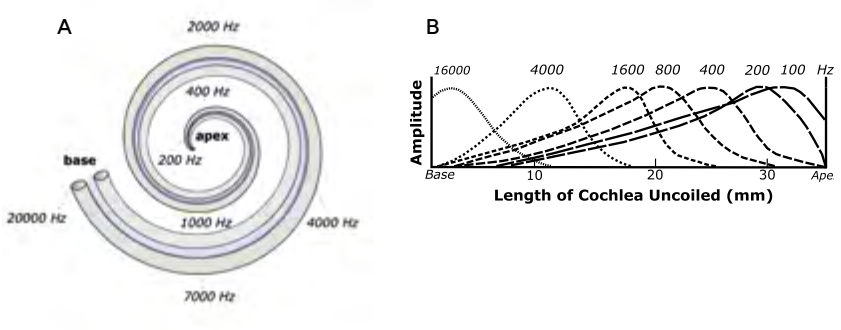

Figure 1.7. (A) The frequency distribution of the cochlea originates due to the differences in mechanical properties and sensitivity along the basilar membrane's length. The human basilar membrane is sensitive to frequencies from ~20 Hz at the apex to ~20.000 Hz at the base. (B) The basilar membrane responds to sound stimulation in a frequency-wise manner. For high frequencies, the peak amplitude of movement can be observed at the base of the basilar membrane. For progressively lower frequencies, this peak occurs closer to the apex. The curves reflect the positive deflections of the envelope of a traveling wave along the basilar membrane.

The tonotopic organization originates in the inner ear and is maintained along the auditory nerve, in the subcortical auditory structures, and the auditory cortex (Brugge and Merzenich, 1973; Rauschecker et al., 1995). The tonotopic map of the human auditory cortex is illustrated in figure 1.8. The human auditory cortex is tuned to high frequencies in the posterior area and anterolaterally to low frequencies.

In the auditory cortex, frequency specificity is maintained by the tuning of the auditory neurons. The receptive fields of neurons that are tuned to tonal or narrow-band sound are shaped according to the preferred frequency and the range of frequencies to which a neuron is responsive. Tonotopic organization of the auditory cortex is not solely shaped by the direct ascending flow of information from the lower level auditory areas; the majority of excitatory synapses in the auditory cortex originate from cortical areas (Lee and Winer, 2005). Additionally, inhibitory interneurons shape the frequency tuning of the auditory cortex via lateral inhibition or surround suppression of activation (Lakunina et al., 2020).

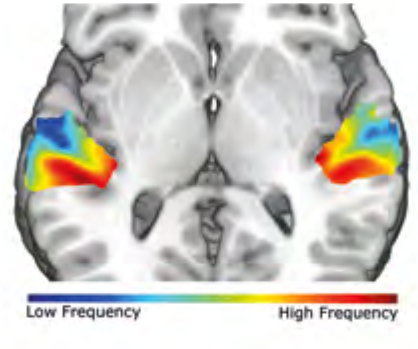

Fig 1.8 Tonotopic map of the human auditory cortex. High frequencies are depicted in red and processed more medial and posterior than the low frequencies depicted in blue. The frequency distribution, or tonotopy, originates in the cochlea and is maintained along the auditory pathway. The auditory (i.e., cochlear) nerve fibers are arranged tonotopically, as are the brainstem and midbrain auditory nuclei, the auditory thalamus, and the auditory cortex.

Modulation of the signals that are forwarded to the auditory cortex from the medial geniculate body (MGB) is accomplished via a feedback loop. The cortex consists of six layers, as depicted in figure 1.9. In the sensory cortices, layer IV receives thalamocortical projections, and layer VI provides feedback from the cortex to the thalamus. Layer VI of the auditory cortex projects to the thalamic reticular nucleus (TRN), a cluster of cells lining the thalamus, which can inhibit the activity of the MGB (Willis et al., 2015). These different processes all contribute to the tonotopic organization of the auditory cortex.

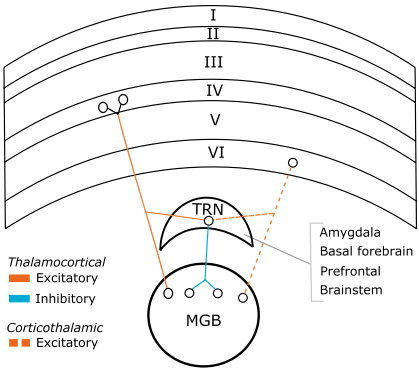

Figure 1.9. The medial geniculate body (MGB) of the thalamus sends projections to (layer IV) and receives feedback from the auditory cortex (layer VI). The thalamocortical and the corticothalamic connections project to the thalamic reticular nucleus (TRN), which sends inhibitory projections to the MGB. The TRN receives additional input from the amygdala, basal forebrain, prefrontal area, and brainstem structures (Caspary and Llano, 2017).

HEARING LOSS

The most prevalent type of hearing loss is acquired or age-related hearing loss, referred to as presbycusis (Yang et al., 2015). Presbycusis is sensorineural hearing loss, likely originating from damage to structures in the inner ear, and it affects high frequencies to a larger extent than low frequencies. The origin of presbycusis in humans is the accumulation of damage to the auditory system related to noise exposure and the degradation of tissues and cells that occur with the complex process of aging. In humans, the pure tone audiogram is the

commonly used metric to describe hearing loss in terms of detection thresholds for different frequencies. An increase in hearing thresholds indicates a decrease in the ability to detect sound, and often sound detection in noise deteriorates before sound detection in quiet is affected. The auditory thresholds are a good representation of the loss or compromise of hair cells. Corresponding to the largest increase in hearing thresholds at high frequencies, the largest decrease in the number of healthy inner and outer hair cells in age-related hearing loss is at the basal end of the basilar membrane (Wu et al., 2020). However, hair cell loss is not the only mechanism implicated in hearing impairment, a notion that was first put forward by Schuknecht (Schuknecht, 1955, 1964; Schuknecht and Gacek, 1993). In addition to hair cell loss, damage to inner hair cell synapses that connect to the cochlear nerve (Viana et al., 2015), atrophy of the stria vascularis (Wu et al., 2020), deterioration of auditory nerve fibers (Felix et al., 1990; Wu et al., 2019), and the loss of spiral ganglion neurons (Makary et al., 2011) are all thought to contribute to hearing loss.

However, auditory impairments are not restricted to the auditory periphery, i.e., the ear, and changes to the central auditory system, i.e., the brain, occur in hearing impairment (Akeroyd, 2008; Houtgast and Festen, 2008; Humes et al., 2013b; Füllgrabe et al., 2014). Peripheral hearing loss instigates neural plasticity of the central auditory system that results in changes in tonotopic maps, spontaneous activity, neural synchronicity, and sound-evoked activity (Robertson and Irvine, 1989; Eggermont and Roberts, 2004).

Contrary to the sound-evoked activity, which is driven by sound stimulation, spontaneous activity is the continuous neuronal activity that is not driven by a stimulus. In the presence of permanent hearing loss, a reduction in the spontaneous activity of auditory nerve fibers was reported in cats (Liberman and Kiang, 1978). Yet, increased spontaneous activity and neural synchrony were reported for hierarchically higher areas of the auditory system. These alterations in spontaneous activity were reported in several different animal models for the ventral cochlear nucleus (Vogler et al., 2011; Martel and Shore, 2020), dorsal cochlear nucleus (Kaltenbach et al., 1998; Finlayson and Kaltenbach, 2009; Pilati et al., 2012), inferior colliculus (Ma et al., 2006; Bauer et al., 2008; Coomber et al., 2014; Vogler et al., 2014), and the auditory cortex (Noreña and Eggermont, 2003; Seki and Eggermont, 2003; Basura et al., 2015). The increase of spontaneous activity observed in hierarchically higher auditory pathway areas after hearing loss induction has been termed central gain (Schaette and Kempter, 2006). Central gain has also been implicated in hyperacusis (Auerbach et al., 2014; Diehl and Schaette, 2015) and tinnitus (Noreña, 2011; Schaette and McAlpine, 2011), where the framework is extended to include sound-evoked activation. A decrease in peripheral input is hypothesized to trigger the upregulation of neuronal activity in central auditory regions to achieve homeostasis (Schaette and Kempter, 2006).

In addition to changes in spontaneous activity, sound-evoked activation of the auditory system is altered in hearing loss.

Neurons in the auditory pathway are distinctly tuned to different frequencies. In hearing loss, changes to the tonotopic maps of the auditory cortex have been described as the shifting of the receptive fields of deafferented high-frequency neurons towards intact receptive fields at lower frequencies (Rajan and Irvine, 1998; Eggermont and Komiya, 2000; Irvine et al., 2001; Muhlau et al., 2006). In contrast, not all animal models of hearing loss have described downward shifts in frequency selectivity. Instead, increased excitability (Kotak et al. 2005) and a decrease in inhibition (Rajan, 1998) of auditory cortical neurons were reported. Previous work collectively indicates that hearing loss is related to plastic changes that extend from the peripheral auditory system to the central auditory system. In humans with hearing loss, cortical and subcortical changes related to acquired hearing loss have not been thoroughly investigated. It may well be that there is a discrepancy between ototoxic or noise-induced hearing loss in animals and human acquired hearing loss that is due to the accumulation of noise exposure and aging processes. Therefore, it is necessary to investigate the effect of acquired hearing loss in a human population to contribute to our understanding of the impact of hearing loss on the brain, thereby possibly expanding treatment options beyond the prescription of hearing aids.

TINNITUS

Tinnitus is the perception of sound in the absence of an external stimulus. It is a common and debilitating symptom with reported prevalence rates of 12 – 30 % in the general population (McCormack et al., 2016). Severe tinnitus is related to a profound reduction in the quality of life (Erlandsson and Hallberg, 2000). Tinnitus is present in the majority of people with hearing loss, and the estimated prevalence of tinnitus increases with increasing age (Tan et al., 2013). In a small subset of tinnitus cases, called objective tinnitus, the etiology of the tinnitus sound can be traced to sound produced by head-related muscle or joint movements or arterial pressure. However, the majority of tinnitus cases are idiopathic, so-called subjective tinnitus, and are not accompanied by an identifiable source of the perceived sound. This thesis is specifically concerned with subjective tinnitus, and future references to tinnitus are intended to reflect information on subjective tinnitus only.

Currently, the specific pathophysiology that results in tinnitus is unclear. In contrast to hearing loss, research focussing on the integrity of peripheral structures in tinnitus is sparse. One animal model of tinnitus indicated a more extensive loss of inner hair cell ribbons in mice with hearing loss and behavioral

signs of tinnitus (Rüttiger et al., 2013). Plastic changes in the central auditory system that relate to tinnitus have been described more extensively. A relation between hearing loss-induced tonotopic reorganization and tinnitus has been hypothesized (Robertson and Irvine, 1989; Mühlnickel et al., 1998; Rauschecker, 1999; Eggermont and Roberts, 2004; Norena and Eggermont, 2005; Eggermont, 2006), although this relation has seldom been investigated directly. Previous experimental studies linked tonotopic map plasticity explicitly to hearing loss and not to tinnitus (Weisz et al., 2005; Wienbruch et al., 2006; McMahon et al., 2016). There is one study that utilized Magnetoencephalography (MEG) in humans which reported a positive correlation between the extent of cortical reorganization and the perceived strength of tinnitus (Mühlnickel et al., 1998).

Conversely, there was no tonotopic plasticity observed in a study investigating tinnitus in humans (Langers et al., 2012), nor in two animal studies (Kotak et al., 2005; Yang et al., 2011). Instead, reduced cortical inhibition and enhanced cortical excitation of the hearing loss affected area were reported in animals with hearing loss and additional behavioral signs of tinnitus. Generally, tonotopic map plasticity is not a well-established cortical trait observed in tinnitus. With the burden of tinnitus leaning heavily on its sufferers, more insight into the neural correlates of tinnitus in humans will aid the development of specific treatments and may pave the way for a cure.

HYPERACUSIS

Hyperacusis is an additional symptom that often co-occurs with hearing loss and is related to altered auditory perception. The perceived loudness of sounds is enhanced in hyperacusis, with soft to moderate intense sounds being perceived as uncomfortable or painfully loud (Anari et al., 1999; Baguley, 2003). Hyperacusis often co-occurs with hearing loss: a reported 59.1 % of people with hyperacusis have hearing loss (Paulin et al., 2016). Tinnitus and hyperacusis also tend to co-occur within individuals: an estimated prevalence of 55 - 86% of people with hyperacusis also report tinnitus (Anari et al., 1999; Dauman and Bouscau-Faure, 2005; Schecklmann et al., 2014). A steep increase of the perceived loudness with sound intensity, measured with a loudness growth function, is characteristic of hyperacusis. This steeper loudness growth results in a reduction in the sound intensity thresholds that produce uncomfortable loudness (i.e., loudness discomfort levels). With lower intensity sounds already producing an uncomfortably loud percept, this results in a compression of the loudness range.

In hearing loss, a reduction in dynamic input range results in a steeper increase in loudness for frequencies affected by hearing loss. This loudness recruitment is evident as a larger increase in loudness with intensity to progress from a

'just audible' sound to 'uncomfortably loud'. Since the intensity level at which someone with hearing loss can perceive a sound is elevated compared to someone who doesn't have hearing loss, but the highest tolerable sound intensity hasn't changed, this results in a steeper growth of loudness with intensity. In hyperacusis, this increase in loudness with intensity is often steeper than in hearing loss alone and can occur without the presence of hearing loss. Previous human and animal neuroimaging studies indicate that hyperacusis is associated with increased cortical and subcortical sound-evoked activity (Gu et al., 2010; Knipper et al., 2013; Rüttiger et al., 2013; Zeng, 2013; Chen et al., 2015). However, research investigating the neural correlates of hyperacusis is sparse.

To date, the neural correlates of hearing loss, tinnitus, and hyperacusis are still uncertain. Since these conditions often co-occur, the separation of their effects on cortical and subcortical structures has proven difficult. Moreover, in human neuroimaging studies investigating tinnitus and hyperacusis, patients with additional hearing loss are underrepresented even though tinnitus, hearing loss, and hyperacusis frequently co-occur.

STRUCTURE AND FUNCTION

A non-invasive method to investigate the brain's plastic changes in the presence of hearing loss and tinnitus is with magnetic resonance imaging (MRI). MRI-scanners use a strong magnetic field to obtain an image of biological tissue. In this thesis, that tissue is the brain. The neuronal cell bodies, their neuropil, and their support system of glial cells appear gray in color and are therefore called gray matter. The gray matter of the brain can be examined with an anatomical MRI-scan that is analyzed with voxel-based or surface-based morphometry. These analysis techniques can identify differences in the gray matter volume and differences in the gyrification and thickness of the cortex. The results presented in chapter 2 rely on this technique. An example of the image obtained with surface-based morphometry is shown in figure 1.10 A.

The projections that send information from the cell body of a neuron to the next neuron are called axons. These axons are coated in myelin, a white fatty lipid, and are therefore called white matter. These axons form the fiber bundles that relay information from one part of the brain to another. The white matter of the brain can be imaged with diffusion-weighted imaging (DWI). This technique investigates the restriction of the movement of hydrogen protons in the brain. Whereas there is no restriction of water diffusion within the ventricles, which are cerebrospinal fluid-filled cavities, there is considerable restriction along myelinated axons, which causes the water to diffuse in one primary direction. Diffusion-weighted analyses can inform on a decrease in axonal density or

atrophy in a specific pathway in the brain. The results reported in chapter 3 are based on this technique. An example of tracked white matter fibers of the acoustic radiation is depicted in figure 1.10 B.

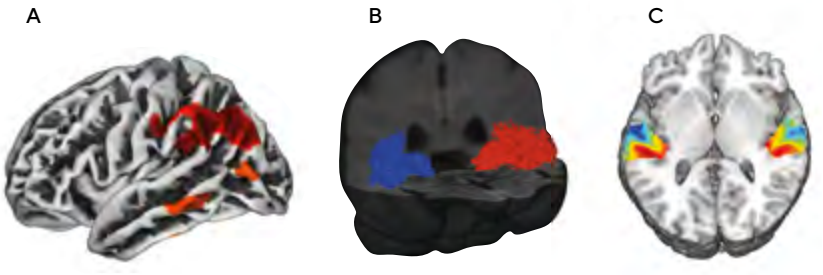

Figure 1.10 Examples of images constructed based on different MRI paradigms. (A) The surface of the left hemisphere. Differences in surface thickness between groups are depicted in color. (B) Tracking of white matter fiber tracts from the medial geniculate body to the auditory cortex. Pictured here are the left (red) and right (blue) acoustic radiation. (C) Functional analyses provide maps of activation of the brain. The responses to different frequencies are depicted in color.

The information relay in the brain happens through action potentials, which set off a cascade of events such as an increase in blood flow, oxygen, and glucose to the active brain area, presumably due to an increased demand for energy. Functional magnetic resonance imaging (fMRI) utilizes this increase in blood flow and volume and the change in the proportion of oxygenated and deoxygenated hemoglobin to measure the functional activity of the brain (Logothetis, 2003). An example of an fMRI activation map based on the blood oxygenation dependency level (BOLD) response is shown in figure 1.10 C. This response relies on the different magnetic properties of oxygenated hemoglobin, which is diamagnetic, and deoxygenated hemoglobin, which is paramagnetic (Pauling and Coryell, 1936). An increase in neural firing will increase the metabolic demand and incite a hemodynamic response. Therefore, fMRI does not create images of neuronal activity. Instead, it images the physiological activity related to neuronal activity (Huettel et al., 2008).

This hemodynamic response is triggered by the synaptic release of glutamate, which causes the calcium levels of near astrocytes to change. These astrocytes then release nitric oxide to dilate the blood vessel and increase blood flow (Ogawa and Sung, 2007). The vascular response takes 2 seconds to occur after the onset of neural events and peaks at around 5 seconds, which is mirrored in the timing of the hemodynamic response (see figure 1.11). The hemodynamic response causes an increase in the level of oxygen that is transported to the active area, and thus the ratio between oxygenated and deoxygenated hemoglobin in that area will change. With a high proportion of oxygenated blood, the T2 MRI signal decreases slowly. In this case, the speed of the signal decay, called the transverse

relaxation time, is decreased. Whereas, with a high level of deoxygenated blood, the signal decreases much faster (Huettel et al., 2008). The eventual image is based on the image intensities obtained with magnetic resonance imaging and the signal decay obtained with the BOLD response. The changes in BOLD signal are sampled per voxel, or volumetric pixel, and all these voxels together form a three-dimensional grid of the brain. For each voxel, the MR or BOLD signal is sampled at a particular frequency or repetition time (TR). The results in chapter 4 and 5 are based on this technique. With the aid of MRI, the changes in the structure and the function of the human brain relating to hearing loss, tinnitus, and hyperacusis can be identified with high spatial resolution.

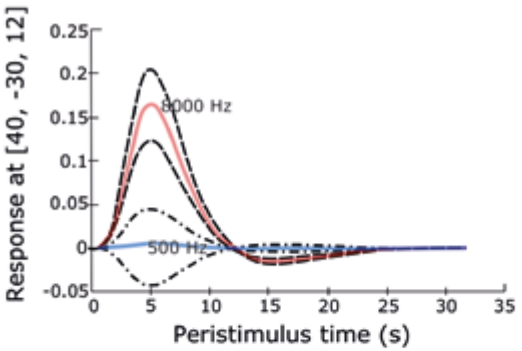

Figure 1.11 Haemodynamic response function of a voxel in the auditory cortex in response to two different frequencies. This specific voxel contains neurons that are, on average, more sensitive to a high-frequency sound (8 kHz, in red) compared to lower frequency sound (500 Hz, in blue). The average signal amplitude in percentage signal change (solid lines) and the corresponding estimated 95% confidence interval (dashed lines) are depicted.

OUTLINE OF THIS THESIS

Perception is generally thought to occur when a peripheral sensory site successfully transfers information to the affiliated cortical area. In auditory perception, a progressive reduction in the quality of this percept occurs with age or noise-exposure, i.e., acquired hearing loss, which relates to the degeneration of the auditory system. The presence of hearing loss increases the chances of developing tinnitus and hyperacusis. Strikingly, a characteristic of these auditory symptoms is not the absence or degradation of a signal, as is the case in hearing loss, but the additional presence of a phantom sound or the enhancement of the loudness of external sounds.

Even though the origin of acquired hearing loss is peripheral, it affects the central auditory system and brain areas that modulate modalities beyond hearing. Similarly, the antecedent of tinnitus and hyperacusis may be peripheral, but the consolidation and continuation of these symptoms are likely grounded in the brain. In humans, the brain correlates are yet to be identified that set apart those that have hearing loss and consequently develop tinnitus and hyperacusis from those that do not. To understand these symptoms, we have to investigate the reorganization of structural and functional brain areas involved in hearing. The challenge is to identify the changes that demarcate one symptom from another and ultimately extract the neural correlates of hearing loss, tinnitus, and hyperacusis.

To investigate the specific effect of hearing loss and the additional impact of tinnitus in this thesis, we ensured that the hearing thresholds of the two hearing-impaired groups were well matched. To disentangle the central characteristics of hearing loss, tinnitus, and hyperacusis, we included two groups with hearing loss, one with and one without tinnitus, and a control group. The hearing loss with tinnitus group was further divided in those with and without scores indicative of hyperacusis to separate brain activity related to tinnitus and hyperacusis. Taken together, this thesis aims to contribute to our knowledge of structural and functional changes of the central auditory system in hearing loss and related symptoms such as tinnitus and hyperacusis.

In chapter 2, we investigate the impact of hearing loss, tinnitus, and age on the gray matter of the brain. Volume-based and surface-based methods are employed to examine their impact on the brain. Of specific interest is the effect on the auditory cortex since this is the location where the transition from sensation to perception is thought to occur. Therefore, the auditory cortex may be a crucial translation point involved in the formation of a phantom sound percept.

In chapter 3, we examine the impact of hearing loss and tinnitus on the largest white matter fiber bundle of the auditory system: the acoustic radiation. We investigate if axonal degeneration or atrophy of the acoustic radiation fibers is a characteristic of hearing loss or tinnitus. We employ a novel technique called fixel-based analysis that has not previously been used to investigate the white matter of the auditory pathway. This method allows for a biological interpretation of the results regarding axonal loss or degeneration of the nerve fiber bundle. An additional important feat of this method is that it can adequately resolve crossing fibers within a voxel and thus reliably track a single fiber tract throughout the brain.

In chapter 4, we study the frequency responses of the auditory cortex in hearing loss and tinnitus. The frequency maps, or tonotopic maps, are derived with a principal component analysis. Furthermore, the frequency responsiveness is determined by the amplitude of responses to different frequencies. In this

chapter, we identify differences in the responsiveness and the tonotopic maps of people who have hearing loss with and without tinnitus.

In chapter 5, we explore the subcortical and cortical activation that is characteristic of hyperacusis. In a subset of hearing loss participants with tinnitus, we contrast the sound-evoked activation of those with and without hyperacusis. We describe the amplitude and extent of the response to sound stimulation of the brainstem and midbrain auditory nuclei, the auditory thalamus, and the auditory cortex. Additionally, we discuss the frequency specificity of these effects.

Chapter 6 provides a general discussion of the work presented in this thesis and places the different chapters within a general framework. Finally, implications for future research, based on the work presented in this thesis, are discussed.

CHAPTER 02

GRAY MATTER DECLINES WITH AGE AND HEARING LOSS, BUT IS PARTIALLY MAINTAINED IN TINNITUS

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ABSTRACT

The impact of age-related hearing loss extends beyond the auditory pathway and impacts brain areas related to cognitive impairment and even dementia. The presence of tinnitus, a sensation of sound that frequently co-occurs with hearing loss, is additionally linked to cognitive decline. Interestingly, structural neuroimaging studies have reported that hearing loss may precede or modulate the onset of cognitive impairment. In this study, we aimed to disentangle the effects of age, hearing loss, and tinnitus on gray matter structure. In total, 39 participants with hearing loss and tinnitus, 21 with hearing loss but without tinnitus, and 39 controls were included in this voxel- and surface-based morphometry MRI study. Whole brain volume and surface thickness measures were compared between the groups. Age-related gray matter volume decline was observed in all groups. Several brain areas showed smaller gray matter volume and cortical surface thickness in hearing loss without tinnitus, relative to controls. This reduction was observed both within and outside of the auditory pathway. Interestingly, these reductions were not observed in participants with tinnitus who had similar hearing loss and were of similar age. Since we have tools to improve hearing loss, hearing screening may aid in the battle against cognitive decline.

INTRODUCTION

Hearing loss is the most prevalent acquired sensory impairment, affecting a large proportion of the ageing population worldwide (Mathers et al., 2000). Hearing loss not only has an impact on the quality of hearing, but it is also associated with an increased likelihood of cognitive impairments and even dementia. Presently, with a globally ageing population, the prevalence of hearing loss is rising (OMS, 2018), together with interest in the impact of age-related hearing loss on the brain. In light of this, it has become clear that sensory impairments are not restricted to the peripheral sensory organs, i.e. the ear, and changes to the brain are involved too (Akeroyd, 2008; Houtgast and Festen, 2008; Humes et al., 2013b; Füllgrabe et al., 2014). Correspondingly, hearing loss-related brain changes appear to affect other faculties than hearing. Several studies have reported a relation between age-related hearing loss, or presbycusis, and poorer cognitive functioning and even dementia (Uhlmann et al., 1989; Lin and Albert, 2014; Panza et al., 2015, 2018; Heywood et al., 2017; Livingston et al., 2017; Uchida et al., 2019). For instance, longitudinal data showed that hearing impairment is associated with a larger likelihood of developing cognitive impairment (Heywood et al., 2017). Furthermore, a recent study identified hearing loss as an independent modifiable risk factor for cognitive decline (Uchida et al., 2019). For a comprehensive overview, see Jafari et al., 2019 (Jafari et al., 2019). It thus appears that hearing loss and cognitive decline do not only appear in the same population, but hearing loss may precede or modulate the onset of cognitive impairment. Therefore, the effect of hearing loss on the brain is a potential window to understand cortical changes related to ageing.

Tinnitus is a symptom that often co-occurs with hearing loss and is characterized by the perception of sound in the absence of an external acoustic stimulus. Since there is a strong link between hearing loss and neural plasticity and a strong association between hearing loss and tinnitus, it has been hypothesized that tinnitus is linked to neuroplasticity as well (Robertson and Irvine, 1989; Mühlnickel et al., 1998; Rauschecker, 1999; Eggermont and Roberts, 2004; Norena and Eggermont, 2005; Eggermont, 2006). Furthermore, similar to hearing loss, tinnitus has been linked to an increased risk of cognitive deficits (Wang et al., 2018). Several studies indicate that the presence of tinnitus has a negative impact on memory, executive functioning, and quality of life (Tegg-Quinn et al., 2016). Interestingly, one study noted that the extent of the cognitive deficits is linked to the perceived severity of the tinnitus (Wang et al., 2018). Thus, in addition to the strong link between tinnitus and hearing loss, it has been reported that tinnitus by itself has a negative impact on cognitive functioning. Therefore, the factor of tinnitus has to be considered when investigating cortical changes in a population with hearing loss.

To date, neuroimaging studies identified several changes in gray-matter brain volume related to presbycusis (i.e. age-related hearing loss), either with Voxel-Based-Morphometry (VBM), Surface-Based-Morphometry (SBM), or other types of automated morphometry. The reported affected areas extend beyond the auditory pathway and correspond to areas related to cognitive impairment and dementia. In both unilateral and bilateral hearing loss, the decrease in brain volume in the auditory cortex, anterior cingulate, and prefrontal cortex correlated with the loss in hearing sensitivity (Wong et al., 2010; Husain et al., 2011; Peelle et al., 2011; Eckert et al., 2012; Wang et al., 2016). Furthermore, VBM studies that investigated cognitive decline and presbycusis reported accelerated cortical volume decline in the presence of presbycusis (Peelle et al., 2011; Eckert et al., 2012; Lin et al., 2014; Profant et al., 2014). In line with this, the results of SBM studies indicated a relationship between cognitive impairment and presbycusis. These surface-based studies identified gray matter atrophy in the primary auditory cortex, the cingulate cortex, the precuneus, the insula, and the parietal cortex in the presence of presbycusis (Profant et al., 2014; Ren et al., 2018). Surprisingly, one study reported an association between hearing loss and an increase in the gray matter of the right angular gyrus (Alfandari et al., 2018). Nevertheless, pervasive decreases in gray matter volume and surface thickness, which are generally associated with cognitive decline, may additionally be linked to age-related hearing loss.

