PERIPHERAL AND CENTRAL FACTORS IN TINNITUS

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Abstract

Tinnitus is the phantom perception of a sound heard in the absence of a physical sound source. One framework that attempts to explain tinnitus is called the *deafferentation model*, which asserts that hearing damage precipitates compensatory neural plasticity in the central auditory system, leading to hyperactivity perceived as tinnitus. While considerable evidence supports this view, the role of deafferentation and its effects on central auditory processing are not fully understood. This thesis addresses two questions raised within the model. First, audiometric hearing loss is not always present in tinnitus subjects, so is there evidence of deafferentation in these individuals? The second question concerns the effect of tinnitus with audiometric hearing loss on central auditory processing. Specifically, is auditory attention affected in tinnitus, and if so, how? Following a background review in Chapter 1, Chapter 2 describes a study addressing the first issue, which found evidence for previously undetected hearing loss in tinnitus subjects that distinguishes them from individuals with normal hearing. Chapter 3 addresses the second question, describing an investigation that found that top-down attention appears to operate normally in tinnitus, but the tinnitus-affected region of auditory cortex is insensitive to the influence of attention because it is busy encoding the tinnitus. Chapter 4 describes a background study using non-tinnitus individuals to test this latter conclusion, finding evidence that auditory cortical neurons reacting to stimulus change are concurrently sensitive to top-down attention. This procedure can be used to assess

if tinnitus-affected auditory cortical neurons are generally insensitive to input. Chapter 5 discusses the implications of the above empirical findings for understanding the role of deafferentation in tinnitus and tinnitus-related changes that occur in central auditory processing.

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List of Abbreviations

A1: Primary auditory cortex
A2: Secondary (nonprimary) auditory cortex
ABR: Auditory brainstem response
AC: Auditory cortex
AM: Amplitude modulation
ANF: Auditory nerve fiber
ANOVA: Analysis of variance
ASSR: Auditory steady-state response
BF: Basal forebrain
BM: Basilar membrane
CF: Characteristic frequency
CN: Cochlear nucleus
dB HL: decibels hearing level
dB SL: decibels sensation level
dB SPL: decibels sound pressure level
DCN: Dorsal cochlear nucleus
DPOAE: Distortion product otoacoustic emission
EEG: Electroencephalogram
EFR: Envelope following response
FFR: Frequency following response
GABA: gamma-Aminobutyric acid
IC: Inferior colliculus
IHC: Inner hair cell
IT: Intermittent tinnitus
ITI: Intertrial interval
Hz: Hertz
MGB: Medial geniculate body
MOC: Medial olivocochlear
ms: millisecond
NBN: Narrowband noise
OAE: Otoacoustic emission
OHC: Outer hair cell
PEST: Parameter estimation by sequential testing
PLV: Phase locking value
PSD: Power spectral density
RI: Residual inhibition
SFR: spontaneous firing rate
SL: Sensation level
SPL: Sound pressure level
SR: Auditory sustained response
STDP: Spike-timing dependent plasticity
STFT: Short-time Fourier transform

TH: Threshold
THQ: Tinnitus Handicap Questionnaire
TFP: Total field power
TFR: Tinnitus frequency region
VCN: Ventral cochlear nucleus

Declaration of Academic Achievement

The material in this thesis includes three empirical manuscripts that each address questions concerning the neural mechanisms involved in tinnitus. Chapters 2 and 4 have been submitted for publication and Chapter 3 has appeared in publication. I am the primary author for each submitted or published manuscript. My specific contributions to the content of this thesis, including submitted or published manuscripts, are as follows:

Chapter 1: Introduction

Author: Brandon T. Paul

Chapter 2: Amplitude modulation encoding deficits suggest hidden hearing loss in individuals with tinnitus and normal audiograms

Authors: Brandon T. Paul, Ian C. Bruce, & Larry E. Roberts
Publication: Submitted
Comments:
For this manuscript I collaboratively developed the research question and design with I.C.B. and L.E.R, solely collected and analyzed data, and was the lead writer with assistance coming from I.C.B. and L.E.R.

Chapter 3: Modulation of Electrocortical Brain Activity by Attention in Individuals with and without Tinnitus

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Chapter 4: Top-down attention modifies a spectral estimate of phase shifts during resets of the auditory steady state response

Authors: Brandon T. Paul, Ian C. Bruce, & Larry E. Roberts
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Chapter 5: Discussion

Author: Brandon T. Paul

Note to the reader: Consistent with the "sandwich" thesis format, Chapters 2, 3, and 4 are written as manuscripts published or submitted for publication. Material in Chapter 1 is often repeated in the introduction of each empirical chapter.