Furthermore, recent neuroimaging studies have tried to identify the central correlate of tinnitus. These studies identified changes in gray matter volume in auditory, prefrontal, and limbic areas that were associated with the presence of tinnitus or its characteristics. More specifically, reductions in gray matter were reported for the prefrontal cortex (Leaver et al., 2011, 2012; Aldhafeeri et al., 2012; Schecklmann et al., 2012a; Boyen et al., 2013; Allan et al., 2016), the subcallosal area (Muhlau et al., 2006; Leaver et al., 2011), precuneus (Allan et al., 2016), supramarginal gyrus (Leaver et al., 2012), orbitofrontal cortices (Mahoney et al., 2011), cingulate (Schecklmann et al., 2012a), insular cortex (Schecklmann et al., 2012a), and the pre- and postcentral gyrus (Schecklmann et al., 2012a). However, the manifestation of this effect in the temporal and thalamic areas is variable in these previously published studies. For the temporal area, it has been reported that an increase in auditory cortex gray matter related to the presence of tinnitus (Mahoney et al., 2011; Boyen et al., 2013), whereas tinnitus distress related to a decrease in auditory cortex gray matter (Schecklmann et al., 2012a). Similarly, for the thalamus, both increases and decreases in volume were reported (Muhlau et al., 2006; Schecklmann et al., 2012a). Overall, the reported changes in cortical structure that are associated with tinnitus vary between studies and are in some instances contradictory. The heterogeneity of the tinnitus population, the confound of hearing loss, differences in age as well as non-corresponding techniques, and lenient statistical thresholds have been named as contributing factors to this discrepancy (Vanneste et al., 2015; Allan et al., 2016).

Since emerging evidence indicates that acquired hearing loss and tinnitus are associated with age-related cognitive impairment, identifying differences in brain structure specific to each of these conditions may help us develop appropriate treatments that not only aid hearing or improve tinnitus but may additionally prevent cognitive decline. A current challenge is to determine if the structural characteristics that might contribute to cognitive impairment should be attributed to hearing loss, tinnitus, or related factors such as age. Hence, the aim of this study was to disentangle the effects of age, acquired hearing loss, and tinnitus on the gray matter of the brain.

METHODS

PARTICIPANTS

One hundred and thirteen participants were included in a larger MRI study at the University Medical Center Groningen, the Netherlands. Three people did not complete all aspects of the research, one data file was corrupted in the transfer process, and in an additional nine scans either the contrast between white and grey matter was insufficient for the segmentation algorithm to work reliably, or movement during anatomical scanning rendered the data with artefacts. This resulted in complete and sufficient data for a total of 99 participants, of which 39 had tinnitus and hearing loss, 21 participants with hearing loss but without tinnitus, and 39 healthy controls with no tinnitus and no or minimal hearing loss. The study was approved by the Medical Ethical Committee of the University of Groningen, performed in accordance with all relevant regulations, and informed consent was obtained from all participants.

AUDITORY THRESHOLDS

The hearing levels of all participants were assessed with pure tone audiometry. Thresholds were determined in a sound-attenuating booth for octave frequencies ranging from 0.25 to 8 kHz and additionally at 3 and 6 kHz. The average hearing loss was quantified as the mean of the hearing thresholds at 2, 4, and 8 kHz of both ears (high-frequency pure-tone average; HF-PTA). A matching procedure was used to estimate tinnitus pitch and loudness. The majority of the participants perceived high-frequency tinnitus (n = 35, >2 kHz), and the remaining four participants reported the perception of broadband noise. None of the participants compensated for their hearing loss with hearing aids or ameliorated their tinnitus with maskers. Participants were required to have hearing thresholds better than 40 dB SPL at 1 kHz to meet the inclusion criteria for a functional task-based scan that was obtained in the same sequence as the anatomical images. These criteria resulted in the inclusion of participants with high-frequency hearing loss only.

MRI DATA ACQUISITION

MRI scanning was performed at the NeuroImaging Center in Groningen on a 3.0 T Philips Intera MRI scanner (Best, the Netherlands). A SENSE 32-channel head coil was used to obtain a whole-brain T1 weighted anatomical image with a voxel size of 1 x 1 x 1 mm (TR 10.4 ms, TE 5.7 ms, Acquisition matrix 256 x 200).

DATA ANALYSIS

All T1-images were aligned according to the individual anatomical anterior-posterior commissures in Vistasoft-Master, software run in Matlab2018a. Further analyses were done in CAT12, a structural imaging-oriented SPM toolbox (Dahnke et al., 2013), and included segmentation, normalization, and smoothing. Total Intracranial Volume (TIV) was estimated for all participants to correct for differences in head and brain size. After these steps were performed, a sample quality check was run on the segmented images to determine if the image quality was sufficiently high; all included images scored above 86%. This score is based on a measure of homogeneity of the sample. It is used to determine image quality after pre-processing (i.e. mean correlation measure), and it was run with TIV as a nuisance variable. Data with a low mean correlation (i.e. low image quality) or large differences to the mean score were manually checked for sufficient image quality. If this confirmed the low image quality, images were excluded from further analysis.

The included images were smoothed with an 8-mm Gaussian kernel. The subsequent analyses were performed on the modulated gray matter volume images (VBM) and the surface-based thickness measures (SBM). Modulated gray matter images were scaled by the amount of contraction induced by non-linear spatial normalization. The value of each voxel was multiplied with the Jacobian determinant obtained with spatial normalization. After the pre-processing and first level analysis, second level whole group and group wise analyses were performed (Mechelli et al., 2005).

A multiple regression model was run on the modulated gray matter images with sex, age, the presence of tinnitus, and average hearing loss as covariates to check the association between these covariates and the gray matter volume data. Then, two-sample t-tests were performed on both the gray matter volume and surface-based thickness measures to test group differences in a pairwise manner. Threshold Free Cluster Enhancement (TFCE) was used to assess the cluster-wise differences in gray matter volume between the groups (Smith and Nichols, 2008). This non-linear method can detect both diffuse low-amplitude signals and sharp focal signals. To test for statistical significance, TFCE computes p-values for each voxel via permutation testing.

RESULTS

The data of 99 participants are presented, of which 39 had tinnitus and hearing loss (THL group), 21 participants had hearing loss but no tinnitus (HL group), and 39 controls with neither tinnitus and no or minimal hearing loss (CO group). Table 1 contains the demographics of the three participant groups.

Table 1. Demographics of participants. The hearing loss groups, with and without tinnitus, did not differ with respect to age (p 0.179). The control group was significantly younger than both hearing loss groups, with and without tinnitus (p < 0.0001).

Group	Mean Age (years)	Standard Deviation	N
Control	45.7	13.5	39
Hearing Loss	62.6	8.8	21
Tinnitus + Hearing Loss	59.2	9.3	39

The mean age of both hearing loss groups differed significantly from the control group, whereas the hearing loss groups did not differ from each other. The hearing loss groups, with and without tinnitus, do not differ significantly on any of the frequencies (corrected for multiple comparisons; see description Fig 1). However, the hearing thresholds of both hearing loss groups differed significantly from those of the control group on all frequencies.

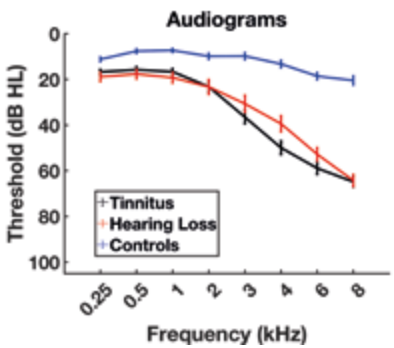


Figure 1. Average hearing thresholds per group with their corresponding standard errors. Pure tone audiometry was used to test octave frequencies from 0.25 to 8 kHz, and 3 and 6 kHz. The two groups with hearing loss, with and without tinnitus, were not significantly different in terms of age (p = .419, t 1.359) and hearing loss (p = .209 (0.25 kHz); p = .3 (0.5 kHz); p = .416 1 (kHz); p = .683 (2 kHz); p = 0.812 (3 kHz); 4 kHz p = .976 (4 kHz); p = .783 (6 kHz); p = .974 (8 kHz)).

Age and average hearing loss (HF-PTA) showed a significant positive correlation across all participants (r = 0.6125, p < 0.0001; Fig 2). Analysis run on group level showed that only in the control group there was a significant correlation between average hearing loss and age (THL: r = 0.20, p=0.22; HL: r = 0.10, p=0.66; CO: r = 0.76, p < 0.0001).

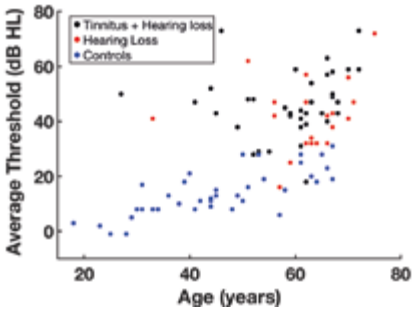


Figure 2. Average hearing threshold of all participants plotted against age. The control group was significantly younger than both the hearing loss group with ($p < 0.0001$, t 5.1) and without tinnitus ($p < 0.0001$, t 5.9). The average threshold was computed for the frequencies of 2, 4, and 8 kHz.

VOXEL-BASED MORPHOMETRY

A voxel-based multiple regression model was used to explain differences in gray matter volume in terms of age, sex, the presence of tinnitus, and average hearing loss (HF-PTA). In addition, Total Intracranial Volume (TIV) was included as a covariate. This analysis demonstrated that age was a significant predictor for gray matter volume in our study sample (family-wise error (FWE) < 0.05, cluster size k > 20; see Table 2). Specifically, age was inversely related to gray matter volume in the cingulate and auditory cortical areas (see Fig 3).

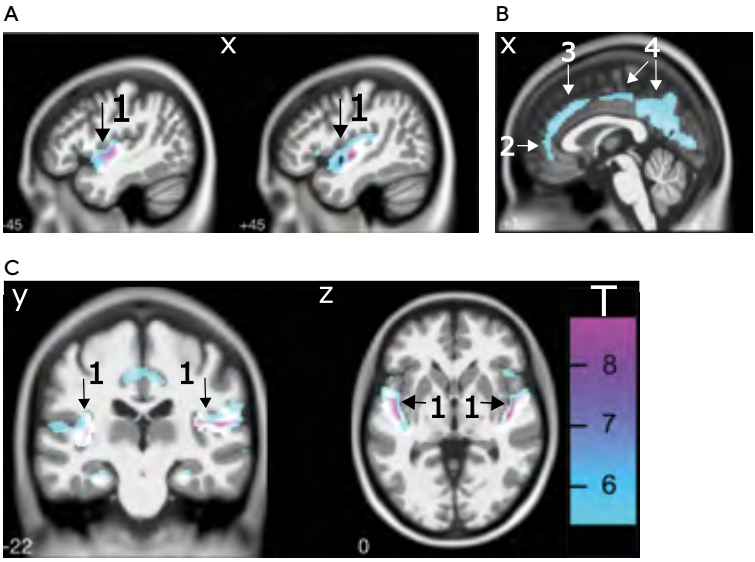


Figure 3. Cortical gray matter volume differences related to age. A multiple regression model with the covariates age, sex, tinnitus, and hearing thresholds at 2, 4, and 8 kHz indicated that increasing age was associated with less gray matter volume in (A) temporal areas (including the bilateral auditory cortices (1)) and (B) anterior (2), middle (3) and posterior (4) cingulate areas, at FWE < 0.05. Scale bar indicates T values.

Table 2 Local maxima coordinates for the whole brain multiple regression analysis. TIV and age were included as covariates, thresholded at cluster-level at FWE < 0.05. Areas identified show a negative correlation with age.

Area	cluster		MNI Coordinates (mm)		
	P (FWE-cor)	*k*	*X*	*Y*	*Z*
Superior Temporal Gyrus (R)	<0.0005	4316	45	-10	-3
Insula			48	6	-8
**Cluster extends to include BA 22 & 41*			56	-2	0
Superior Temporal Gyrus (L)	<0.0005	3125	-45	-6	-10
Insula			-46	-10	2
**Cluster extends to include the insula, BA 22 & 41*			-33	-21	12
Putamen (L)	<0.0005	382	-26	9	-12
**Cluster extends to include the subcallosal gyrus and amygdala*			-26	18	0
Fusiform Gyrus (R)	<0.0005	228	24	-40	-16
**Cluster extends to include the parahippocampal gyrus fusiform gyrus and lingual gyrus*					
Fusiform Gyrus (L)	<0.0005	428	-22	-44	-14
Parahippocampal Gyrus			-26	-28	-18
Lingual Gyrus			-18	-46	-6
Cingulate Gyrus (L)	<0.0005	1264	-14	-44	42
			9	-45	33
			-10	-51	38
Middle Temporal Gyrus (R)	<0.0005	123	58	-57	-3
Hippocampus (R)	<0.0005	144	22	-32	-4
Calcarine sulcus V1 (R)	<0.0005	158	2	-80	6
**Cluster extends to include the lingual gyrus*					
Mamillary Bodies	<0.0005	53	0	-9	-10
Middle Cingulate Gyrus (R)	<0.0005	402	3	27	33
Anterior Cingulate			2	39	21
			4	16	40
Posterior Cingulate Gyrus (R)	<0.0005	222	3	-63	18
Supramarginal Gyrus (R)	0.001	34	40	-46	56
Angular Gyrus (L)	<0.0005	116	-46	-68	32
Olfactory area (R)	<0.0005	104	24	12	-14
Putamen (R)	0.001	31	28	18	2
Middle Temporal Gyrus (L)	0.001	38	-63	-46	8
Inferior frontal gyrus (R)	<0.0005	108	44	20	0
BA 45 (R)			54	18	3
**Cluster extends to include the insula and orbitofrontal cortex*					
Superior Temporal Gyrus (L)	0.001	44	-56	-60	21
BA 7 (R)	<0.0005	67	3	-64	45
			6	-63	36

None of the other predictors was significantly associated with gray matter volume. However, non-significant associations were identified ($p < 0.001$ uncorrected, $k > 20$); The presence of tinnitus was associated with greater gray matter volume in the bilateral lingual gyrus, bilateral crus of the cerebellum, left secondary visual cortex, and the left retrosubicular area. High-frequency hearing loss was associated with less gray matter volume in the right cortical auditory areas and the left cerebellar area. Even though these findings were non-significant, they are mentioned here since other studies identified similar areas as related to hearing loss and tinnitus (see Discussion).

PAIRWISE COMPARISONS BETWEEN GROUPS

Pairwise comparisons on volumetric data of the three participant groups were investigated with Threshold Free Cluster Enhanced (TFCE) two-sample t-tests that were performed on the modulated gray-matter volume. The pairwise group comparisons tested were: tinnitus and hearing loss versus hearing loss (THL vs. HL), hearing loss versus controls (HL vs. CO), and tinnitus and hearing loss versus controls (THL vs. CO). In addition, the inverse of all these comparisons was tested, resulting in a total of six pairwise group comparisons. All of the pairwise comparisons were controlled for age and TIV.

The first analysis revealed a difference in gray matter volume between the two hearing loss groups, with and without tinnitus (THL vs. HL). This group comparison showed significantly higher gray matter volume in the bilateral Lingual Gyrus (BA 37, FWE < 0.05, k = 363(L)/277(R)) for the tinnitus group; see Figure 4 A and Table 3. In this comparison, there were no areas that showed significantly smaller gray matter volume.

Second, in the hearing loss group without tinnitus, there were no areas in which the volume was larger than in the control group (HL vs. CO). However, gray matter volume was smaller in the bilateral middle temporal gyri, left inferior temporal gyrus, right orbitofrontal area, right cingulate cortex, left fusiform gyrus, and right temporopolar area; see Figure 4 B and Table 4.

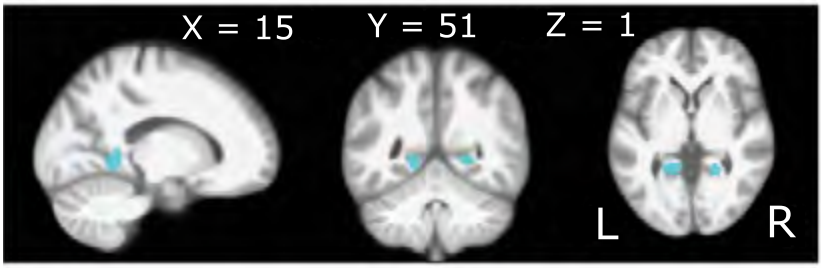
Finally, in the comparison of hearing loss with tinnitus (THL) versus controls (CO), none of the differences reached significance. See Tables 2,3, and 4 for the coordinates and cluster sizes of the reported VBM differences.

Table 3. The coordinates for the local maxima obtained via a two-sample t-test, comparing the hearing loss group without tinnitus to the control group. The areas identified show less gray matter volume in the hearing loss group without tinnitus compared to the controls.

Area	Cluster	Peak-cluster	MNI Coordinates (mm)		
	P (FWE-cor)	*k*	*X*	*Y*	*Z*
Lingual Gyrus (L)	0.019	363	-20	-46	-3
Lingual Gyrus (R)	0.031	277	24	-46	-2
	0.039		18	-44	12

**Cluster extends to include the precuneus*

A



B

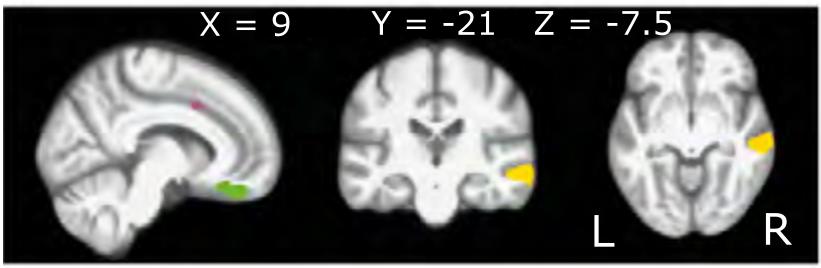


Figure 4. Gray matter volume differences associated with hearing loss and with tinnitus. Threshold free cluster enhancement (TFCE) was employed to investigate group differences in modulated gray matter images. (A) Comparison between the hearing loss groups, with and without tinnitus. In the group with tinnitus, larger gray matter volume of the bilateral lingual gyri was observed. These areas are depicted in blue on a medial view of the sagittal plane of the right hemisphere and a coronal and axial cross-section of the entire brain (B) Comparison between the hearing loss group without tinnitus and the control group. In the hearing loss group without tinnitus, less gray matter volume was observed in the cingulate cortex (pink), the orbitofrontal cortex (green), and the middle temporal gyri (yellow), depicted on a medial view of the sagittal plane of the right hemisphere. Additional clusters with less gray matter volume, not shown here, were observed in the superior and inferior temporal gyri and the fusiform gyrus (see Table 2C). The comparison between the hearing loss group with tinnitus and the controls revealed no significant differences and is therefore not shown in this figure.

Table 4. The coordinates for the local maxima of gray matter volume differences between the hearing loss groups with and without tinnitus. This area shows more gray matter volume in the hearing loss group with tinnitus than in the hearing loss group without tinnitus. The comparison between the tinnitus group and the controls did not reveal a significant group difference and is therefore not shown in this table.

Area	Cluster	Peak-cluster	MNI Coordinates (mm)		
	P (FWE-cor)	*k*	*X*	*Y*	*Z*
Middle Temporal Gyrus (L)	0.016	340	-62	-33	-16
**Cluster extends to include the Inferior Temporal Gyrus*					
Middle Temporal Gyrus (R)	0.019	1144	63	-26	-4
	0.019		57	-26	-10
**Cluster extends to include the insula*	0.020		66	-15	-12
Sub-Gyral Area (R)	0.022	436	10	39	-21
	0.025		9	30	-16
**Cluster extends to include the orbitofrontal cortex and amygdala*					
Middle Cingulate Gyrus (R)	0.044	44	10	12	39
Fusiform Gyrus (L)	0.045	59	-40	-48	-22
	0.049		-34	-40	-18
Superior Temporal Gyrus (R)	0.046	70	52	2	-3
**Cluster extends to include the temporal pole*					
Fusiform Gyrus (L)	0.048	24	-52	-46	-21

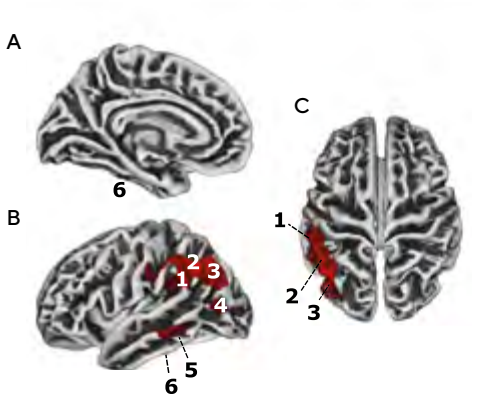


Figure 5. Reduction in cortical surface thickness in the hearing loss group without tinnitus compared to the control group. The three panels show a medial (A) and lateral (B) view of the left hemisphere and a top view (C) of the entire brain. Areas that contained less cortical surface thickness in the hearing loss group without tinnitus compared to the control group (FWE < 0.05, TFCE) are shown in red. These areas include the supramarginal gyrus (1), the angular gyrus (2), the inferior parietal lobule (3), lateral occipital area (4), and the middle temporal (5) and inferior temporal areas (6), all on the left side of the brain.

SURFACE-BASED MORPHOMETRY

In addition to the volumetric measures of the VBM method, surface-based morphometry (SBM) was performed to measure cortical thickness and gyrification (Dahnke et al., 2013). The cortical thickness was significantly reduced in the hearing loss group without tinnitus compared to the control group. This reduction was observed in the left supramarginal gyrus and angular gyrus, extending posteriorly to the inferior parietal gyrus and the left lateral occipital, middle temporal, and inferior temporal areas (TFCE, FWE < 0.05; see Fig 5). No significant differences in cortical thickness were observed between both hearing-impaired groups, with and without tinnitus, and between the hearing loss group with tinnitus and the control group. The gyrification analyses did not show any significant differences between the groups.

POST-HOC ANALYSIS

To assess if the auditory cortex is distinctively affected by age compared to other primary sensory or motor-related areas, post-hoc region-of-interest (ROI) analyses were performed on both the gray matter volume and the surface-based thickness data, inspired by Profant et al. (Profant et al., 2014). Three ROI areas were included: the visual cortex (V1), the motor cortex (M1), and the auditory cortex (A1). ROI determination was performed using the HCP_MMP1 atlas for the thickness parcellations and the atlas provided by Neuromorphometric Inc. for the volume parcellations (Glasser et al., 2016).

The group differences in surface thickness and volume of gray matter that were identified in the three ROIs are depicted in Figure 6. Left dominant laterality was observed for the auditory cortex, both for the volumetric and surface-based methods. No group-related differences in laterality were observed. Therefore, subsequent analyses were performed on the average of left and right ROIs. First, a multivariate analysis of covariance showed a significant decline in gray matter volume and surface thickness with age in all three ROIs; see Table 3. Second, the effect of group on gray matter volume was tested for the three ROIs included, adjusted for multiple comparisons in the strictest sense via a Bonferroni correction, and corrected for age. This analysis showed no significant effect of group on cortical volume or thickness for V1 and M1 after correcting for age (Table 5, Group). Moreover, A1 gray matter volume was not significantly different between the groups. Nonetheless, both the median cortical thickness and the median gray matter volume of A1 were smallest in the hearing-impaired group without tinnitus and largest in the controls. However, only the thickness measures were significantly different between the groups (see Table 5 and Fig. 6A, right panel). To conclude, the effect of age on all of the included cortical areas is strong, and the only group-related difference was observed in the thickness of the auditory cortex.

Table 5. The effect of age and group on gray matter volume and thickness in the visual (V1), motor (M1), and auditory cortices (A1). Significant results of multivariate analysis of variance are shown in bold. Adjustment for multiple comparisons: Bonferroni. Top: the effect of age on gray matter volume and surface thickness. Bottom: the effect of group on the gray matter volume and surface thickness, with age included as a covariate.

	Volume		Thickness	
Age	p-value	F	p-value	F
V1	**0.001**	**12.633**	**0.001**	**12.433**
M1	**0.031**	**4.767**	**<0.0001**	**19.279**
A1	**<0.0001**	**32.247**	**<0.0001**	**17.095**
Group				
V1	0.602	0.511	0.775	0.256
M1	0.767	0.767	0.658	0.420
A1	0.112	2.241	**0.048**	**3.137**

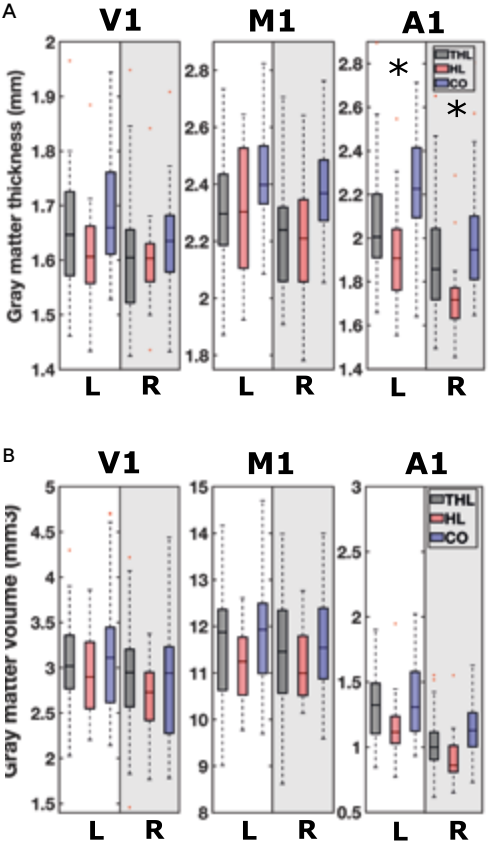


Figure 6. Cortical surface thickness (A) and gray matter volume (B) in primary visual cortex (V1), primary motor cortex (M1), and primary auditory cortex (A1) of the three participant groups: THL (hearing loss with tinnitus); HL (hearing loss without tinnitus); CO (controls without tinnitus or hearing loss). The boxplots show the cortical surface thickness and the gray matter volume for both the left (L) and right (R) hemispheres. Significance, corrected for multiple comparisons at a stringent Bonferroni level, is indicated with an asterisk.

DISCUSSION

The aim of this study was to disentangle the effect of age, hearing loss, and tinnitus on the structure of cortical gray matter. Three groups were included: (1) hearing loss and tinnitus, (2) hearing loss without tinnitus, and (3) controls without hearing loss or tinnitus. The first result is that age was significantly related to gray matter volume decline in all three groups. Second, in the hearing loss group with tinnitus, significantly higher gray matter volume was observed in the lingual gyri compared to the hearing loss group without tinnitus. Third, in hearing loss without tinnitus, significant gray matter reductions relative to controls were observed in the temporal gyri, orbitofrontal area, cingulate cortex, fusiform gyrus, and the temporopolar area. Additionally, the thickness of the cortical surface was significantly less in the supramarginal gyrus, inferior parietal area, lateral occipital area, middle temporal, and inferior temporal areas in hearing loss without tinnitus, compared to controls. These reductions were not observed in hearing loss participants with tinnitus, who were of similar age and had similar hearing thresholds.

AGE-RELATED DIFFERENCES

The multivariate analysis of the association between age, hearing loss, and tinnitus showed that age is significantly related to gray matter volume reductions. This effect was observed in the auditory cortices, the cingulate areas, and the orbitofrontal area. These specific brain areas have been described in other studies on aging and brain volume (Good et al., 2001; Resnick et al., 2003; Hutton et al., 2009; Profant et al., 2014). Our post-hoc analysis on the gray matter volume of the visual, motor, and auditory cortices showed a larger effect of age on the auditory than the visual and sensorimotor cortex. Note that a number of the participants in our study were hearing impaired, either with or without tinnitus. Overall, age appears to have a more detrimental impact on the auditory cortex than on visual or motor cortices. Moreover, hearing loss appears to selectively aggravate the detrimental effect of age on the gray matter of the auditory cortex.

HEARING LOSS RELATED DIFFERENCES

In addition to age, hearing loss has a distinct effect on the gray matter brain volume of the temporal gyri, the orbitofrontal cortex, middle cingulate, and fusiform gyrus. Moreover, in the present study, hearing loss was associated with differences in the cortical surface thickness of the supramarginal gyrus, angular gyrus, inferior parietal area, occipital and temporal areas. Our results are in agreement with previous reports on reduced gray matter volume in the temporal lobes and cingulate area of individuals with hearing loss (Husain et al., 2011; Peelle et al., 2011; Eckert et al., 2012; Lin et al., 2014; Wang et al., 2016). Similarly, our surface-based thickness measure indicated that hearing loss

without tinnitus is related to less gray matter thickness in the parietal cortex and insula, which is in line with the reported results of an earlier study (Ren et al., 2018). In addition to age, hearing loss is thus related to a reduction in the cortical volume and thickness of areas within and beyond the auditory system.

TINNITUS-RELATED DIFFERENCES

Our results support the finding of the study by Husain et al.(Husain et al., 2011) that structural changes are more pronounced in hearing loss without tinnitus than in hearing loss with tinnitus. In line with this, the hearing loss group without tinnitus in our study had significantly lower gray matter volume and surface thickness in several areas compared to controls, whereas the hearing loss group with tinnitus did not. Likewise, Husain et al.(Husain et al., 2011) reported no significant differences in gray matter volume between hearing loss with tinnitus and healthy controls in participants with hearing thresholds similar to our study. Interestingly, this finding suggests that in hearing loss, the additional presence of tinnitus is related to a more limited decline in gray matter and thus better preservation of gray matter in several temporal, frontal, and occipital areas.

In the hearing loss group with tinnitus, the gray matter volume of the lingual gyri was larger than in those without tinnitus. Previously, the lingual gyrus has been connected to tinnitus in studies that investigated resting-state connectivity. These studies reported tinnitus-related increases in the connectivity of the left lingual gyrus with the left auditory cortex (Hinkley et al., 2015) and decreased connectivity of the left lingual gyrus with the auditory resting-state network (Schmidt et al., 2013) compared to controls with normal hearing. Studies that compared tinnitus participants to hearing loss matched controls reported increased connectivity of the bilateral lingual gyrus with the attention and short-term memory networks (Husain et al., 2015), increased connectivity of the lingual gyri with the default mode network, and a trend towards decreased connectivity with the auditory resting-state network (Schmidt et al., 2013). Our results indicate that in the presence of tinnitus, the gray matter volume of the lingual gyrus is altered along with the functional connectivity differences reported in the literature. To our knowledge, there is currently no research published on specific structural preservation of the lingual gyrus in relation to tinnitus. The precise role of the lingual gyrus in tinnitus remains unclear, but other studies reported that the lingual gyrus is linked to better preserved cognitive function in patients with major depressive disorder (Jung et al., 2014). Specifically, larger lingual gyrus volume correlated with better performance on memory tasks (Walhovd et al., 2006; Kalpouzos et al., 2009). In summary, these results suggest a relation between the possible preservation of cognitive functions mediated by the lingual gyrus and the presence of tinnitus.

DIFFERENTIATION WITH OTHER STUDIES ON TINNITUS

The present study showed that larger gray matter volume in the lingual gyri was specifically related to the presence of tinnitus in individuals with hearing loss. In contrast, previous studies reported tinnitus-related reductions in gray matter volume or surface thickness in multiple areas. For instance, differences in the prefrontal cortex (Leaver et al., 2011, 2012; Mahoney et al., 2011; Aldhafeeri et al., 2012; Schecklmann et al., 2012a; Boyen et al., 2013; Allan et al., 2016), the supramarginal gyrus (Leaver et al., 2012), insular cortex (Schecklmann et al., 2012a) and cingulate area (Schecklmann et al., 2012a) were reported. Yet, our study showed that changes in these areas were related to age, and differences in the gray matter of the cingulate area were related to hearing loss. These findings are consistent with the results of Wong et al. (Wong et al., 2010). In their study, it was reported that a significant relation was present between age, not hearing loss, and surface thickness of the superior frontal gyrus. Other studies reported differences in the subcallosal area (Muhlau et al., 2006; Leaver et al., 2011) and the pre and postcentral gyrus (Schecklmann et al., 2012a) in relation to tinnitus. However, in our study, no significant differences in these areas were identified in any of our contrasts. In line with previous reports, the right cluster identified in the lingual gyrus expanded to include the precuneus (Allan et al., 2016). To expand, if age was omitted as a covariate in our analyses or if we used an uncorrected threshold, this resulted in the detection of significant volumetric differences related to hearing loss in the aforementioned areas. Since tinnitus patients often have additional hearing loss and are generally older than control groups, these confounds may explain the uncertainty and contradictory reports on structural differences in tinnitus (Vanneste et al., 2015; Allan et al., 2016; Shore et al., 2016). Therefore, some of the results described in earlier studies may reflect (small) differences in hearing loss or age and are not specifically related to the presence of tinnitus.

BEYOND THE AUDITORY SYSTEM: COGNITION

Our results showed that, regardless of age, several brain areas contained less gray matter in participants with hearing loss. This effect is more pronounced in hearing loss without tinnitus than in hearing loss with tinnitus. The areas identified in our analyses are in line with areas that are reported to show an aggravated decline of cortical volume (Peelle et al., 2011; Eckert et al., 2012; Lin et al., 2014) and surface thickness (Ren et al., 2018) in hearing loss and cognitive impairment. Atrophy of several brain areas beyond the primary auditory system has been related to hearing loss. This widespread loss of cortical gray matter has the potential to affect a wide range of cortical processes that extend beyond auditory processing. The gray matter areas identified in our study are consistent with those reported in previous longitudinal studies. These indicated that in age-related hearing loss, the decline in these gray matter areas is progressive over time and relates to cognitive impairments (Lin et al., 2014; Uchida et al., 2019).

The nature of the relationship between hearing loss and cognitive decline is still debated. It has been suggested that a similar mechanism of progressive loss of brain efficiency can result in both central hearing loss and cognitive decline (Humes et al., 2013a, 2013b; Jafari et al., 2019; Sardone et al., 2019). Directionality of this effect has been suggested. For example, the proposal that a decline in central functioning may contribute to poorer speech understanding and thus that cognition can have a big impact on hearing (Moore et al., 2014). Moreover, presumably the declining processing speed is the common cause of both cognitive decline and hearing loss since the auditory system relies on precise timing, and a reduction in temporal fine structure can hinder speech processing. On the other hand, it has been described that central hearing disorders, or central auditory dysfunctions, often precede cognitive impairment (Gates et al., 2002). The latter finding suggests that central hearing dysfunction can be an early marker of cognitive decline. Based on our results, uncompensated hearing loss without tinnitus is linked to a loss of gray matter in several cortical areas implicated in cognitive decline. In contrast, in the presence of tinnitus, these areas are better preserved.

Overall, it is important to note that the differences between participant groups may have developed as a result of hearing loss or tinnitus. Alternatively, differences may have existed before tinnitus or hearing loss developed. For example, it is conceivable that some pre-existing morphological characteristics correspond to a susceptibility to develop hearing loss or tinnitus. Based on our study, we cannot distinguish between gray matter differences that developed over time and pre-existing conditions. An additional factor that may influence the outcomes is that none of our participants had hearing aids to compensate for their hearing loss or tinnitus maskers to alleviate their tinnitus. Hearing aids and tinnitus maskers may impact the brain by generating more input and hence affect cortical plasticity.

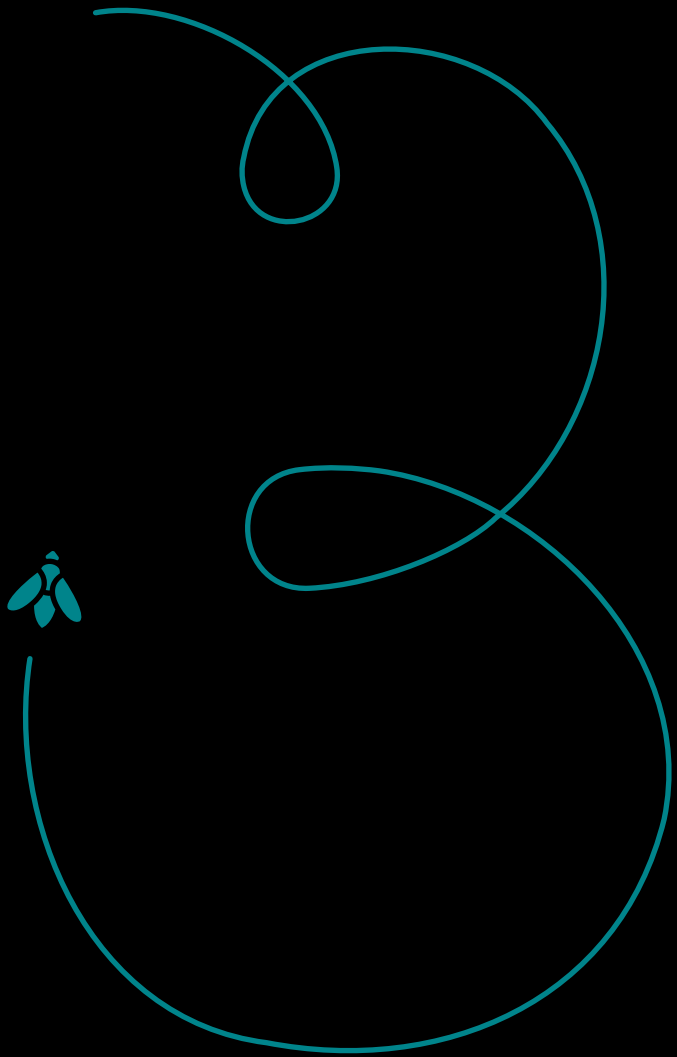
Limitations

The effect of tinnitus on gray matter morphology in this study was derived from the contrast between hearing loss without tinnitus and hearing loss with tinnitus. Our results may thus only pertain to tinnitus associated with hearing loss. The possibility exists that tinnitus without the presence of hearing loss affects the gray matter morphology in a different manner.

CONCLUSION

This study showed age-related gray matter atrophy in several brain areas, both within and outside of the auditory pathway. With the aid of volumetric and surface-based methods, we showed that age-related hearing loss without

tinnitus is related to additional decreases in brain volume of the temporal, frontal, cingular, and parietal areas. The largest differences in gray matter morphometry were caused by age, after which the presence of hearing loss was associated most strongly with a decline in cortical gray matter. Interestingly, the addition of tinnitus in hearing loss individuals appears to reduce the amount of gray matter decline since the gray matter of the hearing loss with tinnitus was not significantly different from that of the control group. The lingual gyrus, in particular, is larger or better preserved in the presence of tinnitus. These findings confirm that hearing loss impacts areas beyond the auditory system and is an important factor to consider in the treatment of cognitive impairments.



CHAPTER 03

MACROSTRUCTURAL CHANGES OF THE ACOUSTIC RADIATION IN HUMANS WITH HEARING LOSS AND TINNITUS REVEALED WITH FIXEL-BASED ANALYSIS

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ABSTRACT

Age-related hearing loss is the most prevalent sensory impairment in the elderly population and is related to noise-induced damage or age-related deterioration of the peripheral auditory system. Hearing loss may affect the central auditory pathway in the brain, which is a continuation of the peripheral auditory system located in the ear. A debilitating symptom that frequently co-occurs with hearing loss is tinnitus. Strikingly, investigations into the impact of acquired hearing loss, with and without tinnitus, on the human central auditory pathway are sparse. This study employed diffusion-weighted imaging to investigate changes in the largest central auditory tract, the acoustic radiation, related to hearing loss and tinnitus. Participants with hearing loss, with and without tinnitus, and a control group were included. Both conventional diffusion tensor analysis and higher-order fixel-based analysis were applied. The fixel-based analysis was used as a novel framework providing insight into the axonal density and macrostructural morphological changes of the acoustic radiation in hearing loss and tinnitus. The results show tinnitus-related atrophy of the left acoustic radiation near the medial geniculate body. This finding may reflect a decrease in myelination of the auditory pathway, instigated by more profound peripheral deafferentation or reflecting a pre-existing marker of tinnitus vulnerability. Furthermore, age was negatively correlated with the axonal density in the bilateral acoustic radiation. This loss of fiber density with age may contribute to poorer speech understanding observed in older adults.

Significance Statement

Age-related hearing loss is the most prevalent sensory impairment in the elderly population. Older individuals are subject to the cumulative effects of aging and noise exposure on the auditory system. A debilitating symptom that frequently co-occurs with hearing loss is tinnitus: the perception of a phantom sound. In this large DWI-study, we provide evidence that in hearing loss, the additional presence of tinnitus is related to degradation of the acoustic radiation. Additionally, older age was related to axonal loss in the acoustic radiation. It appears that older adults have the aggravating circumstances of age, hearing loss, and tinnitus on central auditory processing, which may partly be due to the observed deterioration of the acoustic radiation with age.

INTRODUCTION

Age-related hearing loss is the most prevalent sensory impairment in older individuals (Mathers et al., 2000). In hearing loss, the fast and efficient information transfer in the auditory system is hampered by a reduction in hearing sensitivity related to damage of the peripheral auditory pathway. A challenge in defining the characteristic changes of the auditory system related to hearing loss is that hearing loss often co-occurs with other auditory domain conditions. The most extensively studied co-occurring symptom is tinnitus, the perception of a phantom sound. Tinnitus is a common and debilitating symptom that affects around 12 – 30 % of the general population (McCormack et al., 2016). Unlike hearing loss, research investigating damage to the peripheral auditory system in tinnitus is sparse. Available research suggests that tinnitus relates to distinctive damage to peripheral auditory structures (Rüttiger et al., 2013; Singer et al., 2013). In addition to the damage to the structures of the peripheral auditory system, changes to the structure of the central auditory system have been implicated in both hearing loss and tinnitus.

Most of the information transfer in the auditory system takes place via myelinated axons, i.e., white matter tracts, which facilitate rapid and precise information transfer (Sinclair et al., 2017). In neuroscience, diffusion-weighted imaging (DWI) is the most commonly used method to investigate white matter tracts of humans with hearing loss and tinnitus. DWI scans, obtained with a magnetic resonance imaging (MRI) scanner, reflect the diffusion of water molecules. In the white matter tracts of the brain, water molecules are most likely to diffuse in parallel with the fibers due to restrictions by axonal membranes and myelination. Traditionally, diffusion-weighted images are analyzed with a diffusion tensor model (DTI). Within this model, white matter is quantified by the principle direction of restricted water proton movement (fractional anisotropy) and the more general molecular diffusion rate (mean diffusivity) (Soares et al., 2013). In

voxels with multiple and crossing fiber bundles (i.e., complex fiber populations), DTI cannot readily assign differences in white matter to a specific pathway (Douaud et al., 2011; Jones et al., 2013; Mito et al., 2018). Since up to 90% of white matter voxels have complex fiber populations (Jones et al., 2013), the calculation of only one averaged direction per voxel renders the diffusion tensor model challenging to interpret biologically (Mito et al., 2018).

To resolve multiple fiber populations within voxels, higher-order diffusion-weighted models have led to a fixel based analysis approach that exploits the fact that DWI images contain information on the presence of multiple fiber bundles (fixels) within a voxel (FBA; Raffelt et al., 2017). The approach describes changes in fiber density (FD) and fiber-bundle cross-section (FC) and a combination of these measures (FDC) (Raffelt et al., 2017; Mito et al., 2018). FD reflects microstructural changes in a fiber pathway, with a reduction indicating a decrease in axonal density. FC relates to macrostructural changes of fiber pathways, with a reduction indicating white matter fiber bundle cross-sectional atrophy or pre-existing differences in fiber bundle morphology. Lastly, the FDC measure integrates both the degradation of a fiber tract (FC) and the state of the remaining fibers in a tract (FD) (Raffelt et al., 2017; Mito et al., 2018). In summary, the fixel-based analysis approach is tract-specific and has a biologically meaningful interpretation.

To date, there have been few diffusion-weighted imaging studies that investigated the effect of tinnitus and acquired hearing loss on the central auditory pathway (Tarabichi et al., 2017). Our study aimed to investigate differences in the largest white matter tract of the central auditory system, the acoustic radiation, connecting the auditory thalamus with the auditory cortex. Two groups with hearing loss, with and without tinnitus, were compared to a normal-hearing control group. Additionally, the relation between fixel-based metrics and demographics, hearing level, and tinnitus-related variables was investigated. Finally, the results from conventional voxel-based diffusion tensor analysis were compared to those of fixel based analysis.

MATERIALS AND METHODS

ETHICAL APPROVAL

This study was performed with the approval of the medical ethical committee of the University Medical Center of Groningen, the Netherlands. Participants signed an informed consent form before participation and received reimbursement for their time and travel costs.

PARTICIPANTS

This study included three groups: participants with hearing loss without tinnitus, participants with hearing loss and tinnitus, and a healthy control group. A total of 93 participants were included: 34 participants with hearing loss and tinnitus, 23 with hearing loss but without tinnitus, and 36 healthy controls. Hearing loss participants had to have bilateral symmetrical hearing loss and normal thresholds up to 1 kHz. Pure tone thresholds were obtained in a sound-attenuating booth for frequencies between 0.250 and 8 kHz. At the time of inclusion, none of the participants used a hearing aid to improve their hearing or used a tinnitus masker to alleviate their tinnitus. The tinnitus had to be present for at least six months. Tinnitus variables collected were laterality, pitch, and the duration of tinnitus. All participants were requested to fill in the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983) and the Hyperacusis Questionnaire (Khalfa et al., 2002). Additionally, the tinnitus participants were requested to fill in the Tinnitus Handicap Inventory (McCombe et al., 2001) and the Tinnitus Reactions Questionnaire (Wilson et al., 1991).

DATA ACQUISITION

Diffusion-weighted imaging data were acquired using a 3 T Philips Intera scanner (Best, the Netherlands) with a 32-channel SENSE head coil. All participants were scanned with the parameters: TR 9000 ms; TE 60.6 ms; voxel size = 2.5 x 2.5 x 2.5 mm^3; 61 non-collinear gradient directions; 55 slices; b = 1000 s/mm^2; anterior-posterior phase encoding direction.

PRE-PROCESSING

First, the data were denoised, motion-corrected, and eddy current distortion corrected in FSL (Jenkinson et al., 2012). These general pre-processing steps were followed by apparent fiber density (AFD) specific pre-processing steps of bias field correction and global intensity normalization across participants. Bias field correction eliminates inhomogeneities in the image that are due to low spatial frequency intensity areas (Raffelt et al., 2012b). Then, global intensity normalization was performed using group-wise registration. None of the included DWI-scans were affected by motion to the extent that they had to be excluded from the study.

FIXEL BASED ANALYSES

After pre-processing, fixel-based analyses were performed as outlined in Raffelt et al., 2017, with the aid of MRtrix3.0 (Tournier et al., 2019). Fixels represent the fiber bundle elements within a voxel and are comprised of a set of fibers that are sufficiently similar in orientation. After intensity normalization, single-fiber response functions were estimated for each participant (Tournier et al., 2013). These unique response functions were averaged to obtain a group average response function (Dhollander et al., 2019). The DWI data were up-

sampled to an isotropic voxel size of 1.3 mm to increase contrast, and a whole-brain mask was computed from the up-sampled data. Constrained spherical deconvolution was used to estimate the fiber orientation distribution (FOD) for each participant (Dhollander and Connelly, 2016). For the group comparisons, the relevant population templates were created based on a subset of 15 participants per group, in line with the recommendation of the creators of this method (Tournier et al., 2019), ensuring an equal representation of participants of the groups that were investigated. All individual FOD images were then registered to the FOD population template via nonlinear registration (Raffelt et al., 2011, 2012a). The outcome of this registration was used to construct a template mask that contained white matter voxels present in all participants. Subsequently, individual fixels and their orientation in each voxel were identified by segmenting the FODs. All fixel directions were reoriented using the FOD registration warps, and each identified fixel of the template mask was matched to that of the participant image. Finally, fiber density (FD), fiber-bundle cross-section (FC), and the combination metric FDC were computed.

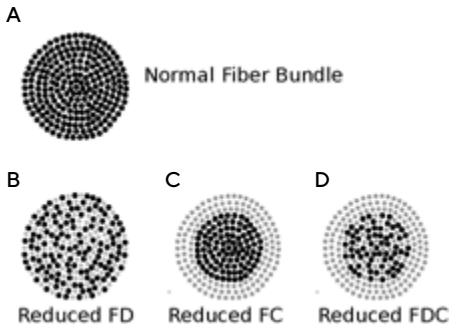

Figure 1. Schematic representation of a decrease in the FBA metrics, in keeping with the original figure displayed in Raffelt et al., 2017. The fixel-based analysis quantifies changes to the intra-axonal volume as three metrics: fiber density (FD), fiber-bundle cross-section (FC), and a combination of both measures (FDC). (A) Normal fiber bundle morphology. (B) A reduction in fiber density corresponds to a loss of axons and reflects a change of the white matter microstructure. (C) A reduction in fiber-bundle cross-section corresponds to the amount of contraction compared to the population template and reflects a shift in the macrostructure of the white matter (D) A combination of reduced fiber density and fiber-bundle cross-section.

The interpretations of a reduction in FD, FC, and FDC for the white matter fiber bundle's structural integrity are visualized in Figure 1. The fiber density (FD) metric reflects a reduction in the volume of restricted water within a given voxel, which can be due to axonal loss. FD is measured on a within-voxel level and reflects the state of the remaining white matter tissue. The fiber-bundle cross-section (FC) metric reflects a change in the cross-sectional area occupied by a

white matter fiber bundle and can reflect cross-sectional atrophy or acquired axonal loss, such as in Alzheimer's' disease (Mito et al., 2018). FDC is a metric that combines the FD and FC metrics into one. All three metrics can reflect the information-carrying capacity of the white matter tissue (Raffelt et al., 2017). To indicate the magnitude of the significant effects in the group-wise comparisons, the effect size of the FBA outcomes was expressed as a percentage decrease in the FBA metric of one group relative to another group.

Whole-brain probabilistic fiber tractography was employed to derive information on local fixel connectivity between neighboring fixels and subsequently apply connectivity-based fixel enhancement to aid statistical analysis (Raffelt et al., 2015). After obtaining the whole brain tractogram, the initially estimated 20 million tracks were reduced to 2 million to decrease tractogram density biases, using spherical-deconvolution informed filtering of tractograms (SIFT; Smith et al., 2013).

ACOUSTIC RADIATION FIBER TRACKING

The focus of the current study was on the white matter changes within the acoustic radiation. The population FOD template was used to perform probabilistic tracking of the acoustic radiation to investigate group differences in the white matter between the auditory thalamus (medial geniculate body) and the primary auditory cortex. First, masks for the auditory cortex and auditory radiations were derived from the Juelich Histological atlas in MNI space, followed by the nonlinear registration of the created masks to the population template space using the Non-linear Image Registration Tool (FNIRT) of FSL. Then, the bilateral medial geniculate body (MGB) regions-of-interest were identified by drawing a 4 mm sphere on the FOD template (MNI coordinates L MGB [-13.4, -3.1, 7.3]; R MGB [13.4, -3.6, -7.3]). Subsequently, tracking of the acoustic radiations in template space was performed by generating 5000 streamlines, with the MGB as seed ROI and the primary auditory cortex as inclusion ROI. The acoustic radiation masks derived from the Juelich Histological Atlas were used to constrain the fiber tracking anatomically.

VOXEL-BASED METRICS

To facilitate the comparison of the FBA metrics with the more conventional DTI derived metrics, voxel-derived tensor-based analyses were performed on the white matter of the acoustic radiation. The two tensor-based metrics computed for each participant were fractional anisotropy (FA) and mean diffusivity (MD), derived with *MRtrix*. These tensor-metrics were transformed to the population template space with the warps earlier calculated for fixel-based analysis. Voxel-masks were constructed for the tracked acoustic radiations (both left and right) to perform voxel-based diffusion tensor analyses in a manner similar to the fixel based analyses.

STATISTICAL ANALYSIS

Differences between groups on demographical variables and questionnaire scores were tested for significance with SPSS 26 (IBM SPSS Statistics for Macintosh, 2019). Group differences for the variable sex were tested with a Chi-Square test of independence. A three-group ANOVA was used to test for group difference for the variable age, and this test was followed by independent pairwise t-tests. A Kruskal-Wallis test was used to test for significant differences between questionnaire scores and hearing thresholds, and this test was followed by a pairwise Mann-Whitney U test. Distributions of hearing thresholds and questionnaire scores were assessed by visual inspection. If the dependent variable distributions were similar for both groups, the differences in medians were reported with the Mann-Whitney U test. However, if the distributions were dissimilar, the Mann-Whitney U test was used to investigate the difference in distribution. A Pearson moment-product correlation was run to assess the relationship between age and high-frequency hearing loss (quantified as the mean of the thresholds at 4,6 and 8 kHz).

Group differences in the derived fixel-based metrics (FD, FC, and FDC) were tested for significance with a general linear model (GLM). Sex and age were added to the model as covariates. The fixel data were smoothed based on the sparse fixel-fixel connectivity matrix derived from the whole-brain streamline tractogram (Raffelt et al., 2015). For each fixel, a family-wise error (FWE) corrected p-value was obtained via permutation testing (n = 5000). For the visualization of the significant fixels, the *mrview* tool in MRtrix3.0 was used. The significant fixels (FWE < 0.05) were then displayed on the whole-brain tractogram and visualized as streamlines. The effect size of the significant FBA metrics was expressed as a percentage difference. Additionally, individual tract-averaged FD and FC metrics were calculated for the acoustic radiation. This approach allowed us to correlate the fixel based metrics with age, sex, hearing level (defined as the average of 1, 2, and 4 kHz), and questionnaire scores.

Threshold free cluster enhancement (TFCE) with permutation testing was used to determine if group differences were significant for the voxel-based tensor derived analysis. Differences in FA and MD metrics were computed on a voxel-by-voxel basis, corrected for sex and age.

RESULTS

In total, the diffusion-weighted imaging data of 93 participants are presented, along with their demographical variables and questionnaire scores (see Table 1). A significantly larger percentage of male participants was present in the hearing loss group with tinnitus compared to the control group ($x^2(1) = 8.6$, $p = 0.003$).

There were no significant differences in the percentage of male participants in the hearing loss group without tinnitus and the control group ($x^2(1) = 0.67$, $p = 0.41$), or between both hearing loss groups ($x^2(1) = 3.4$, $p = 0.07$). There were no significant differences in age ($t=1.88$, $p = 0.13$) between the hearing loss groups, with and without tinnitus. Both the hearing loss group with tinnitus ($t=5.32$, $p < 0.001$) and the hearing loss group without tinnitus ($t=5.97$, $p < 0.001$) were significantly older than the control group.

Table 1. Demographics and questionnaire scores of the three participant groups.

Groups	Controls	Hearing Loss	Hearing Loss + Tinnitus
Demographics	N = 36	N = 23	N = 34
Sex	18 M, 18 F	14 M, 9 F	27 M, 7 F* **
Mean age (years)	45 ± 14 (18 -67)	62 ± 8 (33-75)*	60 ± 8 (41-72)*
Questionnaires			
HADS Anxiety	5 ± 3 (0 - 11)	3 ± 3 (0 - 11)	5 ± 4 (0 - 12)
HADS Depression	3 ± 3 (0 - 9)	2 ± 3 (0 - 10)	4 ± 4 (0 - 16)**
HQ	11 ± 7 (0 - 27)	9 ± 5 (1 - 18)	16 ± 8 (0 - 30)**
THI			39 ± 19 (16 - 82)
Tinnitus			
Mean Duration (years)			14 ± 9 (1 - 33)
Tinnitus Pitch			1 - <4 kHz (n = 9)
			4 - 7 kHz (n = 12)
			≥8 kHz (n = 11)
			Broadband (n = 2)

** indicates that groups differed significantly from the control group ** indicates that hearing loss groups differed significantly from one another at p < 0.001. Chi-square, ANOVA, Kruskal-Wallis, and Mann-Whitney, respectively*

The pure tone audiograms, averaged over both ears, are reported for the three groups in Figure 2. On average, the hearing loss group with tinnitus had higher thresholds than the hearing group without tinnitus. These differences did not reach significance after correction for multiple comparisons (see Figure 2). Both hearing loss groups had significantly higher thresholds than the control group for all frequencies tested.

There was a significant positive correlation between age and high-frequency hearing loss ($r(93)=0.629$, $p < 0.001$). Furthermore, the hearing loss group with tinnitus had significantly higher scores on the Hyperacusis Questionnaire (HQ) ($U=96.5, z=-2.89$ $p = 0.004$) and the depression scale of the Hospital Anxiety and Depression scale (HADS-D) ($U=126.5, z=-2.41$, $p = 0.016$) compared to the hearing loss group without tinnitus, at a level corrected for multiple comparisons.

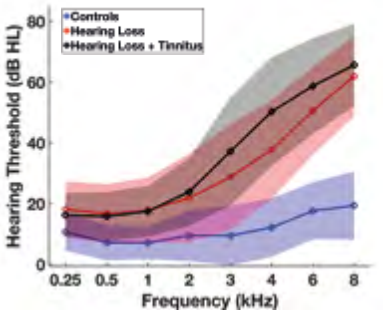

Figure 2. Hearing thresholds for the three groups with their respective standard deviations. After correction for multiple comparisons, the median thresholds of the hearing loss groups were not significantly different (250 Hz p = 0.60; 500 Hz p = 0.95; 1 kHz p = 0.83; 2 kHz p = 0.90; 3 kHz p = 0.09; 4 kHz p = 0.04; 6 kHz p = 0.17; 8 kHz p = 0.42). However, at 4 kHz, the difference in hearing thresholds was significant at an uncorrected level of $p < 0.05$ ($p = 0.04$). Both hearing loss groups, with and without tinnitus, differed significantly on all frequencies from the control group. Higher median thresholds were present in the hearing loss group without tinnitus (250 Hz p = 0.001; 500 Hz to 8 kHz = $p < 0.0005$) and the hearing loss group with tinnitus (250 Hz p = 0.002; 500 Hz to 8 kHz = $p < 0.0005$), compared to controls.

TRACT OF INTEREST FIXEL-BASED ANALYSIS

The auditory radiation was tracked from the medial geniculate body to the primary auditory cortex. The outcomes presented in this study are based on the metrics of these tracts, which are constructed based on 5000 streamlines. These tracts are visualized in Figure 3; the left acoustic radiation is presented in blue and the right in red. The fiber density (FD), fiber-bundle cross-section (FC), and the combined metric (FDC) of the three groups were compared, with the variables of age and sex controlled for within the model. This analysis identified a significantly smaller fiber-bundle cross-section (FC) of the auditory radiation in hearing loss with tinnitus compared to the control group. This effect was significant for the left auditory radiation ($p = 0.024$), and approached significance for the right auditory radiation ($p = 0.056$). Expressed as a percentage change of the hearing loss group with tinnitus compared to the group mean of the control group, this corresponds to an overall decrease in FC of 5.4%. There were no other significant group differences for the FC, FD, and FDC metrics.

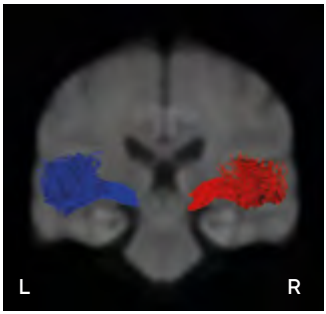

Figure 3. Tracked acoustic radiations for left (blue) and right (red) hemispheres. The tracts are overlaid on a representative coronal slice. Tracking was performed per hemisphere from the medial geniculate body (MGB) to the primary auditory cortex (TE 1.0).

The significant difference in FC occurs in the area of the auditory radiation closest to the medial geniculate area of the thalamus; this is visualized in Figure 4. In panel A, the black lines represent all the fixels identified within the auditory radiation mask at this brain slice. The colored lines represent the fixels where a significant difference was detected between the hearing loss group with tinnitus and the control group. The enlarged inset illustrates that even in voxels with crossing fibers (multiple fixels in a single voxel), the FBA method identifies significant differences between groups only in the fixels belonging to the acoustic radiation, which runs from medial to lateral, that is, from the thalamus in the direction of the auditory cortex (Figure 4 B, C, D). The streamline segments that correspond to the significant fixels (FWE-corrected P-value < 0.05) of the fiber cross-section metric (FC) are displayed in Figure 5.

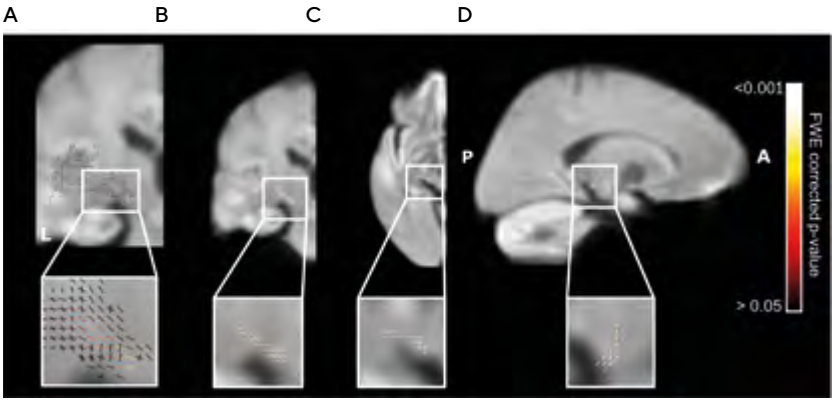

Figure 4. Region of the left acoustic radiation with an altered cross-sectional metric in the left acoustic radiation of participants with hearing loss and tinnitus compared to controls. (A) The line segments indicate fixels, where each fixel corresponds to a population of fibers that pass through the corresponding voxel. The line segments represent all the fixels that were identified within the mask of left the acoustic radiation. The upper picture is an overview to indicate the anatomical location. The zoomed-in inset shows the fixels that belong to a tract with a significantly smaller cross-section in the hearing loss group with tinnitus than the control group. These significantly different fixels are color-coded by their respective FWE-corrected p-values. For black fixels, there was no significant difference between the groups. (B) The non-significant fixels are omitted here. Only the fixels with a significantly decreased cross-section are projected on a coronal slice, (C) an axial slice, and (D) a sagittal slice. Significant differences can be observed in the area of the auditory radiation closest to the medial geniculate area of the thalamus. Note that the FBA analysis identified significant differences in fixels that are part of the acoustic radiation only. Crossing fixels were not significantly different between the groups and are therefore shown in black. All panels show one representative brain slice with the projected fixels. The small line segments in panel D indicate that fixels, and thus the corresponding nerve fibers, are oriented out of the depicted sagittal plane.

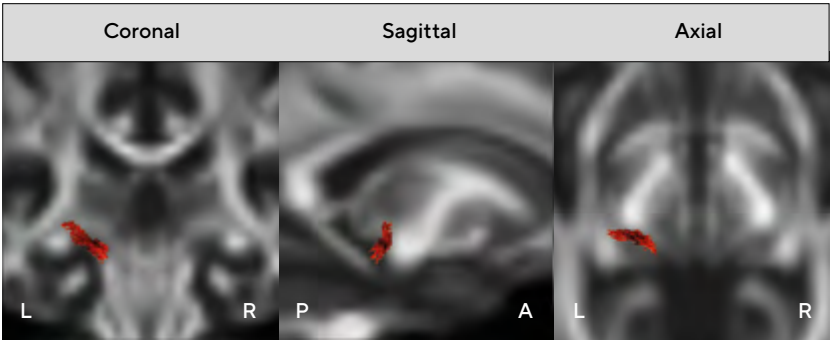

Figure 5. Fiber tracts with a reduction in fiber-bundle cross-section in the hearing loss group with tinnitus compared to the normal-hearing control group. The template tractogram was adjusted to only include the streamline segments that were significantly different in FC between the hearing loss group with tinnitus and the control group (FWE-corrected P-value < 0.05). The significant streamlines are depicted on a coronal, sagittal, and axial slice of the population template map in red. To aid orientation, left (L) and right (R) are indicated for the coronal and axial slices and anterior (A) and posterior (P) for the sagittal slice.

ASSOCIATION TINNITUS-RELATED VARIABLES, QUESTIONNAIRE SCORES, HEARING LEVEL, SEX, AND AGE WITH FIBER DENSITY AND CROSS-SECTION METRICS

As summarised in Table 2, there was a significant association between age and fiber density for both the left and the right acoustic radiation. There was no significant association between age and fiber cross-section. Furthermore, there was no significant association between either the fiber density or fiber cross-section metrics and the THI score, sex, HADS score, or any of the tinnitus characteristics such as tinnitus pitch, duration, lateralization, or hearing level, at a level corrected for multiple comparisons (FWE < 0.05). Since the FDC metric is a combination of the FD and FC metrics, we did not include it in this analysis.

To test if the higher HQ scores in the group with tinnitus affected the results, we split the tinnitus group at an HQ score of 22 (Aazh and Moore, 2017; Koops and van Dijk, 2021) and compared the FC and FD metrics of the two resulting groups (high and low HQ scores) with two-sample t-tests. There were no significant or near significant differences in average FC (Left $p = 0.187$; Right $p = 0.150$) or FD (Left $p = 0.678$; Right $p = 0.904$) of the acoustic radiation between participants with hearing loss and tinnitus with high and low HQ scores. It thus appears that even though the presence of tinnitus is related to an altered FC of the acoustic radiation, additional hyperacusis does not alter this relation.

VOXEL-BASED METRICS OF TRACT OF INTEREST

A significant increase in mean diffusivity (MD) in the left acoustic radiation was observed for the hearing loss group with tinnitus, compared to the control group

(p = 0.03; FWE-corrected). No significant or near significant differences in fractional anisotropy (FA) or mean diffusivity (MD) were observed for any other group comparisons. An overlay of the voxels with significantly different mean diffusivity on the fixel mask with the FC metric shows an anatomical overlap between the voxels with increased MD and the fixels with decreased FC. The voxels with a significant increase in MD are located close to the medial geniculate body and slightly more anterior to the fixels with a significant decrease in FC; see Figure 6.

Table 2. Relation between fixel based metrics and age, hearing loss, and tinnitus-related variables. There was a significant negative correlation between age and fiber density for both the left and the right acoustic radiation (FWE-correct P-value < 0.05). Hearing level, indicated by the mean of 1, 2, and 4 kHz, did not significantly correlate with either FD or FC. Tinnitus-related variables and questionnaire scores were not significantly associated with FD or FC fixel based metrics.

		Left Acoustic Radiation		**Right Acoustic Radiation**	
General variables					
		R	*p*	*R*	*p*
Age	*FD*	-0.38	<0.001*	-0.40	<0.001*
	Log FC	-0.07	0.53	-0.06	0.60
Hearing	*FD*	-0.20	0.06	-0.06	0.59
	Log FC	-0.17	0.11	-0.04	0.71
Sex	*FD*	0.24	0.047	0.12	0.34
	Log FC	-0.09	0.43	-0.04	0.73
Tinnitus related variables					
THI	*FD*	0.29	0.11	0.18	0.33
	Log FC	-0.003	0.99	-0.15	0.41
Duration	*FD*	0.17	0.45	-0.23	0.30
	Log FC	0.21	0.34	-0.27	0.23
Pitch	*FD*	0.23	0.20	-0.20	0.26
	Log FC	0.12	0.51	-0.42	0.02
Laterality	*FD*	0.07	0.68	0.27	0.12
	Log FC	0.23	0.20	0.24	0.17
Questionnaires					
HADS A	*FD*	0.28	0.02	0.29	0.01
	Log FC	0.12	0.32	0.06	0.59
HADS D	*FD*	0.18	0.13	0.13	0.26
	Log FC	0.14	0.26	0.08	0.53

The asterisks denote a significant relation at a p-level level corrected for multiple comparisons (; Bonferroni). The association for sex and laterality with FBA-metrics was tested with a point-biserial correlation. All other associations were tested with a Pearson correlation test.*

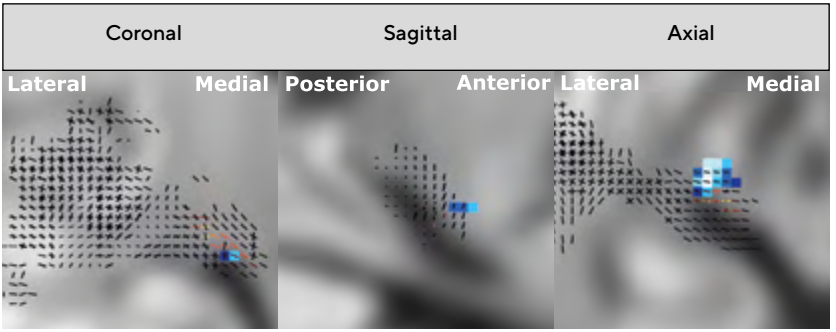

Figure 6. Comparison between fixel-based (FBA) and conventional tensor-based (DTI) measures of fiber tract properties. Both methods identified differences between the group with tinnitus and the controls in the left acoustic radiation. Fixels with a significantly decreased fiber-bundle cross-section (FC) and voxels with increased medial diffusivity (MD, a conventional DTI measure) are overlaid. The significant increase in MD is represented by the blue voxels, whereas the significant decrease in FC is represented by the red/yellow colored fixels (or fibers). There was no significant difference in the white matter of the acoustic radiation between both hearing loss groups or between the hearing loss group without tinnitus and the control group. The significant increase in MD and the significant decrease in FC are overlaid on a coronal, sagittal, and axial slice of the population template. To aid orientation, the lateral and medial side of the brain is indicated in the coronal and axial slices, and the posterior-anterior axis is identified in the sagittal slice. Color-coding of the fixels is identical to Figure 4. This figure illustrates to what degree the outcomes of these two different methods overlap.

DISCUSSION

Fixel-based analysis was used to investigate differences in the acoustic radiation in hearing-impaired participants, with and without tinnitus. In the presence of tinnitus, a significant reduction in the acoustic radiation's cross-section was observed for the left acoustic radiation. Conventional DTI showed a significant increase in mean diffusivity at a similar location in the left acoustic radiation of hearing loss participants with tinnitus compared to controls.

COMPARISON WITH PREVIOUS DWI STUDIES IN ACQUIRED HEARING LOSS

Previous reports on voxel-based tensor metrics in acquired hearing loss or presbycusis are sparse. Only two studies included regions-of-interest in the auditory pathway or reported on its white matter tracts. Two whole-brain voxel-based studies were identified. One reported increased MD in the transverse temporal plane and decreased FA for the middle temporal gyrus (Ma et al., 2016). Since no segmentation or masking of white matter was reported, gray matter may have affected this report's voxel-based measures. The second study reported

decreased FA for the anterior thalamic radiation, specified as acoustic radiation (Husain et al., 2011). In contrast, a diffusion tensor tractography study reported no significant changes in FA or MD in the auditory pathway of hearing loss participants (Profant et al., 2014). In line with the latter study, our tractography results do not indicate significant differences in FA or MD related to hearing loss. Similarly, no hearing loss related differences in the fiber density (FD) or fiber-bundle cross-section (FC) of the acoustic radiation were identified. Whereas earlier studies used voxel-based tensor metrics to infer differences, FBA allows us to firmly place the significant fixels in the fiber tract of interest. Thus, neither conventional DTI nor the improved fiber determination of FBA identified significant differences related to acquired hearing loss in the acoustic radiation tracts.

COMPARISON WITH PREVIOUS DWI STUDIES ON TINNITUS

Diffusion imaging studies on tinnitus that included a control group and reported on or included white matter tracts of the auditory pathway are sparse. Two studies implemented whole-brain analyses (Husain et al., 2011; Seydell-Greenwald et al., 2014), and one of them reported tinnitus-related significant voxels in the white matter of the auditory pathway (Seydell-Greenwald et al., 2014). The data provided in these articles cannot ascertain if the results are specifically located in the acoustic radiation or reflect differences in fiber paths that run in close proximity, such as the longitudinal fasciculus or the anterior thalamic radiation. The application of fiber tractography can give a better indication of the specific tracts. A fiber tractography study reported no significant differences in the voxel-based tensor derived metrics in a group with tinnitus and hearing loss compared to a control group (Crippa et al., 2010). Whereas the former study did not include age and sex in their statistical model, in the current study we did correct for differences in age and sex. In the present study, we identified a difference in MD between the hearing loss group with tinnitus and controls, but there were no significant differences between the hearing loss groups with and without tinnitus. Overall, our findings suggest that in the presence of tinnitus, there is a more pronounced increase in MD in the acoustic radiation, near the medial geniculate body, than in hearing loss without tinnitus.

REDUCTION OF FIBER-BUNDLE CROSS-SECTION AND INCREASE OF MEAN DIFFUSIVITY IN TINNITUS

A significant reduction in fiber-bundle cross-section (FC) was observed in the left acoustic radiation of participants with hearing loss and tinnitus. A similar effect, although non-significant, was observed in the right acoustic radiation. The increase in mean diffusivity (MD) partially overlapped in location with the reduction in FC (Figure 6). Even though we observed a loss of restriction of free diffusion, there was no loss of directionality of this diffusion. The decrease in FC may reflect fiber bundle atrophy or poor myelination of the axons within this area of the acoustic radiation. The degradation of myelination is not expected

to yield a drastic decrease in FA as even the complete absence of myelin decreases FA only by about 20%. However, the absence of myelination causes an increase in MD of ~50% (Gulani et al., 2001). Thus, the FC reduction and MD increase in tinnitus both suggest disruption or thinning of the myelin sheet. The confinement of the observed effect to the thalamic end of the acoustic radiation can reflect that at more distal points along this tract, the axons are less densely packed as the fibers fan-out to project to the larger area of the auditory cortex.

MYELINATION, WHITE MATTER REDUCTIONS, AND EVOKED POTENTIALS IN TINNITUS

Animal research suggested that tinnitus is related to a loss of inner hair cell ribbons (Rüttiger et al., 2013). These ribbons are the electron-dense structures associated with presynaptic active zones at the inner hair cell base. A loss of these ribbons would disrupt the sustained release of neurotransmitters at the first auditory synaptic junction (Matthews and Fuchs, 2010) and thereby impact auditory processing. Such damage may have caused the small but not significantly higher average thresholds of the hearing loss group with tinnitus (Figure 2). Furthermore, it has been suggested that tinnitus may relate to a loss of specific auditory nerve fibers. On the one hand, the loss of low-spontaneous rate fibers (low-SRFs) has been indicated in tinnitus (Schaette and McAlpine, 2011). However, recent evidence suggests that tinnitus in normal hearing doesn't correspond to diminished speech in noise perception (Zeng et al., 2020), which would be expected with the loss of low-SRFs. On the other hand, the specific loss of fast coding high-spontaneous rate fibers (high-SRFs) has also been implicated in tinnitus (Bauer et al., 2007; Knipper et al., 2020) or a combination of both (Paul et al., 2017). The high-SRFs have a thicker myelin sheet than low and medium SR-fibers (Liberman and Oliver, 1984; Gleich and Wilson, 1993), facilitating fast information transfer. This is vital to the functionality of the auditory system since it relies on precise timing. Future work could assess whether damage to specific auditory nerve fibers instigates a Wallerian-like degeneration process that leads to cross-sectional atrophy and increased medial diffusivity in the central auditory tracts.

Overall, myelination in the central nervous system is enhanced by neuronal activity (Demerens et al., 1996), and a reduction in sound-activity has been related to a decrease in the myelin thickness and the number of large diameter axons in the central auditory system (Sinclair et al., 2017). Previously, our group and others have shown that the auditory cortex is less responsive to high-frequency sounds in tinnitus participants with mild or moderate hearing loss than in those without tinnitus (Hofmeier et al., 2018; Koops et al., 2020). The reduction in the acoustic radiation's cross-section in the presence of tinnitus can relate to the previously reported reduction in sound-evoked activity in tinnitus. Critical damage at the peripheral level could result in a thinning of the myelin

sheet of the corresponding central auditory fibers that are no longer stimulated as a consequence. The additional presence of tinnitus in hearing loss may enlarge this effect.

Auditory Brainstem Responses (ABR) can detect changes in the amplitude and time-course of an auditory signal traveling from peripheral to more central auditory areas via the white matter tracts. In tinnitus, the most consistently reported findings are reduced (early) ABR amplitudes (Milloy et al., 2017; Hofmeier et al., 2018) and prolonged wave I and V latencies (Ikner and Hassen, 1990; Ravikumar and Ashok Murthy, 2016; Hofmeier et al., 2018), similar to the changes observed in high-frequency hearing loss (Sand and Saunte, 1994; Watson, 1996; Lewis et al., 2015). A loss of high-SR fibers at the peripheral level, a suggested hallmark of tinnitus, could explain the reported ABR wave latency prolongation (Knipper et al., 2020), which may relate to a reduction in myelination (Kovach et al., 1999). Taken together, the previously reported tinnitus-related ABR features and the current findings of cross-sectional atrophy in the acoustic radiation point towards reduced myelination of the peripheral and central auditory pathway in tinnitus, which warrants further investigation.

AGE-RELATED DECLINE OF FIBER DENSITY

Fiber density (FD) of the acoustic radiation and age were negatively correlated, indicating a progressive loss of thalamocortical axons with increasing age. Consequently, signal transfer from the thalamic medial geniculate body to the primary auditory cortex may be disturbed. Older adults with hearing loss perform worse than younger people with similar hearing loss (Dubno et al., 1984; Fitzgibbons and Gordon-Salant, 1995; Pichora-Fuller et al., 1995; Wingfield et al., 2006; Cardin, 2016) and the loss of axonal density with age may contribute to the poorer speech understanding observed in older adults. A minor decrease in peripheral myelination has previously been related to dramatic degradation of fine temporal structure coding, whereas it hardly affects hearing thresholds (Resnick et al., 2018). Presumably, the reduction of fiber density reported here further contributes to poorer information transfer of auditory information. These findings have implications for the rehabilitation of hearing in older individuals. Peripheral stimulation (i.e., hearing aids) may be supplemented with treatments aimed at stimulating cortical plasticity (e.g., inhibition of neural growth inhibitors in combination with tailored auditory training).

CONCLUSION

Our study did not identify significant changes in the acoustic radiation specifically related to acquired hearing loss. On the other hand, tinnitus was related to macrostructural degeneration of the left acoustic radiation near

the medial geniculate nucleus. In addition, a significant increase in MD was identified in participants with hearing loss and tinnitus, compared to controls. Both the diffusion tensor model and the fixel based analysis results point towards a reduction in the axonal myelination of the acoustic radiation in the presence of tinnitus. Taken together with the peripheral deafferentation reported in animal studies on tinnitus, the current findings suggest impaired integrity of nerve fibers at various levels of the auditory system. Furthermore, age was related to a decrease in fiber density of the acoustic radiation, which relates to axonal loss. This finding suggests a possible relation between acoustic radiation axonal loss and a decline in sound processing in older adults. This latter finding may have implications for the rehabilitation approaches in older individuals.

CHAPTER 04

CORTICAL TONOTOPIC MAP CHANGES IN HUMANS ARE LARGER IN HEARING LOSS THAN IN ADDITIONAL TINNITUS

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ABSTRACT

Neural plasticity due to hearing loss results in tonotopic map changes. Several studies have suggested a relation between hearing-loss-induced tonotopic reorganization and tinnitus. This large functional magnetic resonance imaging (fMRI) study on humans intended to clarify the association between hearing loss, tinnitus and tonotopic reorganization. To determine the differential effect of hearing loss and tinnitus, both male and female participants with bilateral high frequency hearing loss, with and without tinnitus, and a control group were included. In a total of 90 participants, bilateral cortical responses to sound stimulation were measured with loudness matched pure-tone stimuli (0.25 - 8 kHz). In the bilateral auditory cortices, the high frequency sound-evoked activation level was higher in both hearing-impaired participant groups than in the control group. This increase was most prominent in the hearing loss group without tinnitus. Similarly, the tonotopic maps for the hearing loss without tinnitus group were significantly different from the controls, whereas the maps of those with tinnitus were not. These results show that higher response amplitudes and map reorganization are a characteristic of hearing loss, not tinnitus. Both tonotopic maps and response amplitudes of tinnitus participants appear intermediate to the controls and hearing loss without tinnitus group. This observation suggests a connection between tinnitus and an incomplete form of central compensation to hearing loss rather than excessive adaptation. One implication of this may be that tinnitus treatments shift their focus towards enhancing the cortical plasticity on track instead of reversing it.

INTRODUCTION

Peripheral damage causes plasticity to occur in the area of the central nervous system that corresponds to the loss of function. In the auditory domain, hearing loss instigates plasticity that results in changes in tonotopic maps, spontaneous activity, and neural synchronicity (Robertson and Irvine, 1989; Eggermont and Roberts, 2004). Tonotopic maps are a striking feature of the mammalian auditory cortex and underlie the representation of complex sounds such as speech. This spatial separation of frequencies originates in the inner ear, where high frequencies are processed in the base of the cochlea and low frequencies in the apex. This separation is maintained from the cochlea to the auditory cortex (Brugge and Merzenich, 1973; Rauschecker et al., 1995). The tonotopic maps can be disrupted by hearing loss, the most prevalent sensory deficit in the elderly population.

The presence of clinical hearing loss increases the chances of developing tinnitus, the perception of sound in the absence of an external source. To this date, the specific pathophysiology involved in tinnitus remains elusive. However, the tinnitus pitch is often constrained to the frequency regions affected by hearing loss (Schecklmann et al., 2012b; Shekhawat et al., 2014; Sereda et al., 2015; Keppler et al., 2017), or to the border of the intact hearing region (Moore et al., 2010). These findings suggest that hearing loss and tinnitus are intricately related. Excessive or conservative tonotopic reorganization may differentiate between hearing loss with and without tinnitus.

Several papers have suggested a relation between hearing loss-induced tonotopic reorganization and tinnitus (Robertson and Irvine, 1989; Mühlnickel et al., 1998; Rauschecker, 1999; Eggermont and Roberts, 2004; Norena and Eggermont, 2005; Eggermont, 2006), but few have directly investigated this relation. In previous experimental work, the observed tonotopic map plasticity was linked to hearing loss but not to tinnitus (Weisz et al., 2005; Wienbruch et al., 2006; McMahon et al., 2016). In humans, tonotopic map reorganization was reported in one MEG study on tinnitus. A positive correlation was reported between the strength of the perceived tinnitus and the extent of cortical reorganization (Mühlnickel et al., 1998). In contrast, other studies reported no tonotopic plasticity related to tinnitus in humans (Langers et al., 2012) or animals (Kotak et al., 2005; Yang et al., 2011). Instead, these animal studies identified enhanced cortical excitation or reduced cortical inhibition in animals with binaural hearing loss and behavioural signs of tinnitus. The release from inhibition in the hearing loss affected area connects the tinnitus pitch with increased neuronal excitability (Yang et al., 2011). In general, it is not well established that tonotopic map plasticity is a cortical characteristic of tinnitus.

Animal-models of cortical tonotopic reorganization indicate that receptive fields of neurons within the hearing loss affected area shift towards the intact receptors (Rajan and Irvine, 1998; Eggermont and Komiya, 2000; Irvine et al., 2001; Muhlau et al., 2006). This reorganization causes a downwards shift in the characteristic frequency of neurons, in both temporary and lasting hearing loss (Irvine et al., 2000; Norena and Eggermont, 2005, 2006), thus altering the tonotopic map. In contrast, not all animal studies on hearing loss found a downwards shift in tonotopic maps but instead reported increased excitability (Kotak et al., 2005) or decreased inhibition (Rajan, 1998) of the affected frequency regions. In humans, one MEG study reported a shift of the cortical responsive region towards the intact edge-frequency of the audiogram in hearing loss (Dietrich et al., 2001). In summary, different correlates of tonotopic plasticity have been reported in the literature on hearing loss and tinnitus, and the translation of animal-models to human imaging is sparse, especially in tinnitus.

This large fMRI study examined the relation between hearing loss, tinnitus, and tonotopic reorganization with loudness-matched sound stimuli in humans. The inclusion of participants with high frequency hearing loss, both with and without tinnitus, allowed us to investigate to what extent reorganization is a consequence of hearing loss and whether any reorganization is specifically related to tinnitus.

MATERIALS AND METHODS

In accordance with the principles of the declaration of Helsinki (2013), the study was approved by the medical ethical committee of the University Medical Center Groningen, the Netherlands. Written informed consent was obtained, and participants received reimbursement for their participation.

PARTICIPANTS

A total of 113 participants, both male and female, were included in a larger MRI study. In 90 participants, three complete functional runs were obtained. This resulted in 35 participants with hearing loss and tinnitus, 17 participants with hearing loss without tinnitus, and 38 healthy controls without hearing loss or tinnitus (Table 1). None of the participants used hearing aids to compensate for their hearing loss or ameliorate their tinnitus. Pure tone audiometry was performed in a sound-attenuating booth to determine hearing thresholds for all participants at octave frequencies ranging from 0.125 to 8 kHz. Tinnitus pitch and loudness were estimated with a matching procedure. In addition, the participants completed the Tinnitus Handicap Inventory (McCombe et al., 2001), the Tinnitus Reactions Questionnaire (Wilson et al., 1991), the Hyperacusis Questionnaire (Khalfa et al., 2002) and the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983).

Group differences were tested with a Chi-square test of independence for the variable sex and a three-group ANOVA followed-up by independent pairwise t-tests for the variable age. The questionnaire scores were assessed by means of a Kruskal-Wallis test and followed up by a pairwise Mann-Whitney test.

EXPERIMENTAL DESIGN

Data acquisition

All MRI data were obtained with a 3.0 T Philips Intera MRI scanner (Best, the Netherlands) at the Neuro Imaging Center Groningen. The scanner was equipped with a SENSE 32-channel head coil. Both structural and functional images were obtained for each participant. The structural image was a whole-brain T1 weighted image (voxel size 1mm x 1mm x 1mm). The functional images were acquired in a sparse imaging sequence (Hall et al., 1999), as single shot EPI: 47 slices; no gap; scan matrix 72 x 67; descending slice order; TR of 10 seconds, TE 22 ms, Flip Angle 90°. For each participant, a total of three runs, of each 65 EPI volumes, were acquisitioned.

Sound stimuli

During the fMRI experiments, loudness matched auditory stimuli were presented. Prior to the MRI session, participants performed a binaural loudness matching task in which the stimulus tones at 0.25, 0.5, 2, 4, and 8 kHz were all matched in perceived loudness to a 1-kHz tone at 40 dB SPL. This loudness matching compensates for loudness distortion present in sensorineural hearing loss (Moore and Glasberg, 2004). In addition, studies indicate that sound-evoked cortical activation correlates better with loudness rather than the level of sound stimuli (Hall et al., 2001; Langers et al., 2007). A two alternative-forced-choice, 1-up-1-down loudness matching procedure was used to approximate equal loudness sensation over all frequencies. An interleaved staircase method was applied, with a maximum of 15 trials per frequency, seven reversals, and a step size of [10,5,5,3,3,1] dB SPL. This method yielded an equal loudness contour for each participant.

Procedure MRI

The individually loudness-matched auditory stimuli were presented during the relatively silent scanner intervals in the sparse sampling protocol. The auditory stimuli were 245 ms in length and were repeated at a 4-Hz repetition rate. Every volume acquisition consisted of 7.5 seconds of sound stimulation with one frequency, followed by 2 seconds of scanning. In addition to the sound stimuli, there was a silence condition. Stimulus conditions were presented binaurally in a quasi-random order via an MR Confon Sound System (Baumgart et al., 1998). Sound levels in the MRI were calibrated with a B&K 4134 microphone, inserted in the ear of a KEMAR dummy. To control for effects of attention, participants were instructed to perform a visual valence task similar to the task used by

Langers and van Dijk (2012). Participants were instructed that the sound stimuli were irrelevant and asked to concentrate on the visual task.

STATISTICAL ANALYSIS

Data Pre-processing

The fMRI data analysis was performed in MATLAB (version 2018a) and with the aid of SPM12 (Statistical Parametric Mapping). Functional images were pre-processed, realigned, and co-registered to the anatomical image, then normalized to fit a standard brain (MNI) and resliced to a voxel-width of 2 mm. The images were smoothed with a Gaussian kernel with full width half maximum of 5mm. During pre-processing, a logarithmic transformation was applied to the fMRI volumes to convert the output to units of percentage signal change (Langers and van Dijk, 2012).

A second level analysis was performed to assess the response to sound, voxel-by-voxel, on a group level, by means of an F-test on the six coefficients of the sound-frequency related regressors. A minimum cluster size of k > 1000 was used to exclude smaller activation clusters of no interest to tonotopic mapping. The remaining activation clusters were used to construct a Region-of-Interest (ROI) for further analyses (n = 5141 voxels).

Group comparisons

Group differences in median activation levels and corresponding Bayes Factors were calculated for each frequency. Differences in activation patterns between the groups were obtained by calculating the Euclidean distance per frequency, based on the mean signal change in all voxels:

$$d_{ab} = \sqrt{(\sum_i^n (x_{ai} - x_{bi})^2)},$$

where *a* and *b* refer to the two groups being compared, and the sum is taken over all n=5141 voxels in the cortical regions of interest. This distance was computed for each stimulus frequency. It is a measure of the difference in activation patterns between groups *a* and *b*. The voxels were assigned to the different frequencies according to their peak activation responsiveness. Permutation testing was performed to assess statistical significance of the group differences.

Principal Component Analysis

To obtain a robust measure for tonotopic map changes, a principal component analysis was performed by means of singular value decomposition, without centering (similar to Langers et al. (2012a)). The participant matrices (5141 × 6) were concatenated to form an aggregate matrix A of 462690 × 6 (90 participants

× 5141 voxels × 6 frequencies). The principal components (X_i) were extracted from this matrix A. Frequency-wise analyses were performed on the aggregate matrix A, expressing percentage signal change instead of principal component loadings. The advantage of performing PCA on one concatenated matrix containing data of all participants is that all PCA derived component maps are based on the same principal components and can therefore be compared across participants (Langers et al., 2014).

Assessment of the statistical significance of these principal component scores was done by calculating, for each pairwise group comparison, the Mahalanobis distance to quantify the magnitude of separation between the principal component clusters of the different groups. The method described here was coined by Goodpaster and Kennedy (Goodpaster and Kennedy, 2011). The Mahalanobis distance definition used was: $D_M(PC1, PC2) = \sqrt{d' C_W^{-1} d}$ based on the median voxel response per participant. With d expressed as the difference vector between the centroids of two groups according to $d = [C_{PC12} - C_{PC11}, C_{PC22} - C_{PC21}]$ and C_W^{-1} as the pooled variance-covariance matrix between two groups. A Hotelling's T^2 statistic was calculated to test if the cluster separation was significant between groups. The following equation was used: $T^2 = \frac{n1n2}{n1+n2} d' C_W^{-1} d$. The n values indicate the sample sizes of the two groups. A larger T^2 statistic indicates a larger distance between the PCA score centroids of the two groups. Next, an F-test was performed, and the F-value, the ratio of between group versus within group variance, computed according to: $F(p, n1 + n2 - p - 1) = \frac{n1+n2-p-1}{p(n1+n2-2)} T^2$, with p being the discriminator variables (the two PC's). The critical F-value was determined in a look-up table, based on the numerator and denominator degrees of freedom at $\alpha = 0.05$. This critical F value determines if the variance between the centroids of the two groups is significant. Finally, a p-value was calculated for each group comparison to determine the probability of this finding is small enough to reject the null-hypothesis, i.e. there are no differences in PC scores between the groups.

RESULTS

To assess differences in cortical responsiveness to sounds, sparse-sampled sound-evoked cortical activation was obtained for 38 control participants, 17 participants with hearing loss but without tinnitus, and 35 participants with hearing loss and tinnitus (Table 1). The participant groups with hearing loss were well matched on hearing loss (Fig 1A). There are no significant differences between the hearing loss groups at the included octave frequencies, except at 500 Hz (Mann-Whitney test, $p = 0.05$). The control group differs significantly from both hearing loss groups on all frequencies ($p < 0.05$). Accordingly, the mean equal loudness contours of the stimuli indicate that both hearing loss groups

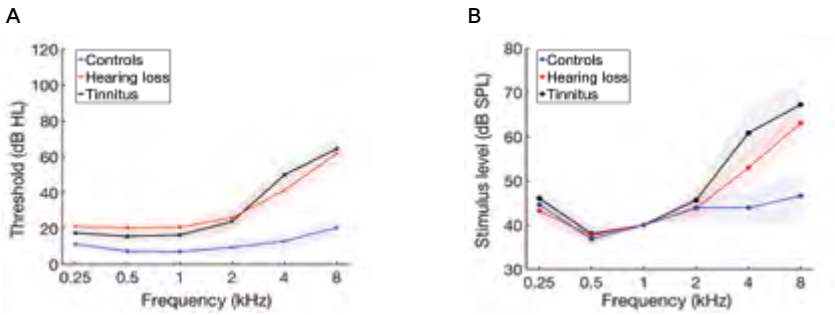

Fig 1. Hearing characteristics of participants. (A) Audiometric thresholds used in the MRI scanning protocol are indicated here, with their corresponding SE. (B) During MRI scanning, stimuli were presented at loudness levels equal to the 40-phon loudness curve. All stimuli were thus matched in loudness to a 1-kHz pure tone at 40 dB SPL. The average levels of the stimuli are depicted per group for the six frequencies presented along with their corresponding SE.

Table 1. Demographics and questionnaire scores of the three participants groups in this fMRI study.

Groups	Controls	Hearing loss	Tinnitus + Hearing loss
Demographics	N = 38	N = 17	N = 35
Sex	20 M, 18 F	9 M, 8 F	29 M, 6 F* **
Mean age (years)	46 ± 13 (22-67)	61 ± 10 (33-75) *	59 ± 10 (26-73) *
Questionnaires			
HADS Anxiety	4.6 ± 3 (0-11)	2.5 ± 3.1(0-11)	5.2 ± 3.7 (0-12)
HADS Depression	2.8 ± 2.7 (0-8)	1.8 ± 2.4 (0-10)	4.9 ± 3.9 (0-16)
HQ	11.7 ± 6.6 (3-27)	8.8 ± .8.5 (1-18)	18.5 ± 9.3 (0-33) * **
TRQ			32.1 ± 21 (0 - 78)
THI			37 ± 21 (4- 82)
Tinnitus			
Mean duration (years)			12.3 ± 8.6 (1-33)
Tinnitus Pitch			2 - 4 kHz (n =11)
			4 - 8 kHz (n = 3)
			≥8 kHz (n = 17)
			White noise (n = 4)

* indicates that the group differed significantly from the control group ** indicates that de hearing loss groups differed significantly from one another at $p < 0.001$. Chi-square, ANOVA, Kruskal-Wallis and Mann-Whitney, respectively

needed higher sound intensities to perceive equal loudness at 4 and 8 kHz than the control group (Fig 1B).

The groups differ significantly in terms of sex distribution ($p = 0.014$), with a significantly larger proportion of men in the tinnitus group. A significant difference in age ($F_{14,72}$, $p < 0.001$) existed between the groups, which is due to the difference between the tinnitus and control group ($p< 0.001$) and the hearing loss and control group ($p < 0.001$). There is no significant difference in age ($p = 0.529$) between the groups with hearing loss, with or without tinnitus. HADS subscales did not show significant group differences. HQ score distributions differed significantly between the groups ($p = 0.002$). Post-hoc testing showed that the hearing loss and control groups did not differ significantly ($p = 0.238$), nor the tinnitus and control comparison ($p = 0.057$) in contrast to the tinnitus and hearing loss comparison ($p = 0.002$). In the hearing loss group with tinnitus, 5 participants had HQ scores that could indicate a reduced tolerance to sound. The exclusion of these participants did not alter any of the measures displayed, and hence they were included in the analyses.

SOUND-EVOKED ACTIVATION

To determine the sound-evoked cortical activation, regions of interest (ROIs) were constructed based on the overall significantly activated voxels in response to sound across all 90 participants (FWE < 0.05, cluster size k > 1000; Fig 2A). These group-based ROIs were obtained by the equal weighting of the six sound-stimulus regressors in an omnibus F-test. All subsequent second-level analyses were performed on these 5141 voxels corresponding roughly to the bilateral auditory cortices. For each stimulus frequency, the average signal change was computed across all voxels in the ROI. The cortical response to 8 kHz is significantly larger in the tinnitus (Mann-Whitney test, $p = 0.025$, $Z = 2.25$, $BF_{10} = 1.82$) and the hearing loss ($p = 0.003$, $Z = 2.94$, $BF_{10} = 5.24$) groups compared to the control group, and this response is large in comparison to voxels with different preferred frequencies (Fig 2B).

Nevertheless, the Bayes Factors (BF_{10}) indicate that this effect is more robust for the hearing loss group without tinnitus. A one-way ANOVA indicated that the differences in percentage signal change between participants was not explained by age ($F(2,41) = 1.167$, $p = 0.341$), or sex differences ($F(2,1) = 0.287$, $p = 0.599$), but confirmed the significant differences for group ($F(2,2) = 4.17$, $p = 0.026$).

Similarity in cortical activation patterns was investigated by means of a Euclidean distance measure, calculated for all three group comparisons. A small Euclidean distance between the two groups implies that their cortical activation patterns are similar. The cortical activations patterns of the group with tinnitus and the control group are most similar to each other, except at 8 kHz (Fig 2C). At

8 kHz, the activation pattern of the hearing loss group without tinnitus diverged strongly and significantly ($p < 0.0028$) from the control group. In the group with tinnitus, a similar but non-significant shift was observed.

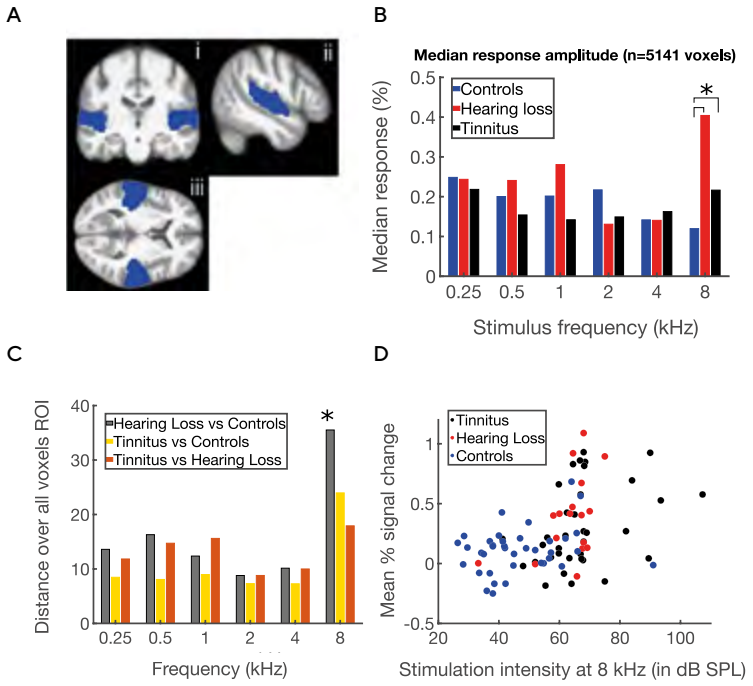

Fig 2. Sound-evoked activation levels. (A) Regions-of-interest based on overall activated voxels (n = 5141) in response to sound across all 90 participants. (B) Group level responsiveness profile, based on percentage signal change in ROI voxels in response to the six presented frequencies. At $p < 0.05$, a significant difference in the responsiveness levels is observed for both hearing loss groups, with and without tinnitus, compared to the control group, in response to 8 kHz stimulation ($p = 0.02$ and $p = 0.003$). However, significance remains when corrected for multiple comparisons (Bonferroni corrected 0.05/6=0.008), only for the hearing loss group without tinnitus. (C) Euclidian distance between response profiles of participant groups, per frequency. The distance was computed using the response amplitudes of all voxels as spatial response profile. A smaller distance indicates more similar voxel responses on that frequency. The statistical significance of the distances was determined by permutation testing (n = 50000). The distance between hearing loss without tinnitus and controls is significant for 8 kHz ($p < 0.0028$, Bonferroni corrected). (D) Mean percentage signal change per group during 8 kHz stimulation. Per participant, the level of stimulation (in dB SPL) at 8 kHz is plotted against the mean percentage signal change over all voxels in the region-of-interest. Even though the absolute and mean highest percentage signal change occurred in the hearing loss group, the highest stimulation levels were applied in the tinnitus group.

Additional analyses were performed to investigate if the highest stimulation levels could explain the highest responsiveness levels at 8 kHz. Due to the presence of high-frequency hearing loss, both hearing loss groups with and without tinnitus were stimulated at higher intensities in the high frequencies than the control group. For each participant, the percentage signal change in response to 8 kHz stimulation was plotted against stimulation intensity (Fig 2D). The highest stimulation levels occurred in the tinnitus group, whereas the highest percentage signal change occurred in the hearing loss group. The over-representation of high frequencies persists when only moderate hearing losses (≤60 dB HL at 8 kHz) or mild stimuli levels (< +1SD control mean) are considered. This suggests that higher levels of activation are not merely the direct result of higher levels of stimulation.

PRINCIPAL COMPONENT ANALYSIS

To obtain robust tonotopic response maps, a principal component analysis was used (PCA). The first and second principal component's response profiles, overall voxels, were obtained by an analysis that included all three participant groups (Fig 3A, B). We included the first two principal components, with the first principal component explaining 73% of the signal's variance and the second component an additional 11%. The first principal component reflects overall responsiveness to sound stimulation (Fig 3A), as a direct comparison to the overall activation confirmed.

The tonotopic maps could be inferred from the cascaded response profile of the second principal component, which shows a stage wise increase from negative loadings on low frequencies to positive loadings on high frequencies (Fig 3B). The aggregate responses were portioned into individual spatial response maps to compute the average group maps (Fig 3C). This analysis showed that the high frequencies are more dominant in the spatial frequency group maps of both hearing loss groups than in the maps of the controls. This high frequency dominance is strongest for the hearing loss group without tinnitus (Fig 3C).

Assessment of the differences in principal component scores of the first and second principal components was done by calculating the Mahalanobis distance, Hotelling's T^2, F-statistics, and p-values (see Table 2). These analyses showed that the principal component scores, both for the first and the second principal components, of the hearing loss group without tinnitus were significantly different from those of the control group, as indicated by the critical F value and p-value ($p = 0.012$) at a level of p for multiple comparisons ($p=0.0167$). The difference between the principal component scores of the hearing loss group with tinnitus and the control group nearly reached significance ($p=0.0175$). In contrast, the hearing loss groups, with and without tinnitus, were not significantly different from one another ($p=0.5864$).

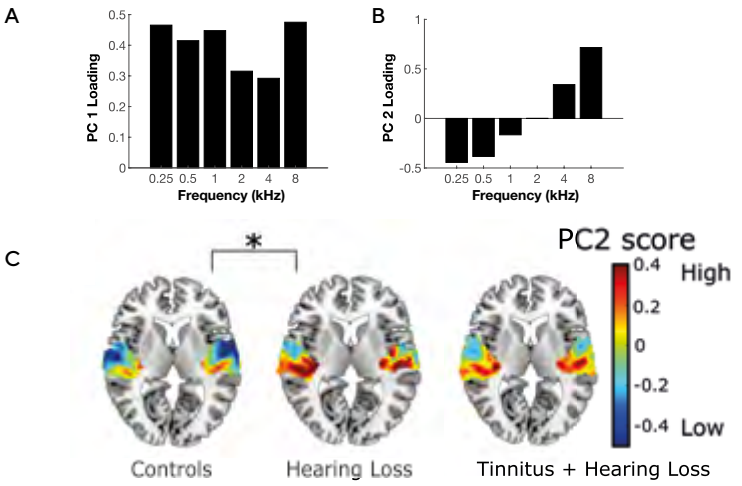

Fig 3. Characterization of tonotopic organization by principal component analysis (PCA). (A) Frequency dependent response profile of the first and (B) second principal component. (C) Spatial frequency group maps, based on the component strength of the second principal component. Positive component scores indicate high frequency responsiveness (i.e. more responsive to high than low frequencies), whereas a negative score indicates responsiveness to low frequencies. A Hotelling's T^2 statistic was calculated to compare the principal component clusters and indicated a statistically significant difference between the second principal component scores of the hearing loss group without tinnitus compared to those of the control group ($p = 0.012$).

Table 2. Summary of pairwise cluster separation of the first and second component given by Mahalanobis distances, Hoteling's T2 statistic, F0-statistics and p-values.

	Mahalanobis distance	Hotelling's T^2 statistic	F-value	p
Hearing loss vs Controls	0.9145	9.82	4.82	0.0120*
Tinnitus + Hearing loss vs Controls	0.6912	8.70	4.29	0.0175
Tinnitus + Hearing loss vs Hearing loss	0.3102	1.10	0.54	0.5864

* indicates that the groups differed significantly from one another on a p level corrected for multiple comparisons.

DISCUSSION

Our findings show that functional reorganization of the auditory cortex is less pronounced in hearing loss with tinnitus than in hearing loss without tinnitus. Both the response amplitudes and the tonotopic map characteristics in participants with tinnitus were intermediate to those of normal hearing control participants and hearing loss participants without tinnitus. Thus, the reorganization is a consequence of hearing loss and is more conservative in hearing loss with tinnitus. In other words, the presence of tinnitus in hearing loss appears not to relate to excessive cortical plasticity but instead to more diminished adaptation than in hearing loss alone.

The increased response amplitudes in both hearing loss groups were present only at 8 kHz, the highest frequency tested. The hearing loss was at its largest at this frequency for the majority of our hearing loss participants (75%). This profile is typical for (age-related) high-frequency sensorineural hearing loss (Gates and Mills, 2005). It is worth noting that the stimuli in our experiments were loudness matched across frequency for each participant individually. This loudness matching ensured that all stimuli were audible and perceived as equally loud, regardless of raised hearing thresholds. Consequently, the stimulus intensity levels at higher sound frequencies were increased in the hearing loss groups, with and without tinnitus, compared to the normal hearing participants (Fig 1). In the tinnitus group, this effect was not related to the tinnitus frequency. Even though most tinnitus participants had high frequency tinnitus (see Table 1), the tinnitus pitch was not significantly correlated with the frequency eliciting the highest percentage signal change ($R = -.217$, $p = 0.276$). The lack of significant correlation suggests that the increased responsiveness at 8 kHz is not related to the tinnitus itself but rather to the accompanying hearing loss. This is in line with the finding that this increase in responsiveness is present in both the hearing loss group with and without tinnitus.

Generally, the stimulus levels were similar in the two hearing loss groups. Although in some instances, the intensities were larger in the hearing loss group with tinnitus (Fig 2C; data points at 80-110 dB SPL). Hence, it is quite remarkable that the cortical responses were largest in the hearing loss group without tinnitus, despite that the stimulus intensities did not surpass those of the hearing loss group with tinnitus. Similarly, the largest differences in the tonotopic map were found when contrasting the hearing loss group without tinnitus to the normal hearing participants. Conversely, the tonotopic map of the hearing loss participants with tinnitus was more similar to those of normal hearing participants (Fig 2 and 3). Since these differences cannot simply be accounted for by the differences in stimulus intensities, it may reflect different degrees of (re)organization of the auditory system for participants with hearing loss and tinnitus compared to those without tinnitus.

The majority of tinnitus related fMRI studies included participants with normal hearing thresholds or mild hearing losses. The results across these studies are variable. Gu et al. reported elevated auditory cortex activation in tinnitus participants with normal hearing (Gu et al., 2010). Unfortunately, their hyperacusis controlled design resulted in relatively small participant groups (n = 7 with tinnitus, n = 5 without tinnitus). In a similar fMRI study by Langers et al., cortical response amplitudes were similar between normal hearing participants with and without tinnitus, except for a small region in the lateral portion of the left Heschl's gyrus (Langers et al., 2012). Similarly, Lanting et al. reported no differences in cortical response amplitudes in relation to unilateral tinnitus and mild to moderate hearing loss (Lanting et al., 2008). In contrast, Hofmeier et al. showed a pronounced reduction of the cortical responses in tinnitus participants with mild hearing loss in a study that excluded hyperacusis (Hofmeier et al., 2018).

The present study included participants with moderate to profound high-frequency hearing loss. In both hearing loss groups, with and without tinnitus, increased responsiveness to 8-kHz stimulation was observed compared to the normal hearing control group. These findings are in line with Ghazaleh et al., who reported no tinnitus-related differences in tonotopic map characteristics in participants with unilateral hearing loss and tinnitus (Ghazaleh et al., 2017). Boyen et al. also found no differences in cortical responses between hearing loss with and without tinnitus (Boyen et al., 2014). Even though the hearing loss in the Hofmeier study was very mild, up to 40 dB per frequency, the results are very similar to that of the current study. There is no obvious explanation for the variability across these studies; however, the studies with larger participant groups (Lanting et al., 2008; Langers et al., 2012; Hofmeier et al., 2018) suggest that response amplitudes are either similar of reduced in tinnitus.

The reduced sound-evoked cortical amplitudes in hearing loss with tinnitus (Fig 2 B; (Hofmeier et al., 2018)) have been interpreted as a failure to increase response gain (Knipper et al., 2013; Hofmeier et al., 2018). This failure to increase response gain in the presence of heightened spontaneous activity presumably results in tinnitus. The cortical inability in tinnitus to adapt sufficiently to hearing loss finds a rational in reduced levels of Arc, a cytoskeletal protein involved in long-term synaptic plasticity (Nikolaienko et al., 2018), as reported in the auditory cortex of tinnitus animals (Tan et al., 2007; Rüttiger et al., 2013). Whereas generally, Arc is mobilized after inducing hearing loss (Kapolowicz and Thompson, 2016), the expression of Arc is significantly reduced in animals that develop tinnitus (Rüttiger et al., 2013). These findings support the notion that tinnitus in the presence of hearing loss is associated with insufficient adaptation to hearing loss at a cortical level.

The enhanced representation of high frequencies in hearing loss appears to contrast with some animal models of tonotopic reorganization. Several animal studies reported the absence of high frequency responsiveness in the auditory cortex and over-representation of low-frequencies in animals with induced high frequency hearing loss (Rajan and Irvine, 1998; Irvine et al., 2000; Norena and Eggermont, 2005). The differences between these animal studies and our human data presumably relate to differences in techniques used to assess cortical neural activity. The animal models were based on the best- or characteristic frequencies of cortical neurons measured with near-threshold stimuli. This method is especially informative of the spatial localization and extent of the cortical area that preferentially responds to a particular frequency. In our study, we measured BOLD-responses at supra-threshold levels. The BOLD response is informative of the cortical area that responds to sound stimulation and the intensity or amplitude of this response. Therefore, these findings may not contrast each other but instead investigate a different aspect of the cortical responses to sound.

Finally, although our results show group differences in the auditory cortex, it is not clear whether these differences arise due to changes in the function of the cochlea or the brain. Naturally, sensorineural hearing loss involves cochlear pathology. However, the differences observed between the hearing-impaired participants with tinnitus and those without tinnitus may be due to both cochlear and central differences. Recent evidence suggests that tinnitus is associated with reduced ribbon synapse density in the cochlea (Rüttiger et al., 2013; Zhang et al., 2014) and reduced ARC expression in the cortex (Rüttiger et al., 2013; Singer et al., 2013). With the measures of the present study, i.e. pure tone audiometry and MRI, it is not possible to identify differences in cochlear pathology between the hearing loss groups.

Limitations

In earlier studies by Profant et al., the authors described that with increasing age, stronger sound evoked responses were observed in the auditory cortex (Profant et al., 2015; Profant et al., 2014). To investigate if age differences did not cause the observed group differences in the present study, we plotted per group the age of participants against their high frequency evoked cortical activation to observe any correlation. This demonstrated that none of the groups showed any significant or near significant correlation between age and high-frequency evoked cortical activation levels (THL R = -.105, p = 0.547; HL R = .119, p = 0.650; CO R =0.246, p = 0.137). However, it is worth noting that our hearing loss group without tinnitus has fewer younger people than the hearing loss group with tinnitus.

CONCLUSION

In conclusion, hearing loss was associated with higher levels of sound-evoked cortical responsiveness, and this increase was most pronounced in the group with hearing loss but without tinnitus. Both in terms of response amplitudes and tonotopic map characteristics, the participants with hearing loss and tinnitus appear intermediate to the controls and the hearing loss participants without tinnitus. This finding suggests that tinnitus is related to an incomplete form of central compensation to hearing loss rather than excessive adaptation. As a consequence, treatments for tinnitus may need to enhance the cortical plasticity rather than reversing it.

CHAPTER 05

HYPERACUSIS IN TINNITUS PATIENTS RELATES TO ENLARGED SUBCORTICAL AND CORTICAL RESPONSES TO SOUND EXPECT AT THE TINNITUS FREQUENCY

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ABSTRACT

Hyperacusis, hypersensitivity to sounds of mild to moderate intensity, has been related to increased neural gain along the auditory pathway. To date, there is still uncertainty on the neural correlates of hyperacusis. Since hyperacusis often co-occurs with hearing loss and tinnitus, the effects of the three conditions on cortical and subcortical structures are often hard to separate. In this fMRI study, two groups of hearing loss and tinnitus participants, with and without hyperacusis, were compared to specifically investigate the effect of the latter in a group that often reports hyperacusis. In 35 participants with hearing loss and tinnitus, with and without hyperacusis, as indicated by a cut-off score of 22 on the Hyperacusis Questionnaire (HQ), subcortical and cortical responses to sound stimulation were investigated. In addition, the frequency tuning of cortical voxels was investigated in the primary auditory cortex. In cortical and subcortical auditory structures, sound-evoked activity was higher in the group with hyperacusis. This effect was not restricted to frequencies affected by hearing loss but extended to intact frequencies. The higher subcortical and cortical activity in response to sound thus appears to be a marker of hyperacusis. In contrast, the response to the tinnitus frequency was reduced in the group with hyperacusis. This increase in subcortical and cortical activity in hyperacusis can be related to an increase in neural gain along the auditory pathway and the reduced response to the tinnitus frequency to differences in attentional resources allocated to the tinnitus sound.

INTRODUCTION

Hyperacusis is characterized by the experience of uncomfortable loudness for sounds that are not uncomfortably loud to most people (Anari et al., 1999; Baguley, 2003). In other words, this heightened sensitivity to sound intensity occurs in response to soft and moderate sounds. Hyperacusis often co-occurs with hearing loss; 59.1% of people with hyperacusis also have hearing loss, according to a physician established incidence report (Paulin et al., 2016). In hearing loss, loudness perception is altered due to a reduction in the dynamic input range that results in a diminished loudness output range. Consequently, a steeper increase in loudness ensues, or loudness recruitment, for frequencies affected by the hearing loss. In hyperacusis, loudness recruitment is often steeper than in hearing loss alone and can be present without a reduction in the dynamic input range. In addition to comorbidity with hearing loss, hyperacusis often co-occurs with tinnitus, with an estimated prevalence of 55 - 86 % of hyperacusis in tinnitus patients (Anari et al., 1999; Dauman and Bouscau-Faure, 2005; Schecklmann et al., 2014). Tinnitus is the perception of sound in the absence of an external source. It is a common symptom that occurs in 12 – 30 % of the general population, although prevalence estimates of tinnitus vary (McCormack et al., 2016). The prevalence rises to higher estimates with increasing age, and tinnitus is present in the majority of people with hearing loss (Tan et al., 2013). Both hyperacusis and tinnitus are debilitating symptoms, and even though several treatment options are available, there is presently no cure for either condition.

Currently, there is no comprehensive knowledge of the mechanisms behind tinnitus and hyperacusis. Hyperacusis and hearing loss have been explained by non-linear neural gain models (Diehl and Schaette, 2015) and investigated with animal experimental work (Auerbach et al., 2019). According to the neural gain model, neural gain in the central auditory pathway is triggered by a decrease in peripheral input (Schaette and Kempter, 2006). This reduction in input corresponds to hearing loss. Within this model, hyperacusis is hypothesized to result from abnormal gain along the auditory pathway in response to sound-evoked activation (Gu et al., 2010; Diehl and Schaette, 2015). In contrast to sound-evoked activation, tinnitus is explained by the amplification of spontaneous neural activity in the central auditory system in a manner similar to sound-evoked activity, causing the spontaneous activity to cross the threshold of perception (Auerbach et al., 2014; Diehl and Schaette, 2015). However, whereas this framework of neural gain can incorporate that tinnitus and hyperacusis do not always co-occur (Penner, 1986a), it does not incorporate that hearing loss is not always present in hyperacusis or tinnitus (Tan et al., 2013). Other models of tinnitus proposed that tinnitus results from increased central noise, a different mechanism from the increased non-linear gain implicated in hyperacusis and hearing loss (Knipper et al., 2013; Zeng, 2013). In this view, tinnitus is associated

with reduced gain in the auditory pathway (Hofmeier et al., 2018) and reduced connectivity along the auditory pathway (Boyen et al., 2014; Lanting et al., 2016). Therefore, two different pathways are proposed for the origins of tinnitus and hyperacusis, whereas central gain is specifically related to hyperacusis, tinnitus may be related to increased central noise in the auditory system.

To date, there is still uncertainty on the neural correlates of hearing loss, tinnitus, and hyperacusis. Since these conditions often co-occur, this hampers the separation of their effects on the central auditory system. Previous neuroimaging studies indicate that both subcortical and cortical sound-evoked activity is increased in the presence of hyperacusis (Gu et al., 2010; Knipper et al., 2013; Rüttiger et al., 2013; Chen et al., 2015). In other studies, both tinnitus and hyperacusis were co-occurring, and the effects of both conditions proved challenging to disentangle since their co-occurrence was not controlled for (Lanting et al., 2008; Melcher et al., 2009). Previous studies that specifically focused on hyperacusis were performed with individuals with no or minimal hearing loss. In general, tinnitus patients with hearing loss are underrepresented in these fMRI studies on hyperacusis, even though tinnitus, hearing loss, and hyperacusis often co-occur.

The current study aims to specifically evaluate subcortical and cortical responses in tinnitus patients with and without hyperacusis. A distinctive characteristic of this study, and our previous study (Koops et al., 2020), is the focus on individuals with moderate sensorineural hearing loss, a group that is often encountered in tinnitus clinics. Subcortical responses to sound stimulation were investigated for tinnitus patients with and without hypersensitivity to sound, as indicated by a score of ≥ 22 on the Hyperacusis Questionnaire (Aazh and Moore, 2017). In addition, cortical sound-evoked responses, the cortical response to the presentation of the tinnitus frequency, and the tuning of cortical voxels were investigated in the auditory cortex of tinnitus patients with and without hypersensitivity to sound.

MATERIALS AND METHODS

The medical ethical committee of the University Medical Center of Groningen, the Netherlands, approved this study. The study was performed in accordance with the principles of the declaration of Helsinki (2013), and participants received reimbursement for their time and signed a written informed consent document.

PARTICIPANTS

In the context of a larger MRI study (Koops et al., 2020), 35 participants with hearing loss and tinnitus were included. Hearing thresholds were obtained in a sound-attenuating booth for octave frequencies 0.125 to 8 kHz and additional for 3 and 6 kHz. None of the participants used hearing aids to compensate for

their hearing loss or improve their tinnitus. All participants were requested to fill in the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983), the Hyperacusis Questionnaire (Khalfa et al., 2002), the Tinnitus Handicap Inventory (McCombe et al., 2001), and the Tinnitus Reactions Questionnaire (Wilson et al., 1991). Hyperacusis was defined as an HQ score of 22 or higher, in line with the recommendation of Aazh and Moore (2017).

Care was taken to prevent discomfort for participants during participation in this study. The recruitment advertisement specifically noted that MRI research is rather noisy. During contact with the researcher, either via e-mail or a phone call, it was stressed that although the sound levels within the MRI are not harmful with the earphones on, the scanner noise is still loud. Despite our precautions, two participants expressed discomfort during the scanning procedures described below.

For the variable sex, group differences were tested with a Chi-square test of independence. For the variables age and tinnitus duration, an independent pair-wise t-test was used. Group differences in hearing thresholds, stimulation intensity, questionnaire scores, and tinnitus loudness and pitch were tested using an independent-sample Mann-Whitney U test.

EXPERIMENTAL DESIGN

Data acquisition

A 3.0 T Philips Intera MRI scanner (Best, the Netherlands), equipped with a SENSE 32-channel head coil, situated at the Neuro Imaging Center in Groningen, was used to acquire the MRI scans. A sparse imaging paradigm was used to obtain the functional volumes and minimize scanner noise interference with the auditory task (Hall et al., 1999). A whole-brain structural T1 weighted scan (1mm x 1mm x 1mm) was obtained in the same session to facilitate co-registration and normalization of the functional MRI scans. The functional images were acquired in 47 slices, single-shot EPI with no gap, in descending order with a scan matrix of 72 x 67, FOV 210x210x141, and a TR of 10 seconds, TE 22 ms, Flip Angle 90°. A total of three runs of 65 EPI volumes, lasting 10 minutes per run, were acquired for each participant. A single brain volume acquisition consisted of 2 seconds of scanning and was preceded by a stimulus of 7.5 seconds duration. This sparse sampling protocol was employed to ensure that the sound presentation coincided with the relative quiet of the interscan intervals.

Sound stimuli

Each participant performed a binaural loudness matching task prior to the MRI scanning, where they matched the perceived loudness of tones at 0.25, 0.5, 2, 4, and 8 kHz to that of a 1-kHz tone at 40 dB SPL. To obtain an equal-loudness contour for each participant, a two alternative-forced-choice interleaved

staircase procedure was used with 15 trials per frequency, 7 reversals, and a step size of [10,5,5,3,3,1,1] dB. All participants performed the loudness matching task twice to ensure proper understanding of the task. The thresholds from the second trial of the loudness matching task were used to set the intensities for the stimuli presented during the MRI scanning. Both the headphones used in the MRI and the headphones used in the sound-attenuating booth were calibrated with a B&K 4134 microphone inserted in the ear of a KEMAR dummy. Loudness matching was performed to improve comparability between participants since both participants with and without hearing loss were included in the larger fMRI-study. Thus, the use of loudness matching established audibility of the stimuli for all participants, with and without hearing loss, and equal loudness over frequencies within a participant. The use of loudness-based stimuli builds on the finding that sound-evoked cortical activation correlates well with the perceived loudness of a tone in both normal-hearing participants and hearing-impaired participants (Langers et al., 2007). Additionally, loudness correlates better with the Blood Oxygenation Response Levels used in fMRI, than sound intensity (Hall et al., 2001; Langers et al., 2007).

Procedure MRI

All sound conditions, i.e. loudness-matched tones at frequencies ranging from 250 – 8000 Hz and a silence condition, were presented binaurally in a quasi-random order. The stimuli consisted of tones of 245 ms in duration at a 4 Hz repetition rate, with the total duration of sound stimulation lasting 7.5 seconds for each volume acquisition. An MR Confon Sound System (Baumgart et al., 1998) was used to deliver the sound stimuli in the MRI during the sparse-sampling protocol. Simultaneously with the presentation of the auditory stimuli, participants performed a visual valence task (Langers et al., 2012). In order to control for attention, participants were instructed to focus on and respond to the visual valence task.

STATISTICAL ANALYSES

Data Preprocessing

Data analysis was performed with SPM12 (Statistical Parametric Mapping) and MATLAB (version 2020a). The functional MRI images were first realigned, then co-registered to the anatomical image, and normalized to fit a standard MNI brain which resulted in the reslicing of the images to an isotropic voxel-size of 2 mm. Smoothing was performed with a full-width half-maximum Gaussian kernel of 5mm. A logarithmic transformation was used to convert the fMRI output into percentage signal change (Langers and van Dijk, 2012).

Subcortical Regions-of-Interest

The subcortical auditory regions incorporated in the subcortical mask were drawn in Mrtrix (Tournier et al., 2019) on the anatomical SPM12 MNI-template.

Included areas are the Cochlear Nucleus (CN), Superior Olivary Complex (SOC), Inferior Colliculus (IC), and Medial Geniculate Area (MGB) of the thalamus. The MGB and IC are recognizable on an anatomical template, whereas the CN and SOC are not. For the CN and SOC, we based the location of our ROIs on a recent functional imaging study identifying activation in these areas (Sitek et al., 2019). Masks were drawn larger than the actual structure to ensure that all of the intended areas were included. FSL was used to combine these regions into a single template. Group differences were tested with a two-sample t-test.

In addition to the ROI analysis, the average percentage signal change in response to sound was calculated for each subcortical region for all voxels that showed a significant response to sound at FDR < 0.05. Differences in sound-evoked responses were tested with a repeated-measures ANOVA to investigate differences within subjects over auditory areas and to compare the subcortical and cortical activation of participants with high (≥ 22) and low scores (<22) on the Hyperacusis Questionnaire. For each voxel that showed a significant difference in activation, it was determined with the MNI template and FSLeyes (0.26.1; McCarthy, 2020) if this voxel was indeed part of the auditory subcortical structures.

Furthermore, two-sample t-tests were performed to investigate the presence of frequency-specific differences in subcortical activation between the groups with high and low HQ scores. Finally, the subcortical response to the tinnitus frequency, or the frequency closest to the tinnitus frequency, was compared between the groups with high and low scores on the HQ. This was done by means of a two-sample permutation t-test (n =5000), permuting the participants over the groups.

Cortical Regions-of-Interest

The cortical region-of-interest analyses were masked by the anatomical Brodmann areas 41, 42, and 22 that correspond to the auditory cortex. These masks were defined with WFU Pickatlas (Maldjian et al., 2003). Brodmann area 41 corresponds to the primary auditory cortex, Brodmann area 42 to the secondary auditory cortex, and Brodmann area 22 is the association auditory cortex. Average percentage signal change in response to sound was calculated for the primary, secondary, and association auditory cortex. Furthermore, for these areas, the average response per frequency was calculated. Two-sample t-tests were performed per frequency response to test for frequency-specific differences in the auditory cortex (BA41, BA42, BA22) between tinnitus participants with high and low scores on the HQ. Finally, the cortical response to the tinnitus frequency, or the frequency closest to the tinnitus frequency, was compared between the groups with high and low scores on the HQ. Statistical testing was performed with a permutation (n=5000) two-sample t-test, permuting the participants over the groups.

Tuning of cortical voxels

For all participants, a voxel tuning measure was derived for the cortical region of Brodmann area (BA) 41, based on a 'best frequency' tonotopic map (Berlot et al., 2020). For every voxel, the frequency condition that elicited the highest response was obtained, and the voxels were classified according to this peak-frequency responsiveness. This was performed for the voxels that were significantly activated within the anatomical mask of BA 41 in response to sound at an FWE p-value of < 0.05. The tuning of each voxel was then determined by the responses of the voxels in BA 41 to the six presented frequencies (i.e. both for the amplitude in response to the preferred frequency and the amplitude in response to non-preferred frequencies), in line with the method proposed in the paper of Berlot et al., 2020. The largest response, or best frequency, was normalized to 1. A permutation two-sample T-test (n = 5000) on the average of the non-preferred frequencies, where the participants were permuted over the groups, was used to compare the voxel tuning of the tinnitus group with high HQ and low HQ scores. This analysis was performed for the non-preferred frequencies, i.e. the 2nd and 3rd frequency away from the preferred frequency. The permutation testing was done by extracting the responses to the best and non-preferred frequencies on a per frequency level. These responses were normalized and pooled to obtain a matrix with responses to the non-preferred frequencies for each BF. If there was a second frequency away from the BF on either side of the BF, the average of these was taken.

RESULTS

BEHAVIOURAL RESULTS

In total, 11 of the participants had a hyperacusis (HQ) score ≥ 22. For the remaining 24 participants, the hyperacusis score was below 22. Hearing thresholds were not significantly different between the groups with high and low HQ scores, as shown by an independent-samples Mann-Whitney U-Test (see Figure 1 A). In line with this, there were no significant differences in the intensity of the stimuli presented during scanning (see Figure 1 B).

Additionally, the groups were not significantly different in terms of sex distribution (p = 0.392), age (t = 0.159, p = 0.875), or tinnitus pitch (p = 0.91) and loudness (p = 0.88). The group with higher HQ scores had a significantly longer duration of tinnitus (t = 2.3, p = 0.031), and higher THI total scores (p = 0.005). There were no significant differences in terms of scores on the HADS Anxiety (p = 0.195) or Depression scale (p = 0.08), although the effect on the latter approached significance. See Table 1.

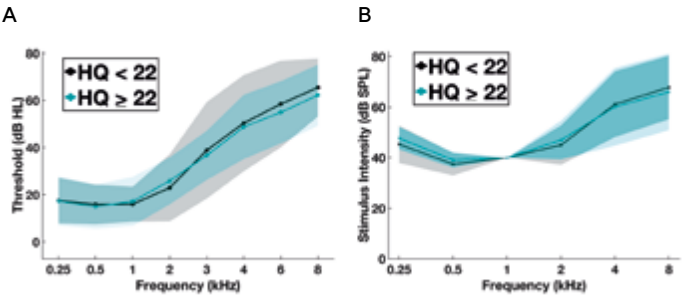


Figure 1. (A) Mean audiometric thresholds of participants. Shading indicates group standard deviations. In black, the mean thresholds of participants with HQ scores < 22, and in blue, the mean thresholds of participants with HQ scores < 22. There are no significant differences between the groups on any of the frequencies (250 Hz p = 0.64; 500 Hz p = 0.77; 1 kHz p = 0.69; 2 kHz p = 0.47; 3 kHz p = 0.79; 4 kHz p = 0.71; 6 kHz p = 0.52; 8 kHz p = 0.39). (B) Intensity of loudness matched stimuli presented during MRI scanning. All stimuli were matched in loudness to a 1 kHz tone at 40 dB SPL, resulting in a 40-phon loudness contour. Depicted are the averaged intensities of the presented stimuli and the corresponding group standard deviations.

Table 1. Demographical information and questionnaire scores of the two participant groups.

Groups	HQ < 22	HQ > 22
Demographics	N = 24	N = 11
Sex	19 M, 5 F	10 M, 1 F
Mean age (years)	59 ± 11 (26-72)	59 ± 9 (41-73)
Questionnaires		
HADS Anxiety	4 ± 3 (0-11)	6 ± 4 (0-12)
HADS Depression	4 ± 3 (0-8)	7 ± 4 (0-16)
HQ	12 ± 6 (0-21)	28 ± 5 (22-37)*
THI	27 ± 19 (4- 76)	48 ± 19 (20- 82)*
Tinnitus		
Mean duration (years)	10 ± 6 (2-20)	17 ± 9 (1-33)*
Tinnitus Pitch	1 - 4 kHz (n = 8)	1 - 4 kHz (n =5)
	5 - 7 kHz (n = 3)	5 - 7 kHz (n = 2)
	≥8 kHz (n = 9)	≥8 kHz (n = 4)
	Broad Band (n = 4)	
Tinnitus loudness	60 dB HL ± 17 (30-100)	61 dB HL ± 16 (40-85)

* indicates that groups differed significantly from one another at p < 0.001. Chi-square, ANOVA, Kruskal-Wallis and Mann-Whitney, respectively

SUBCORTICAL RESPONSES INCREASED IN HYPERACUSIS

In the subcortical auditory pathway, the comparison of sound-evoked activation in participants with high versus low HQ-scores showed that higher hyperacusis scores were related to higher sound-evoked responses in the area of the bilateral superior olivary complex, the right inferior colliculus, and right medial geniculate body. See Table 2 and Figure 2.

Table 2. Region-of-interest analysis comparing high vs low HQ scores in hearing loss and tinnitus participants. Significance, cluster size, T values, and MNI coordinates of the region of interest analyses are displayed. A mask was drawn on the MNI template and included the bilateral cochlear nucleus, superior olivary complex, inferior colliculus, and medial geniculate. The significant differences are reported in the table.

	Cluster level		*Peak level*				*Area*	
FWE-corrected	*k*	*p*	*T*	*MNI Coordinates*			*Lat*	*Region*
				x	*y*	*z*		
High HQ> Low HQ	3	0.023	5.7	14	-18	-4	R	Medial Geniculate Nucleus
	3	0.011	4.9	6	-32	-8	R	Inferior Colliculus
	4	0.008	4.4	8	-34	-36	R	Superior Olivary Complex
	2	0.016	4.3	-4	-34	-34	L	Superior Olivary Complex

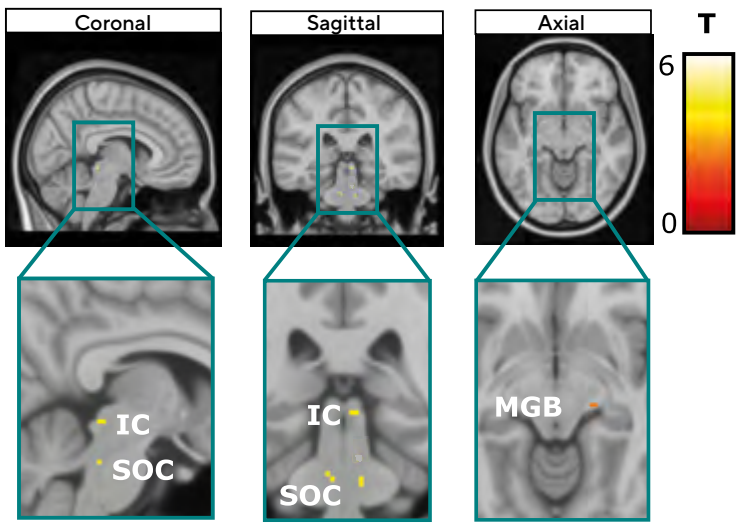


Figure 2. Increased sound-evoked activation in higher HQ scores vs lower HQ scores. In the panels of the sagittal, axial, and coronal view, an enlarged area of the increased activation is shown. All voxels indicated here show a significant difference in activation at an FWE-level of 0.05. See also Table 2.

This region of interest analysis identified specific voxels that showed a difference in responsiveness. Whereas we could obtain significant responses in the subcortical areas when we investigated the responses to all sound conditions together, we could not robustly identify significant activation in all subcortical ROIs if we included only one frequency at a time. Therefore, we could not specify if there were frequency-specific differences in subcortical sound-evoked activation between the groups.

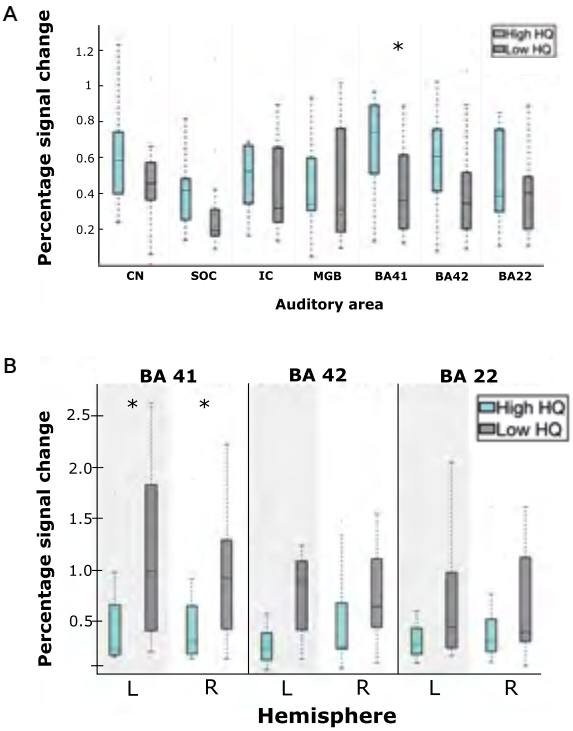


Figure 3 (A) Average sound-evoked responses in brain areas along the central auditory pathway. The averaged responses to sound of the various auditory areas included in our analyses are presented for the group with low and high HQ scores. (B) Response of the auditory cortex to a sound stimulus at the tinnitus frequency. For both groups, the average sound-evoked responses to the individual tinnitus frequency, or the frequency closest to that, are depicted for the left and right hemispheres for both groups. The group with low HQ scores has a significantly higher response in BA 41 to the presentation of the tinnitus sound, indicated by the asterisks.

In light of the central gain theory, we investigated if a clear increase in response to sound could be observed along the auditory neuraxis. In Figure 3 A, the average response to all sound conditions is depicted for each auditory area. To test for both within-participant and group differences in activation over subcortical and cortical areas, a repeated-measures ANOVA was applied. The assumption of sphericity was violated according to Mauchly's Test of Sphericity ($x^2(20) = 97.4$, $p < 0.005$, and therefore a Greenhouse-Geisser correction was applied. There was no significant effect of area on activity levels within participants, $F(3.083,102) = 2.481$, $p = 0.064$). There was a significant effect of group on percentage signal change in the auditory areas after Bonferroni correction for multiple comparisons ($F = 10.25$, $p = 0.003$). The average sound-evoked response for each group and auditory area is depicted in Figure 3 A. The previous ROI analysis showed that specific voxels within the auditory subcortical structures showed a significant increase in activity in response to sound for the group with high HQ scores. Similarly, the group with high HQ scores had higher average activity over all auditory subcortical and cortical regions.

FREQUENCY SPECIFIC CORTICAL RESPONSES

On a cortical level, high HQ scores resulted in significantly higher activation in BA 41, BA 42, and BA 22 in response to sound if the combined responses of all sound conditions were considered (FWE (RFT) < 0.05; Figure 4 A). In a frequency-wise analysis, it appeared that the amplitudes of the frequency responses in BA 41 are almost twice that of those in BA 22 (see Figure 4 B and D). These differences in amplitude between BA 41 and BA 22 were significant for the group with high HQ-scores ($t = 3.5$, $p_{perm} = 0.0054$) but not for the group with low HQ-scores ($t = 0.38$, $p_{perm} = 0.72$). A frequency-specific difference in amplitude was identified at 250 Hz, with the high HQ-score group having significantly increased responses in BA 41 ($p = 0.018$), BA 42 ($p = 0.0289$), and BA 22 ($p = 0.024$) (Figure 4 B, C, and D). In addition, in BA 41, higher responses to 4 kHz were observed in the group with high HQ-scores ($p = 0.024$). These effects are not significant after correcting for multiple comparisons in the strictest sense ($p < 0.0083$). Even though there are significant group differences in overall responsiveness to sound for all three auditory cortex areas, there are no frequency-specific differences that remain after stringent correction for multiple comparisons.

Furthermore, we tested for group differences in the average cortical sound-evoked response to the tinnitus frequency (or the closest match). In response to the tinnitus frequency, a significantly higher response was observed for the tinnitus group with low HQ-scores, for both the left primary auditory cortex ($t = 22.4$, $p_{perm} = 0.0352$) and the right primary auditory cortex ($t = 16.95$, $p_{perm} = 0.0412$), see Figure 3 B. Similarly, a higher response to the tinnitus frequency was observed for the group with low HQ scores in the secondary, and association auditory cortices, although this effect did not reach significance (L BA42: T =

2.9, p_{perm} = 0.6; RBA42: T = 7.8, p_{perm} = 0.1; L BA22: T = 9.3, p_{perm} = 0.3; R BA22: T = 10.9, p_{perm} = 0.14); see Figure 3 B. To test if this significant group difference in the response of the primary auditory cortex to the tinnitus frequency is related to the reported difference in the duration of tinnitus, duration was included as a continuous covariate of no interest before rerunning the tinnitus response analysis. This did not alter the results. Therefore, the difference in response to the tinnitus frequency is not explained by the difference in tinnitus duration and is likely related to the presence or absence of hyperacusis.

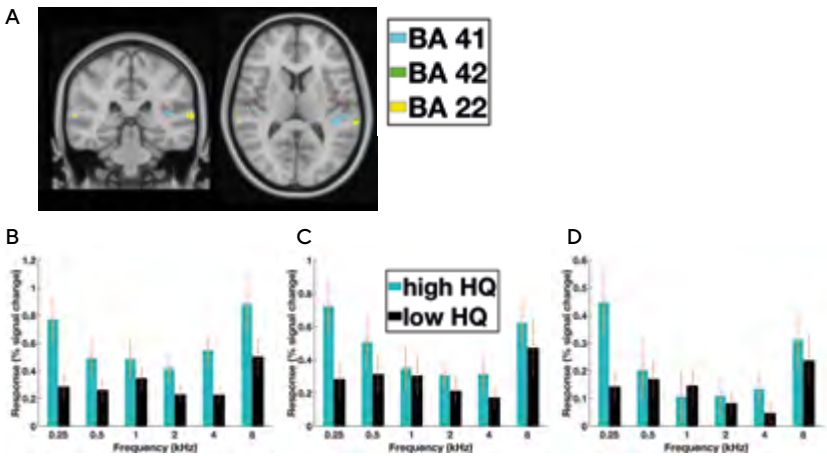
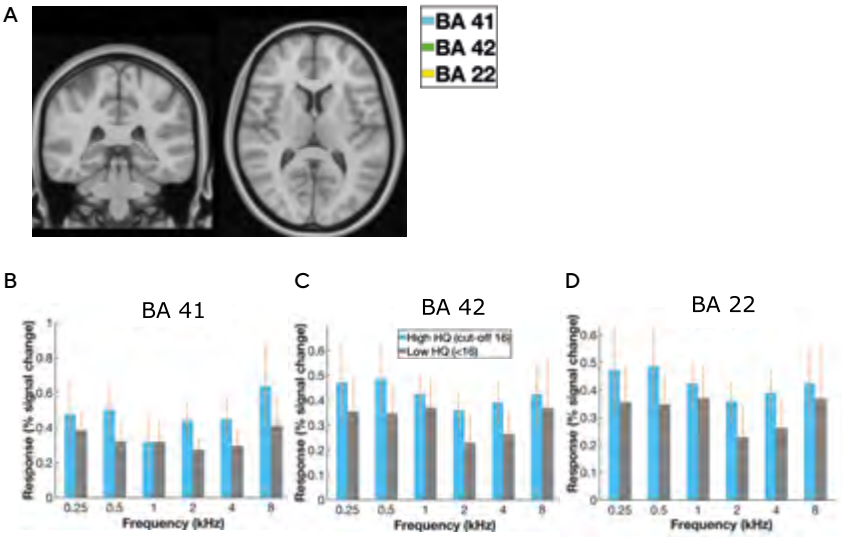


Figure 4. Cortical sound-evoked activity was higher in tinnitus participants with high HQ scores than with low HQ scores. (A) In colour, the voxels with overall higher activity in response to sound in the group with high HQ scores (FWE < 0.05). Here, the combined response to all sound conditions was considered. In blue, for those with higher HQ-scores, the voxels with higher activity in BA 41, in green for BA42, and in yellow for BA22. (B) Responses in the bilateral primary auditory cortex (BA41). (C) Responses in the secondary auditory cortex (BA 42). (D) Responses in the association auditory cortex (BA 22). Mean responses and their standard errors are shown for the group with high HQ scores and the group with low HQ scores. The amplitude of responses is plotted per frequency.

We performed an additional sensitivity analysis using an HQ cut-off score of 16, as proposed by Fioretti et al., 2015. The results of this analysis show that with an HQ cut-off score of 16, there are fewer voxels in the auditory cortex (BA41, BA42, and BA22) that show a statistically significant difference in the group comparison, see suppl. Fig 1 A. The average responses in the cortical areas are still higher in the group with high HQ-scores (≥ 16) than those with lower HQ-scores. However, this difference between the groups is smaller than in the group comparison with an HQ cut-off score of 22, see suppl. Fig 1 (B, C, D). This sensitivity analysis shows that whereas an HQ cut-off score of 22 can be related to a significant increase in cortical responsiveness to sound, an HQ cut-off score

of 16 does not reflect a significant increase in activity. In light of the hypothesis that an increase in central gain is related to hyperacusis, an HQ cut-off score of 22 does reflect this, whereas an HQ cut-off score of 16 does not.



Supplementary Figure 1. Group comparison of cortical sound-evoked activity in response to sound, employing the alternative HQ cut-off score of 16 (as proposed by Fioretti et al., 2015) to indicate hyperacusis. (A) In colour, the voxels with overall higher activity in response to sound in the group with high HQ scores (≥16) (FWE < 0.05). Here, the combined response to all sound conditions was considered. Voxels with higher activity in the group with higher HQ scores (≥16) for BA 41 in blue, in green for BA42, and in yellow for BA22. (B) Responses in the bilateral primary auditory cortex (BA41). (C) Responses in the secondary cortex (BA 42). (D) Responses in the association auditory cortex (BA 22). Mean responses and their standard errors are shown, comparing the group with high HQ scores (≥16) and the group with low HQ scores. The amplitude of responses is plotted per frequency.

CORTICAL TUNING IN RESPONSE TO SOUND IN HYPERACUSIS

The tuning curves of voxel responses in the primary auditory cortex, where the response to the frequency that elicited the largest response was normalized to 1, are displayed in Figure 5. This frequency is referred to as the best frequency (BF). Below and above the BF, the responses were by definition smaller than 1 (see Figure 5 A and B). A two-sample permutation t-test on the average of the non-preferred frequencies (2nd and 3rd frequency away from the preferred frequency of a voxel) showed that this difference was not significant (L BA41: t = 1.94, p_{perm} = 0.055; R BA41 t = 1.54, p_{perm} = 0.13). Thus, these results do not provide evidence for a difference in the cortical tuning of the auditory cortex in tinnitus with and without hyperacusis.

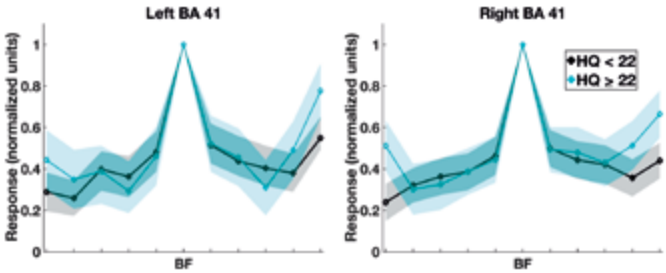


Figure 5. Average tuning curves of voxels in the primary auditory cortex. For each voxel, the response to the stimulus frequency that elicited the largest response was normalized to 1. Subsequently, responses were averaged across voxels. On the x-axis, the BF is centred, and the distance to non-preferred frequencies are indicated in octave wise steps. Depicted are the median normalized responses and the corresponding 95 % confidence intervals. There was no significant difference in cortical frequency tuning between the groups.

DISCUSSION

We investigated the effect of hyperacusis on cortical and subcortical sound-evoked auditory activity in participants with tinnitus and hearing loss. The specific impact of hyperacusis was investigated by comparing two participant groups with similar tinnitus and hearing loss, one with and one without hyperacusis. On a subcortical level, increased responses were observed in the right medial geniculate, inferior colliculus, and the bilateral superior olivary complex of participants with hyperacusis. On a cortical level, our results show a relation between hyperacusis and increased overall sound-evoked activation in the primary, secondary, and association auditory cortex. Altogether, higher subcortical and cortical activity in response to sound thus appears to be a marker of hyperacusis.

SUBCORTICAL AND CORTICAL RESPONSES TO SOUND IN THE PRESENCE OF HYPERACUSIS

These findings replicate the findings of previous publications on human and animal studies (Gu et al., 2010; Knipper et al., 2013; Rüttiger et al., 2013; Zeng, 2013; Chen et al., 2015; Auerbach et al., 2019). In our study, tinnitus participants had additional and pronounced hearing loss, which contrasts with the previous human studies that included participants with no or minimal hearing loss. To account for differences in hearing loss within our study, we carefully loudness matched all stimuli on an individual basis. This loudness matching implies that each participant perceived the different frequencies as equally loud, regardless of their hearing loss. Nonetheless, the observed increased responses to sound in

participants with higher HQ-scores suggest that in the presence of hyperacusis, the overall perceived loudness of the stimuli may have been higher. Generally, similar to the study of Gu et al. (2010), our participants had mild hyperacusis, as it was not their primary complaint and was only rarely mentioned during the interview. In the current study, increased responses to sound were present in hyperacusis and overt hearing loss, which is in line with former studies that reported increased activity in hyperacusis with minimal hearing loss. Thus, it appears that even in milder forms and in the presence of hearing loss and tinnitus, the subcortical and cortical responses to sound are increased in the presence of hyperacusis.

RELATION BETWEEN LOUDNESS AND INCREASED ACTIVATION IN AUDITORY BRAIN AREAS

To expand on the possible increase in perceived loudness in the presence of hyperacusis, it is worth noting that both increases in intensity and broadening of bandwidth increase the perceived loudness of sound stimuli, as described by Gu et al., 2010 (Zwicker et al., 1957; Hawley et al., 2005). Normally, a stimulus that excites several frequency channels of the auditory system is likely to result in larger loudness. Conversely, if the frequency channels themselves have a reduced frequency selectivity, even a narrow-band stimulus will excite more of those channels. Consequently, a tone may be perceived as relatively loud when it excites a large number of frequency channels. In our study, we did not find evidence for a broadening of cortical tuning. Therefore, the increase in perceived loudness in hyperacusis may not be related to a loss of cortical frequency specificity. In line with this, in normal-hearing listeners, increases in loudness result in increased midbrain activation (Harms and Melcher, 2002; Sigalovsky and Melcher, 2006), even when sound energy is constant. For the primary auditory cortex, it has been established that with increased loudness, increased activation is observed in both normal-hearing and hearing-impaired participants (Hall et al., 2001; Langers et al., 2007; Behler and Uppenkamp, 2016). The relation with increased activation is stronger for the loudness than for the intensity of stimuli, and this relation is similar in participants with and without hearing loss. Thus, the link between loudness and fMRI response amplitude is well established. Hence, the increased responses observed in participants with hyperacusis presumably reflect an increase in the perceived loudness of sounds.

CENTRAL GAIN AND THE DISTINCTION BETWEEN LOUDNESS RECRUITMENT AND HYPERACUSIS

In light of the central gain theory, we observed increased activation in response to sound in participants with moderate hearing loss and tinnitus and the additional presence of hyperacusis. This increased activation was present in the subcortical auditory structures and in the primary, secondary, and association auditory cortices. In line with previous findings on hyperacusis, the increased

cortical responses were not restricted to the hearing loss area and instead were present for the entire range of frequencies tested (Noreña and Chery-Croze, 2007; Diehl and Schaette, 2015). This frequency independence of loudness perception mirrors the findings of previous studies that reported that the attenuation of high frequencies via earplugging can lead to altered loudness perception in low frequencies (Formby et al., 2003; Munro et al., 2014), and that stimulation at high frequencies decreased the loudness of low frequencies (Noreña and Chery-Croze, 2007). Changes in loudness perception thus appear to affect the entire range of frequencies and are present in areas not directly affected by attenuation or stimulation. This is in line with a report that in patients with hyperacusis complaints, the loudness discomfort levels are decreased over the whole range of tested frequencies and not restricted to the hearing loss region (Sheldrake et al., 2015). Therefore, it appears that this heightened reactivity to sound is a phenomenon that occurs separately from loudness recruitment observed in hearing loss. Whereas in hearing loss, steeper growth of loudness is limited to the frequencies where hearing loss is present, in hyperacusis the growth of loudness is present over the whole range of frequencies. In summary, our results show an increase in activation in subcortical and cortical parts of the auditory pathway, where the cortical increase in activation affects the entire frequency range despite hearing loss primarily at high frequencies.

THE CORTICAL RESPONSE TO THE TINNITUS FREQUENCY IN THE PRESENCE OF HYPERACUSIS

Stimulation of the auditory cortex with a frequency similar to the tinnitus frequency resulted in a significantly smaller response for the group with hyperacusis. The finding that the brain responds differently to the presentation of the tinnitus frequency in the presence of hyperacusis may indicate that tinnitus with and without hyperacusis reflect different types of tinnitus. Previous work indicates that enhanced responses in the auditory cortex are related to sustained over-attention to the auditory domain (Krumbholz et al., 2007; Paltoglou et al., 2011). It may be that in tinnitus without hyperacusis, there is specific over-attention to the tinnitus frequency band. Whereas in the presence of hyperacusis, attentional resources are drawn by the increased loudness of all sound frequencies. Since both groups in our study experience tinnitus of similar loudness, this suggests that the difference in primary auditory cortex activation in response to the tinnitus frequency is not shaping the tinnitus percept.

Presently, we can only speculate about the cause of the reduced BOLD-response at the tinnitus frequency in hyperacusis. There is currently no research to inform us if external sound stimuli and internal tinnitus activity add up to result in enhanced cortical activation or whether external stimulation normalizes the tinnitus-related activity. The reduced contrast at the tinnitus frequency observed in the presence of hyperacusis could potentially relate to a saturation effect if

the hyperacusis related increase in activity and the response at the tinnitus frequency, as observed in the group without hyperacusis, would add up. This summing of activation could result in a decreased contrast when the activity is already driven to near saturation by the hyperacusis related increase in sound-evoked activity. Alternatively, the presence of hyperacusis related neural hyperactivity in response to sound may cause the external sound to interact with the tinnitus frequency in a different manner than in tinnitus without this subcortical and cortical hyperactivity. Future research will have to inform us about the precise relation between hyperacusis related neural hyperactivity, a reduction in the response to the tinnitus frequency, and the recruitment of auditory attentional networks.

THE CHALLENGE OF DEFINING HYPERACUSIS

In the current paper, the definition of hyperacusis is based on the paper of Aazh and Moore (2017), who showed that an HQ cut-off score of 22 matches well with the lower end of the 95% confidence interval identifying patients with reduced loudness discomfort levels (<77 dB HL), thus capturing the majority of patients that present with hyperacusis complaints. Hereby, we deviate from the cut-off score of 28 that was suggested by the developers of the HQ in their original article (Khalfa et al. 2002), which was intended to indicate severe cases of hyperacusis. The original diagnostic criterion of hyperacusis based on an HQ cut-off score of 28 has been challenged (Fackrell et al., 2015; Fioretti et al., 2015; Aazh and Moore, 2017). Apart from the alternative proposed cut-off score of 22 used in the current study, a cut-off score of 16 was proposed by Fioretti et al. (2015) to reflect the presence of hyperacusis based on the comparison of the area under the Receiver Operator Characteristics (ROC) curve of the HQ and uncomfortable loudness levels. However, Sheldrake et al. showed that the use of loudness discomfort levels alone to diagnose hyperacusis results in a large number of false positives (Sheldrake et al., 2015). Similar to a previous report (Gu et al., 2010), not hyperacusis but tinnitus was the primary complaint of participants in the current study. Please note that patients with severe hyperacusis are unlikely to participate in an fMRI study due to the high sound levels. Our study shows a clear difference in the responsiveness of the auditory areas in the group with an HQ-score of 22 and higher compared to those with lower scores. It thus appears that this group, with milder complaints of hyperacusis, can provide us with important insights into the subcortical and cortical changes that are related to hyperacusis.

CONCLUSION

Hyperacusis was related to an increase in sound-evoked activity in the subcortical and cortical auditory pathway. For the auditory cortex, this increase was not restricted to the hearing loss frequencies but was present for frequencies outside

of the range affected by hearing loss. This result was obtained by comparing two groups, with and without hyperacusis, where both groups had hearing loss and tinnitus. On a subcortical level, hyperacusis was related to higher responses in the MGB, IC, and SOC. On a cortical level, hyperacusis was related to an increase in overall sound-evoked activation in the primary, secondary, and association auditory cortex. We did not identify a hyperacusis related loss of tuning specificity for the primary auditory cortex. In the presence of hyperacusis, responses to the tinnitus frequency were reduced. In summary, higher subcortical and cortical activity in response to sound thus appears to be a marker of hyperacusis.

CHAPTER 06

GENERAL DISCUSSION

ELOUISE KOOPS

In this thesis, the distinctive neural correlates of hearing loss, tinnitus, and hyperacusis have been teased apart with the aid of Magnetic Resonance Imaging. This differentiation was based on both structural and functional measures of neural plasticity in humans. For hearing loss and tinnitus, the impact on the processing hubs, or gray matter, and the connections that join the subcortical and cortical auditory areas, the white matter, were investigated. In addition to exploring the structure of the brain, the changes in the functional activity that relate to hearing loss, tinnitus, and hyperacusis were studied. The gray and the white matter of the auditory pathway and their functional activity are vital to complex auditory perception. Different aspects of auditory perception are altered in the presence of hearing loss, tinnitus, and hyperacusis, suggesting the possibility of distinct neural signatures. Therefore, this thesis aimed to identify and specify the distinctive neural correlates of hearing loss and associated perceptual symptoms such as tinnitus and hyperacusis.

CHANGES IN THE GRAY MATTER RELATED TO HEARING LOSS AND TINNITUS

The investigation of the gray matter morphology provided insight into the structural composition of the brain when hearing loss is present. In chapter 2, hearing loss related reductions in gray matter volume and surface thickness were identified in several auditory and non-auditory brain areas. Hearing loss was related to a reduction in the gray matter of the auditory cortex, an area essential for hearing and speech perception. Furthermore, this structural analysis revealed reductions in the gray matter of several brain areas outside of the auditory pathway. The areas identified in our study are in line with previous longitudinal studies that reported a progressive decline of gray matter over time in the presence of hearing loss and related this decline to cognitive impairments (Lin et al., 2014; Uchida et al., 2019). Interestingly, it thus appears that the impact of hearing loss goes beyond the bounds of the auditory system. In that manner, hearing loss can affect cognitive abilities that do not directly rely on the quality of hearing. Alternatively, the loss of gray matter with hearing loss and the poorer performance on hearing and cognitive tasks can result in concert from an overarching progressive loss of brain efficiency (Humes et al., 2013a, 2013b; Jafari et al., 2019; Sardone et al., 2019).

Strikingly, chapter 2 outlines that in contrast to the group with hearing loss but without tinnitus, there were no differences in gray matter volume or surface thickness in the hearing loss group with tinnitus compared to the control group. Previously, very similar findings were presented by a different research group (Husain et al., 2011). In addition to contrasting the tinnitus group to a control group without hearing loss, in our current work we contrasted two groups with hearing loss, one with and one without tinnitus. This contrast indicated that the additional presence of tinnitus with hearing loss is associated with the preservation of gray matter volume and thickness in temporal, frontal, and occipital areas.

Furthermore, in chapter 2, we show that the additional presence of tinnitus in those with hearing loss related to a larger volume of the lingual gyri. Earlier research has identified altered connectivity of the lingual gyrus in tinnitus, yet this is the first report on a structural difference of the lingual gyrus in relation to tinnitus. The precise role of the lingual gyrus in tinnitus remains unknown, but a larger volume of the lingual gyrus has been linked to better performance on memory tasks (Walhovd et al., 2006; Kalpouzos et al., 2009) and the preservation of cognitive functioning in patients with major depressive disorder (Jung et al., 2014). The findings of chapter 2 provide an exciting addition to our knowledge of the impact of tinnitus on the brain. Overall, whereas hearing loss is associated with less gray matter in several brain areas implicated in cognitive decline, the additional presence of tinnitus is associated with better preservation of these cortical areas.

The observed preservation of gray matter may relate to a reduction in the cytoskeletal protein Arc, a protein involved in cortical neuroplasticity, as observed in an animal model of noise-induced hearing loss and tinnitus (Rüttiger et al., 2013). Normally, injury or sensory deprivation is followed by dynamic changes to the cortex, which occur even though this plasticity rarely restores function. If this ability of the cortex to adapt is diminished, as a reduction of the cortical expression of Arc would suggest, this may result in better preservation of the original state of the system. Especially in the absence of direct injury to the central nervous system, as is the case in hearing loss. A deviation from the normal course of this adaptation process may relate to a vulnerability to tinnitus. In this case, these cortical differences can reflect pre-existing genetic or environmental vulnerabilities that increase the chances of developing tinnitus after hearing loss onset.

Alternatively, the absence of differences in the cortical gray matter in the presence of tinnitus may not necessarily indicate the conservation of gray matter in the sense of preservation but instead reflect that the process of cortical reorganization looks different in the presence of tinnitus. Whereas the observed sum of the cortical layers of gray matter is not significantly different in those with tinnitus compared to those without tinnitus, there can exist differences across these layers. The spatial resolution of human neuroimaging techniques is currently not good enough to confidently examine the different cortical layers. Lastly, we cannot rule out that these cortical differences between people with hearing loss, with and without tinnitus, are driven by small differences in peripheral deafferentation.

CHANGES IN THE WHITE MATTER RELATED TO HEARING LOSS AND TINNITUS

The neurons in the gray matter of the brain are connected by dense networks of white matter fiber tracts that relay information between spatially different locations. The largest white matter tract within the auditory pathway is the acoustic radiation; this thalamocortical tract connects the medial geniculate nucleus of the thalamus to the auditory cortex. Thus, this tract is the last passage an auditory signal travels along before it reaches the cortex. This makes the acoustic radiation an important connection in the investigation of the phantom sound percept of tinnitus, since the activation of the auditory cortex is generally thought to result in conscious sound perception. In chapter 3, macrostructural and microstructural changes of the acoustic radiation were investigated in relation to hearing loss and tinnitus. Existing diffusion-weighted imaging studies on acquired or age-related hearing loss are sparse, and the results are occasionally contradictory. These contradictory results may be explained by the limitations of

the diffusion tensor model, which estimates one primary direction of restricted water movement per voxel.

Improved precision of fiber tract determination was achieved by employing a higher-order diffusion model in the findings presented in this thesis. The great advantage of this fixel-based analysis is that it enables the identification of multiple fiber tracts within one voxel, which precludes the distortion of the outcomes by crossing fiber tracts that are not of interest. To facilitate the comparison of our work with existing literature, we also used the more conventional diffusion tensor model to infer hearing loss related diffusion characteristics of the acoustic radiation. Neither the conventional DTI analysis method nor the fixel-based analysis identified differences in the white matter of the acoustic radiation that related to hearing loss.

Contrary to the observed preservation of white matter in acquired hearing loss without tinnitus, there was degeneration of the acoustic radiation in hearing loss with additional tinnitus. In chapter 3, it is described that both conventional diffusion tensor analysis and the novel fixel-based analysis indicate atrophy of the acoustic radiation, especially in the left hemisphere, and in the vicinity of the medial geniculate body. The tinnitus-related macrostructural changes may reflect a difference in myelination or a reduction in the number of myelinated axons in the acoustic radiation in the presence of tinnitus. Previous diffusion-weighted imaging studies that reported on the auditory system and included a control group are sparse. An apparent limitation of these existing studies is the challenge of determining if the results reflect changes to auditory pathways or other fiber tracts that run through the same capsule (Husain et al., 2011; Seydell-Greenwald et al., 2014). This uncertainty was resolved in chapter 3 using higher-order fixel-based analysis, which is tract-specific and has a biologically meaningful interpretation.

It is worth pointing out that these results do not identify the mechanism behind these changes to the structure of the acoustic radiation. On the one hand, the presence of an auditory phantom percept in itself may be related to fiber-tract-specific alterations. On the other hand, these macrostructural changes may be the result of subtle differences in peripheral deafferentation between hearing loss participants with and without tinnitus. Taken together with the possibility of distinct peripheral deafferentation profiles, as reported in animal studies on tinnitus (Rüttiger et al., 2013), the current findings suggest that the impaired integrity of nerve fibers exists at various levels of the auditory system in the presence of tinnitus. Future research will have to inform us on the precise association of tinnitus with these observed differences in the white matter of the acoustic radiation

FUNCTIONAL DIFFERENCES RELATED TO HEARING LOSS AND TINNITUS

The gray matter of the auditory system provides processing capacity, and the white matter facilitates effective communication between different brain areas. Nonetheless, the state of the auditory system is not only determined by its structural features but also by its functionality. To that end, in chapters 4 and 5, the sound-evoked activity in cortical and subcortical areas related to hearing loss, tinnitus, and hyperacusis was investigated. The findings reported in chapter 4 show that hearing loss is related to increased response amplitudes in the auditory cortex in response to supra-threshold loudness-matched high-frequency stimulation. Furthermore, the results demonstrated an overrepresentation of the high frequencies in the tonotopic maps of participants with hearing loss, measured with the same stimulation paradigm.

A key finding of the functional MRI study was that in hearing loss with tinnitus, the response amplitudes of the auditory cortex and tonotopic map changes are less pronounced than in hearing loss without tinnitus. It thus appears that the observed reorganization is a consequence of hearing loss, and the adaptation is more conservative in the additional presence of tinnitus. Therefore, we conclude that tinnitus appears related to a more conservative form of adaptation instead of excessive adaptation to hearing loss. The less pronounced responsiveness of the auditory cortex to high-frequency sounds in hearing loss participants with tinnitus could be related to the reduction in the cross-section of the auditory radiation reported in chapter 3. In hearing loss induced tinnitus, the critical damage in the inner ear could result in a thinning of the myelin sheet of the corresponding central auditory fibers that are no longer stimulated as a consequence of this deafferentation.

The findings on brain activity in this thesis are based on the BOLD-response to supra-threshold stimulation levels, which is informative on the intensity or amplitude of the sound-evoked responses and the extent of the cortical area that preferentially responds to a particular frequency. The work on hearing loss-related tonotopic organization in animal models utilizes best- or characteristic frequencies of cortical neurons. This method informs on spatial localization and the extent of the cortical area that is responsive to a particular frequency at threshold level. Existing reports on tonotopic map changes that relate to tinnitus vary. Whereas earlier studies suggested that the observed tonotopic reorganization related explicitly to tinnitus (Rajan and Irvine, 1998; Irvine et al., 2000; Norena and Eggermont, 2005), more recent studies (Langers et al., 2012; Miyakawa et al., 2019; Koops et al., 2020) indicate that tonotopic reorganization is a characteristic of hearing loss, and not of tinnitus.

The variation in the results of previous studies might also reflect that different study types recorded activity from various cortical layers, some of which receive information via corticocortical or thalamocortical feedback loops in addition to ascending or lemniscal auditory input. Similarly, measurements may have included various cell types, which receive input from several peripheral fiber types such as low, medium, or high spontaneous rate fibers. For example, it is thought that stimulation at threshold and low intensities activates high spontaneous rate fibers, whereas moderate or high-level supra-threshold stimulation additionally activates medium- and low spontaneous rate fibers (Liberman, 1978). Besides, these specialized fiber types might be non-uniformly affected by various types of hearing loss. Therefore, the current and previous studies may investigate different aspects of sound-evoked cortical responsiveness and different types of hearing loss.

HYPERACUSIS RELATED CHANGES IN SOUND-EVOKED RESPONSES

In chapter 5, responses to sound in the subcortical and cortical regions of the auditory pathway were investigated in relation to hyperacusis. On a subcortical level, hyperacusis was related to higher sound-evoked responses in the superior olivary complex, the inferior colliculus, and the medial geniculate body. On a cortical level, this hyperreactivity was present in the primary, secondary, and association auditory cortices of tinnitus participants with hyperacusis compared to those without hyperacusis. In contrast, the responses to the tinnitus frequency were reduced in the participants with hyperacusis, both compared to other frequencies and compared to tinnitus participants without hyperacusis.

Earlier studies showed that the loudness growth in hearing loss is limited to the frequencies affected by hearing loss. However, in hyperacusis, the loudness growth is steeper for all frequencies (Noreña and Chery-Croze, 2007; Diehl and Schaette, 2015). Our findings are consistent with these reports and indicate an increase of activation for both the frequencies affected and unaffected by the loss of auditory sensitivity, i.e., hearing loss. The relation between increased functional MRI response amplitudes and increased perceived loudness is well established (Hall et al., 2001; Harms and Melcher, 2002; Sigalovsky and Melcher, 2006; Langers et al., 2007; Behler and Uppenkamp, 2016). In line with patient reports that soft to moderately-intense stimuli are perceived as incredibly loud, the increased auditory cortex activation in hyperacusis presumably reflects the increase in perceived loudness.

The central gain model has been employed to unravel the steeper loudness growth curves that are observed in hyperacusis (Zeng, 2013). This central gain enhancement theory explains hearing loss and hyperacusis in terms of homeostatic

upregulation of sound-evoked activity in the central auditory pathway (Diehl and Schaette, 2015). In hearing loss, this upregulation is instigated by a reduction in peripheral input, whereas the mechanisms that instigate increased loudness growth in hyperacusis remain shrouded. In line with the central gain theory, we observed increased subcortical and cortical activation in response to sound in participants with hyperacusis. Nonetheless, it remains unclear if homeostatic upregulation of central activity is the mechanism via which hyperacusis arises.

Whereas the framework of neural gain can incorporate that tinnitus and hyperacusis do not always co-occur (Penner, 1986b), it does not incorporate that hearing loss is not always present in hyperacusis (Tan et al., 2013). Nevertheless, in cases of hyperacusis that co-occur with hearing loss, the homeostatic upregulation model could apply. In contrast to the hyperacusis-related hyperactivity in response to various sound-frequencies, there was a reduced response to the tinnitus frequency in the presence of hyperacusis. Since there were no differences in the characteristics of the tinnitus percept between those with and without hyperacusis, this result suggests that whereas hyperacusis may be related to neural gain, tinnitus may not be. Similarly, the results reported in chapter 4 indicate that tinnitus is related to less pronounced hearing loss related hyperactivity.

CONSIDERATION OF LIMITATIONS FMRI

Functional MRI has a methodological limitation that pertains to the reported results on brain activity. Functional MRI analysis relies on the contrast between a baseline measurement and a task-evoked response measurement. Consequently, the resulting activation maps depend on the neural activity at baseline and during sound stimulation. Animal studies have shown that in the presence of hearing loss, an increase in spontaneous activity can be observed in the auditory cortex (Noreña and Eggermont, 2003; Seki and Eggermont, 2003). From these studies it has been inferred, but not experimentally proven, that tinnitus may relate to increased spontaneous activity in the auditory cortex. If these inferences are correct, differences in sound-evoked activation between individuals with and without tinnitus may relate to a difference in baseline neural activity.

Melcher et al. (2000; 2009) have discussed the implications of the hypothesized heightened spontaneous activity in tinnitus in relation to functional MRI data. In short, the first proposed theory is that a tinnitus-related increase in spontaneous or baseline activity causes the sound-evoked activity in the auditory cortex to appear repressed due to the existence of a ceiling or saturation effect. Within this theory, it is hypothesized that sound-evoked activity builds on the tinnitus-related increased baseline activity level. The total response, the sum of baseline and sound-evoked activity, cannot exceed a maximum amplitude due to neural or hemodynamic response saturation. Consequently, the measured BOLD-response and the derived contrast between baseline and sound-evoked activation may be

reduced (figure 6.1, A & B). However, there is evidence that in the human auditory cortex, sound intensities up to 96 dB SPL (Hart et al., 2002), 97 dB SPL (Behler and Uppenkamp, 2016), and up to 100 dB SPL (Langers et al., 2007) do not result in a saturation effect in participants with normal hearing and hearing loss. In our study, in only one participant, the stimulation at 8 kHz exceeded 100 dB SPL (i.e., 107 dB SPL). To double-check if this participant disproportionally affected our results, the analyses of chapter 4 were re-run while excluding this particular participant. The removal of this participant did not alter the results of this study. Consequently, it is unlikely that the sound-evoked responses measured in Chapters 4 and 5 are affected by saturation of the neural or hemodynamic response. The responses observed in chapter 4 can be obtained in the presence of increased baseline activity if there is no saturation effect present, as depicted in figure 6.1, C.

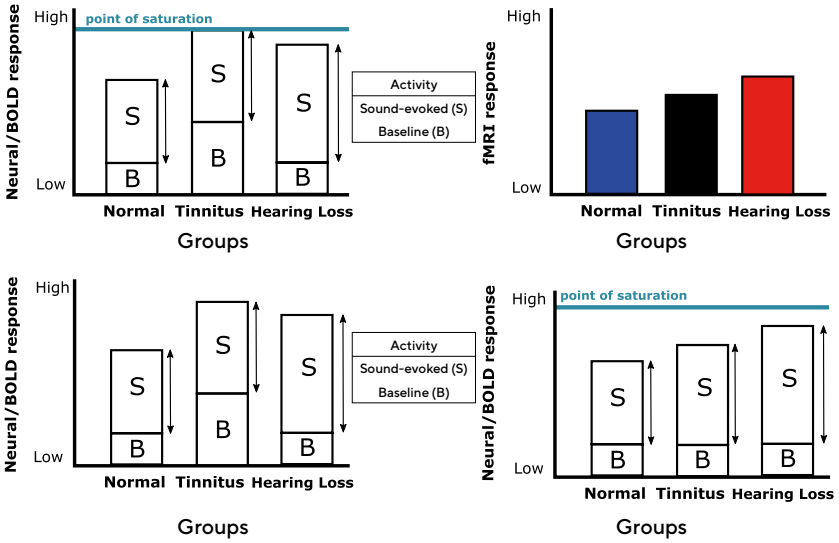


Figure 6.1 Different scenarios that would lead to the results described in Chapter 4. (A) An increase in tinnitus-related baseline activity could reduce the contrast between baseline and sound-evoked activity in the tinnitus group. This scenario would occur if the sum of activity cannot exceed an absolute maximum due to a ceiling or saturation effect. (B) The magnitude of the fMRI response results from the contrast of baseline and sound-evoked activity. The pattern depicted here would be obtained with the example of panel A, but also with the examples of panels C and D. (C) If there is no neural or hemodynamic saturation present, the increased baseline activity can be present without affecting the fMRI contrast. (D) Alternatively, the same fMRI responses would be obtained if the increased baseline activation in the tinnitus group is reduced during sound stimulation due to the noisy scanner environment. The scenarios in panels C and D reflect a true effect of cortical responses to sound. In other words, in the examples of panels C and D, a reduced BOLD-response in the hearing loss group with tinnitus indicates a reduction of sound-evoked activity compared to the hearing loss group without tinnitus.

Alternatively, the second proposed theory by Melcher et al. (2000; 2009) is that sound-evoked responses may not be building on the increased tinnitus-related activity. Instead, the tinnitus-related activity is repressed or normalized during sound-stimulation; this is illustrated in figure 6.1, D. In the relatively noisy environment of the MRI-scanner, the baseline condition is never measured in silence. Therefore, it does not reflect spontaneous but sound-evoked activity in response to the background noise. This second theory of sound-evoked reduction of tinnitus-related activity would result in similar baseline levels for those with and without tinnitus in an MRI study.

These arguments, together, indicate that the difference in activity observed in chapter 4 between the hearing loss groups with and without tinnitus is due to a difference in sound-evoked subcortical and cortical responses and does not reflect a saturation effect.

AGE-RELATED CHANGES

Aging is a complex biological process that includes the gradual accumulation of pernicious changes to our cells (Cole et al., 2019). Over time, these changes become apparent in the brain and affect its ability to function efficiently. Interestingly, while aging is a natural process, it affects us at different rates, with some people aging faster than others. Especially, the rate of aging of the brain can be out of step with the chronological age. On the one hand, normal aging has been implicated in the emergence of brain-related disorders. On the other hand, these disorders have been explained as occurrences of accelerated aging. In this thesis, the effect of age on structural and functional measures of the brain was investigated. It is important to separate age and hearing loss effects since these factors are intricately related, and therefore their impact is easily misattributed.

The gray matter of the brain declines with increasing age, and the auditory, cingulate, and orbitofrontal areas are affected in particular, as described in chapter 2. Previous studies on aging and brain volume have reported a decline in the same brain areas (Good et al., 2001; Resnick et al., 2003; Hutton et al., 2009; Profant et al., 2014). In our study, aging effects were particularly prominent in the auditory cortex, as depicted in figure 3 of chapter 2. A reduction in the gray matter of the brain has been related to cognitive impairments and dementia. Hearing loss is also associated with a decline in the gray matter of several brain areas implicated in cognitive impairments. The additive decline of gray matter with advancing age may be an additional risk factor for those with hearing loss to develop cognitive impairments or even dementia.

In addition to the age-related gray matter changes described in this thesis, age-related differences in the connecting white matter were identified. Concretely, the axonal density of the acoustic radiation declines with increasing age, as described in chapter 3. A consequence of this progressive loss of axons is the potential disruption of signal transfer between the auditory thalamus and the auditory cortex. With the knowledge that hearing loss is the most prevalent acquired sensory disorder worldwide, the decline in gray matter and white matter with age may reflect a specific vulnerability of the auditory system that aggravates the impact of peripheral hearing loss on speech understanding and communication. In line with this, previous research indicated that older adults with hearing loss perform worse than younger people with similar hearing loss (Dubno et al., 1984; Fitzgibbons and Gordon-Salant, 1995; Pichora-Fuller et al., 1995; Wingfield et al., 2006; Cardin, 2016). The deterioration of the gray matter of the auditory cortex and the white matter of the acoustic radiation with age may well be the neurological correlates that reflect these behavioral consequences. In conclusion, the group that is most affected by hearing loss are older individuals, and they are disadvantaged by the impact of both age and hearing loss on central auditory structures.

CONCLUSION

The aim of this thesis was to investigate and separate the neural correlates of hearing loss, tinnitus, and hyperacusis. Our results provide evidence for distinct neural correlates, both structural and functional, of hearing loss, tinnitus, and hyperacusis. Hearing loss has a profound impact on the gray matter of the brain, on the tonotopic map, and the magnitude of the activity of the auditory cortex in response to sound. The additional presence of tinnitus reduced the extent of the gray matter decline and the extent of functional adaptations observed in hearing loss without tinnitus. Furthermore, hyperacusis was related to increased sound-evoked responses in the subcortical auditory areas and the auditory cortex. In the auditory cortex, this increase was observed for frequencies within and outside of the hearing loss affected frequency range. Moreover, whereas hearing loss did not relate to distinct micro- or macro-structural changes of the acoustic radiation, the additional presence of tinnitus related to a decline in the fiber-bundle cross-section near the medial geniculate body of the thalamus. Collectively, our results show that tinnitus is related to a more conservative form of plasticity in response to peripheral hearing loss, whereas hyperacusis relates to excessive responses to sound in central auditory areas. Overall, advancing age was related to a reduction in the gray matter of limbic, frontal, primary sensory, and primary motor cortices and reduced axonal density in the acoustic radiation. Therefore, older individuals are disadvantaged by the impact of both age and hearing loss on central auditory processing.

FUTURE DIRECTIONS

The identified differences in the brain's gray and white matter and the auditory system's activity in hearing loss, tinnitus, and hyperacusis can occur due to peripheral pathology or central mechanisms. The differences reported in this thesis can result from neural plasticity instigated by sensory deprivation or reflect biomarkers that signal pre-existing vulnerabilities. Therefore, the results of this thesis provide a good starting point for future investigations into the biomarkers of hearing loss in humans and related symptoms such as tinnitus and hyperacusis.

Interestingly, the previously reported preservation of cognitive functions with larger lingual gyrus volume may have a bearing on the current findings of tinnitus-related preservation of the lingual gyrus. Future research can determine the extent to which the lingual gyrus volume correlates with tinnitus characteristics and the preservation of cognitive functions. In tinnitus, effective cortical adaptation to sensory deprivation might be hindered by a pre-existing plasticity deficit. In that sense, the preservation of gray matter may point to a vulnerability that puts an individual at risk of developing tinnitus. In general, the preservation of gray matter may provide a better buffer against the onslaught of aging. The technique used to investigate the gray matter in this thesis is informative on the sum of the cortical layers. More work is needed to define whether there is true preservation of gray matter in tinnitus or if these findings reflect a difference across cortical layers. Currently, human neuroimaging methods do not have the spatial resolution to investigate the structure and function of specific cortical layers. However, the development of MRI-scanners with increased magnetic field strengths for human research is promising. Since the gray matter appears conserved in tinnitus, this provides a promising scaffold that can be used to treat auditory domain conditions. These treatments could capitalize on the intact processing capacity by selectively promoting auditory plasticity to reduce the impact of tinnitus.

It is certainly of interest to determine how this more conservative reorganization in the presence of tinnitus affects other modalities and faculties. Similarly, it is yet unclear if tinnitus, hearing loss, and age affect the composition of the brain's gray matter in distinct ways. One of the formers may affect the number or size of surviving neurons, whereas the other may affect the dendrites and unmyelinated axons or the glial cells. These examples would all result in a reduction of gray matter volume. And, these effects may be more prominent in specific cortical layers for the various conditions. The broad implication of our findings in hearing loss is that the rehabilitation of hearing in older individuals via hearing aids may be supplemented by treatments aimed at stimulating cortical plasticity to recuperate hearing effectively.

Research questions that can be derived from the tinnitus-related white matter differences revolve around determining whether these differences reflect pre-existing distinctions that point towards a tinnitus vulnerability. It is unclear whether these white matter differences were present before the onset of hearing loss or tinnitus or if they emerged secondary to these conditions. To expand on these questions, it is still unclear if peripheral deafferentation can affect central white matter integrity. In other words, can peripheral deafferentation alone cause changes in the central white matter? And, if these central changes are inherited from the peripheral system, are these changes restricted to the auditory pathway and thus modality-specific? Or, can peripheral damage to the auditory system instigate a more global degeneration process that affects white matter beyond the auditory pathway? Alternatively, the vulnerability to peripheral damage and central degradation may have a common cause. For instance, related to changes in metabolic processes, which simultaneously increases the likelihood that both peripheral damage and central changes occur.

An essential next step in terms of functional brain activity in relation to tinnitus and hearing loss is the investigation of the hypothesized saturation of the BOLD response in different types of hearing loss and in the presence of tinnitus. Hopefully, this experiment will give a definitive answer on whether the decreased BOLD response observed in hearing loss participants with tinnitus compared to those without tinnitus is not affected by a saturation effect of the hemodynamic or neural response. This knowledge may prove important for the interpretation of the BOLD-response in general. Furthermore, if this saturation response does occur, it still needs to be determined if this can be traced to an increase in baseline BOLD-activity, and whether this relies on a tinnitus-related increase in spontaneous activity. Ultimately, studies that couple measurements of spiking activity of auditory neurons and functional MRI will be necessary to shed light on this hypothesized interdependency of a reduced BOLD-contrast in the presence of increased baseline or spontaneous activity.

To date, there is no evidence that the peripheral auditory system is involved in hyperacusis, and little research has been done to investigate this association. Future research can help us establish the amount of deafferentation and peripheral damage in people with hyperacusis. Furthermore, if a particular type of peripheral deafferentation is observed in relation to hyperacusis, it will need to be determined if this difference can reliably differentiate those with and without hyperacusis. Consequently, the next step will be to determine if this difference in deafferentation is large enough to instigate the observed abnormal central gain. In addition to the afferent auditory pathway, the efferent auditory pathway is of particular interest in hyperacusis-related research since there are direct down-stream projections from the central auditory system to the cochlea's outer hair cells that modulate the gain of the output of the inner ear. On the other hand,

the loudness related neural response enhancement observed in hyperacusis may not rely on a cochlear trigger mechanism but on a central mechanism. The neural system regulating perceived loudness is yet obscure and warrants further investigation in general.

Overall, future research will have to bridge the gap between the identified cochlear pathology and central correlates of hearing loss, tinnitus, and hyperacusis obtained with animal models and human research. Similarly, common ground needs to be established between tinnitus models based on different types of noise-related deafferentation and ototoxic drug-related hearing impairment. Ideally, a great step towards this goal will be to combine the precise measures employed in animal models with a neuroimaging method that is translatable to human work on tinnitus. In that manner, we can use techniques that can inform us on the precise cell types and fiber types involved and have the ability to directly compare these results to those obtained in human participants where we have the great advantage that they can unambiguously report the presence of tinnitus.

SUMMARY

The auditory system is fine-tuned by auditory experience and is plastic throughout life. Whereas the peripheral auditory system is functional prenatally (Sininger et al., 1999) and develops to maturity shortly after birth (Moore, 2002), the central auditory system is shaped by auditory experience throughout the first years of life (Sininger et al., 1999) and up to adolescence (Ponton et al., 2000). Beyond this developmental fine-tuning, the central auditory system has the ability to adapt after exposure to harmful environmental factors that compromise the peripheral auditory system (Syka, 2002; Gold and Bajo, 2014). Consequently, sensory deprivation or other damage to the auditory system incites dynamic changes to both the wiring of fiber tracts and the condition and firing of neurons. For instance, neuronal responses to sound can be altered by a reduction in input from the peripheral auditory system (Syka, 2002), as is the case in hearing loss. Conditions that often co-occur with hearing loss, tinnitus and hyperacusis, are thought to affect the auditory pathway in a distinct manner. Since these conditions often co-occur, there is considerable overlap in their reported impact on the auditory system, which may hinder the development of specific treatments. Therefore, it is essential to investigate structural and functional aspects of the auditory system that are distinctive for hearing loss, tinnitus, and hyperacusis.

In **chapter 2**, we investigated the gray matter of the brain, which consists of the neurons and their dendrites and glial cells. An anatomical scan was obtained for each participant, and these scans were analyzed with voxel- and surface-based morphometry. These analyses showed that hearing loss was associated with reductions in gray matter volume and surface thickness of both auditory and non-auditory brain areas. The gray matter of the auditory cortex was particularly affected compared to other primary sensory and motor cortical areas. The presence of additional tinnitus related to better preservation of gray matter. Finally, we showed that age has a significant impact on the gray matter of the brain, particularly on the auditory cortex.

In **chapter 3**, with diffusion weighted imaging, we investigated the structure of the acoustic radiation. We employed fixel-based analysis to identify macro- and microstructural changes to the acoustic radiation, which is the largest fiber pathway in the auditory system, connecting the auditory thalamus to the auditory cortex. In addition, we used the conventional diffusion tensor model. Both types of analyses showed no relation between acquired hearing loss and micro- or macro-structural changes of the acoustic radiation after rigorously controlling for the effects of age and tinnitus. However, hearing loss with additional tinnitus was related to macro-structural alterations of the acoustic radiation. To specify, tinnitus was associated with a decrease in fiber-bundle cross-section of the

acoustic radiation in the vicinity of the medial geniculate body. Furthermore, with increasing age the axonal density of the acoustic radiation decreased, which may be linked to a loss of efficient information transfer in the aging brain.

In **chapter 4**, we identified that increased response amplitudes and more severe tonotopic map changes in the auditory cortex were related to hearing loss. A key finding was that the altered response amplitudes and the tonotopic map changes were less pronounced in the presence of tinnitus. Therefore, we concluded that this observed reorganization is a consequence of hearing loss, whereas this adaptation is less pronounced in the presence of tinnitus.

In **chapter 5**, we explored whether the activity of the central auditory areas was changed in the presence of hyperacusis. We identified increased responses to sound in the subcortical auditory pathway in tinnitus participants with additional hyperacusis. This increase of activation was not restricted to the frequencies affected by hearing loss. These findings provide neural correlates of the perceptual increase in loudness that is characteristic of hyperacusis. Furthermore, our findings revealed a decreased response to the tinnitus frequency in the presence of hyperacusis. Taken together, these findings suggest that hyperacusis is related to increased neural gain, whereas tinnitus may not be.

In this work, we characterized that hearing loss, tinnitus, hyperacusis, and by extension, age have a distinct impact on the auditory system. Whereas hearing loss relates to pronounced changes in auditory and non-auditory gray matter and the reorganization of the tonotopic map of the auditory cortex, tinnitus relates to more a conservative form of plasticity. Conversely, tinnitus was associated with macro-structural changes of the acoustic radiation, whereas hearing loss was not. This difference may be due to subtle variations in peripheral deafferentation or reflect a pre-existing vulnerability to tinnitus. Furthermore, hyperacusis was related to hyperactivity in response to sound, which provides a neural correlate of the perceived increase in loudness that is characteristic of hyperacusis. Moreover, age has a pronounced impact on the gray and white matter of the brain, and older individuals are disadvantaged by the impact of both age and hearing loss on the brain. As a consequence, rehabilitation methods may need to consider promoting neural plasticity to improve the cortical aspects of auditory processing in addition to the conventional peripheral amplification of sound.

NEDERLANDSE SAMENVATTING

Het auditieve systeem wordt nauwkeurig afgesteld via auditieve ervaring, een dynamisch proces dat levenslang bijgesteld wordt. Terwijl het perifere auditieve systeem prenataal al functioneel is (Sininger et al., 1999) en kort na de geboorte volgroeid is (Moore, 2002), ontwikkelt het centrale auditieve systeem zich door auditieve ervaring vanaf de eerste levensjaren (Sininger et al., 1999) tot aan de adolescentie (Ponton et al., 2000). In aanvulling op deze ontwikkelingsgebonden aanpassingen is het centrale auditieve systeem in staat om zich aan te passen aan schade aan het perifere auditieve systeem (Syka, 2002; Gold and Bajo, 2014). Schade aan het auditieve systeem zet aan tot dynamische verandering van zowel de structuur van de vezelbanen als de conditie en functionaliteit van neuronen. Wanneer er minder signalen vanuit het perifere auditieve systeem worden doorgegeven, zoals het geval is bij gehoorverlies, treden er veranderingen op in de respons van neuronen op geluid (Syka, 2002). Aandoeningen die vaak samen met gehoorverlies voorkomen, tinnitus en hyperacusis, beïnvloeden het auditieve systeem waarschijnlijk op verschillende manieren. Doordat gehoorverlies, tinnitus en hyperacusis vaak samen voorkomen is het moeilijk om de relevante oorzaak van de veranderingen in het auditieve systeem te onderscheiden. Daarom is het van belang om de verschillende structurele en functionele veranderingen in het auditieve systeem te onderzoeken.

In hoofdstuk 2 beschrijven we de veranderingen in de grijze massa, die bestaat uit neuronen en hun dendrieten en ondersteunende glia-cellen. Dit onderzoek vond plaats door middel van een anatomische scan. Verschillen in volume en de corticale dikte die gerelateerd zijn aan gehoorverlies en tinnitus zijn berekend met behulp van verschillende analysetechnieken. Deze analyses laten zien dat gehoorverlies samenhangt met een verlies aan volume van de grijze massa en van corticale dikte in zowel auditieve als niet-auditieve gebieden, waarbij de grijze massa in de auditieve cortex bij gehoorverlies sterker was aangedaan dan de grijze massa bij gehoorverlies met tinnitus. Er is een relatie tussen de additionele aanwezigheid van tinnitus en het behoud van grijze massa die bij gehoorverlies zonder tinnitus verloren zou zijn gegaan. Daarnaast laten we zien dat leeftijd een significante impact heeft op de grijze massa van de hersenen.

In hoofdstuk 3 is de structuur van de akoestische radiatie onderzocht middels een 'fixel-based' analyse. Deze analyse stelde ons in staat om de micro- en macroveranderingen in de akoestische radiatie te onderzoeken. De akoestische radiatie is de grootste witte vezelbundel in het auditieve systeem. Deze verbindt de auditieve kern in de thalamus met de auditieve cortex. Om de vergelijking met eerdere onderzoeken te vergemakkelijken, hebben we ook het conventionele

'diffusion tensor' model gebruikt om de data te analyseren. Beide analysetypen laten zien dat gehoorverlies niet gerelateerd is aan micro- of macrostructurele veranderingen van de akoestische radiatie. Tinnitus was echter wel gerelateerd aan macrostructurele veranderingen van de akoestisch radiatie. In het bijzonder bleek er een relatie te bestaan tussen tinnitus en een afname van de doorsnede van de akoestische radiatie ter hoogte van de auditieve kern in de thalamus. Verder laten we zien dat in de akoestische radiatie met een toename in leeftijd een afname in de dichtheid van axonen wordt waargenomen. Dit kan duiden op een afname van efficiënte informatieoverdracht in het ouder wordende brein.

In hoofdstuk 4 laten we zien dat toegenomen respons-amplitudes en grotere veranderingen aan de tonotopische map van de auditieve cortex zijn gerelateerd aan gehoorverlies. Een belangrijke bevinding was dat de respons-amplitudes en de veranderingen in de tonotopische map in mindere mate aanwezig waren in deelnemers met tinnitus. Om die reden concluderen we dat de waargenomen reorganisatie een gevolg is van gehoorverlies en niet van tinnitus.

In hoofdstuk 5 onderzoeken we in welke mate de activiteit van subcorticale en corticale auditieve gebieden gerelateerd is aan hyperacusis. Onze bevindingen laten zien dat hyperacusis samenhangt met een frequentie-onafhankelijke toename van activiteit in de primaire, secundaire en associatie-auditieve cortices in mensen met gehoorverlies en tinnitus. Verder vonden we in deelnemers met hyperacusis een verminderde respons op de tinnitus frequentie. Deze bevindingen kunnen geïnterpreteerd worden als de neurale correlaten van de toegenomen luidheid van geluid die kenmerkend is voor hyperacusis.

Samenvattend tonen we in dit proefschrift aan dat gehoorverlies, tinnitus, hyperacusis en leeftijd een verschillende invloed hebben op het auditieve systeem. Terwijl gehoorverlies gerelateerd is aan veranderingen in de grijze massa in auditieve en niet-auditieve gebieden en aan corticale reorganisatie, is tinnitus gerelateerd aan een meer behouden vorm van reorganisatie. Er is daarentegen juist wel een relatie tussen tinnitus en veranderingen in de macrostructuur van de akoestische radiatie. Deze verschillen kunnen verklaard worden door subtiele verschillen in de perifere schade of het gevolg zijn van een onderliggende kwetsbaarheid voor tinnitus in de hersenen. Hyperacusis is gerelateerd aan een toename van subcorticale en corticale activiteit en deze toename is niet beperkt tot het frequentiegebied waar gehoorverlies is opgetreden. Deze bevinding zou de neurale grondslag voor de toename in waargenomen luidheid kunnen zijn. De oudere populatie ondervindt nadeel van de gecombineerde invloed van leeftijd en gehoorverlies op de structuur en activiteit van de hersenen. Het is het overwegen waard om naast behandelmethoden met hoortoestellen ook therapieën te ontwikkelen die de plasticiteit van de hersenen vergroten.

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