Chapter 1: Introduction

1.1 Overview and introduction to the deafferentation model of tinnitus

Chronic tinnitus is the phantom sound perception experienced in the absence of a physical sound source, often described as a "ringing" or "hissing" in the ears. As of 2013, 41% of surveyed Canadians have experienced tinnitus, and 20% of these individuals found it severe enough to negatively affect daily function (Stats Canada, 2015). Although many of those afflicted may seek medical assistance to relieve their tinnitus, they will find there is no cure or generally effective treatment. The path to developing effective therapies for tinnitus relies on developing a scientific understanding of the causes and basic mechanisms of tinnitus. A starting point is that tinnitus is not generated in the ear as people commonly experience; cutting the peripheral auditory nerve does not effectively stop tinnitus in most cases (House & Brackmann, 1981). Rather, evidence suggests that tinnitus is a consequence of neuroplastic changes in the brain (Noreña & Farley, 2013; Shore et al., 2016). This does not suggest that the auditory periphery (including the cochlea, the primary hearing organ in the inner ear) is not involved. It is clear that most tinnitus cases occur when input to the brain from the ear is significantly diminished.

In agreement with this view, 85% of tinnitus sufferers have evidence of hearing loss in their audiogram, a standard clinical test that assesses thresholds of audibility for tones in quiet (Henry et al., 2005). The normal range of human hearing is for sound frequencies between 20 Hz and 20 kHz, and on average,

threshold shifts in those with tinnitus begin around 2 kHz and further increase into the high frequency hearing range up to 16 kHz (Roberts et al., 2008). If tinnitus subjects with this or a similar profile of audiometric loss are asked to rate the similarity of their tinnitus sensation to a range of pure tones, similarity ratings also begin to increase around 2 kHz and peak between 5 and 8 kHz. In other words, the frequencies where people hear their tinnitus are where there are audiometric threshold shifts that indicate hearing loss. If noise maskers covering the tinnitus and hearing loss frequency region are played to these individuals for about 30 seconds or longer, they commonly report a short-duration suppression of the tinnitus sound after the masker ceases, a phenomenon termed "residual inhibition" or RI (Roberts et al., 2008). These findings suggest that aberrant activity among neurons tuned to the hearing loss frequencies (herein called the "tinnitus frequency region," TFR) may be generating the tinnitus, and suppressing that activity with noise maskers briefly stops the tinnitus. The challenge remains to determine what these neurons are doing to generate the tinnitus, and to identify where in the auditory pathway that this activity is occurring (Roberts et al., 2010; Shore et al., 2016).

To meet this challenge, complementary human and animal research has attempted to establish the cause and neural correlates of tinnitus as well as the consequences of tinnitus on general cognitive function. Broadly, this research suggests that damaging effects of noise exposure, aging, and ototoxic drugs diminish peripheral input to the central auditory system, leading to widespread

hyperactivity in neurons tuned to the hearing loss frequencies (Noreña & Farley, 2013). The mechanism behind hyperactivity is not fully understood, but could reflect either loss of inhibition in auditory pathways (Eggermont & Roberts, 2004), neuromodulators released when phantom sounds are experienced (Roberts et al., 2013), homeostatic mechanisms that are rapidly employed to compensate for loss of input and keep neuronal activity operating in a normal range (Turrigiano & Nelson, 2004), or an increase in activity-dependent plasticity (such as spike-timing dependent plasticity) that increases the excitability of deafferented neurons (Koehler & Shore, 2013). A possible and unwanted consequence is that hyperactivity can potentiate the formation of coherent or synchronous neural activity across large neuronal ensembles in central auditory structures (Shore et al., 2016). It has been proposed that synchronous activity (especially in auditory cortex) may be the direct correlate of the tinnitus sound (Eggermont & Roberts, 2004; Shore et al., 2016), since synchronous activity is theorized to be necessary for the formation of an auditory object that emerges into conscious perception (Brette, 2012). The neural changes in tinnitus extend beyond the auditory system to non-auditory networks, specifically affecting attention, memory, emotion, and somatosensory function (Husain, 2016). However, it is currently not known whether non-auditory changes are necessary and/or sufficient for the development and perception of tinnitus (Shore et al., 2016).

The idea that reduced peripheral input can lead to central hyperactivity underlying tinnitus is referred to as the *deafferentation model* of tinnitus, with

deafferentation referring to a cessation of peripheral input to the central nervous system. Here, deafferentation specifically arises due to auditory peripheral damage. To understand the model and evidence that supports it, this chapter will present a basic overview of the auditory system, common types of auditory deafferentation, and human and animal studies revealing tinnitus correlates after deafferentation. This chapter will highlight two questions raised under the deafferentation model, the first dealing with peripheral factors and the second with central factors, which are the topics of Chapters 2, 3, and 4. Peripheral factors here refer to the structure and function of the auditory periphery, including the cochlea and auditory nerve. Central factors refer to the central auditory nervous system and non-auditory brain regions, beginning at the cochlear nucleus in the brainstem.

1.2 Basic overview of the auditory system

The basic components of the classical auditory pathway are presented in Figure 1.1. Depictions in Figure 1.1 and descriptions below represent a small but core subset of auditory connections between the cochlea and the cortex. The review emphasizes the structure and function of mechanosensory hair cells and the auditory nerve that are important to understand material in Chapter 2, as well as the auditory cortex which is the focus of Chapters 3 and 4. Unless otherwise referenced, material in this section is attributed to Yost (2007).

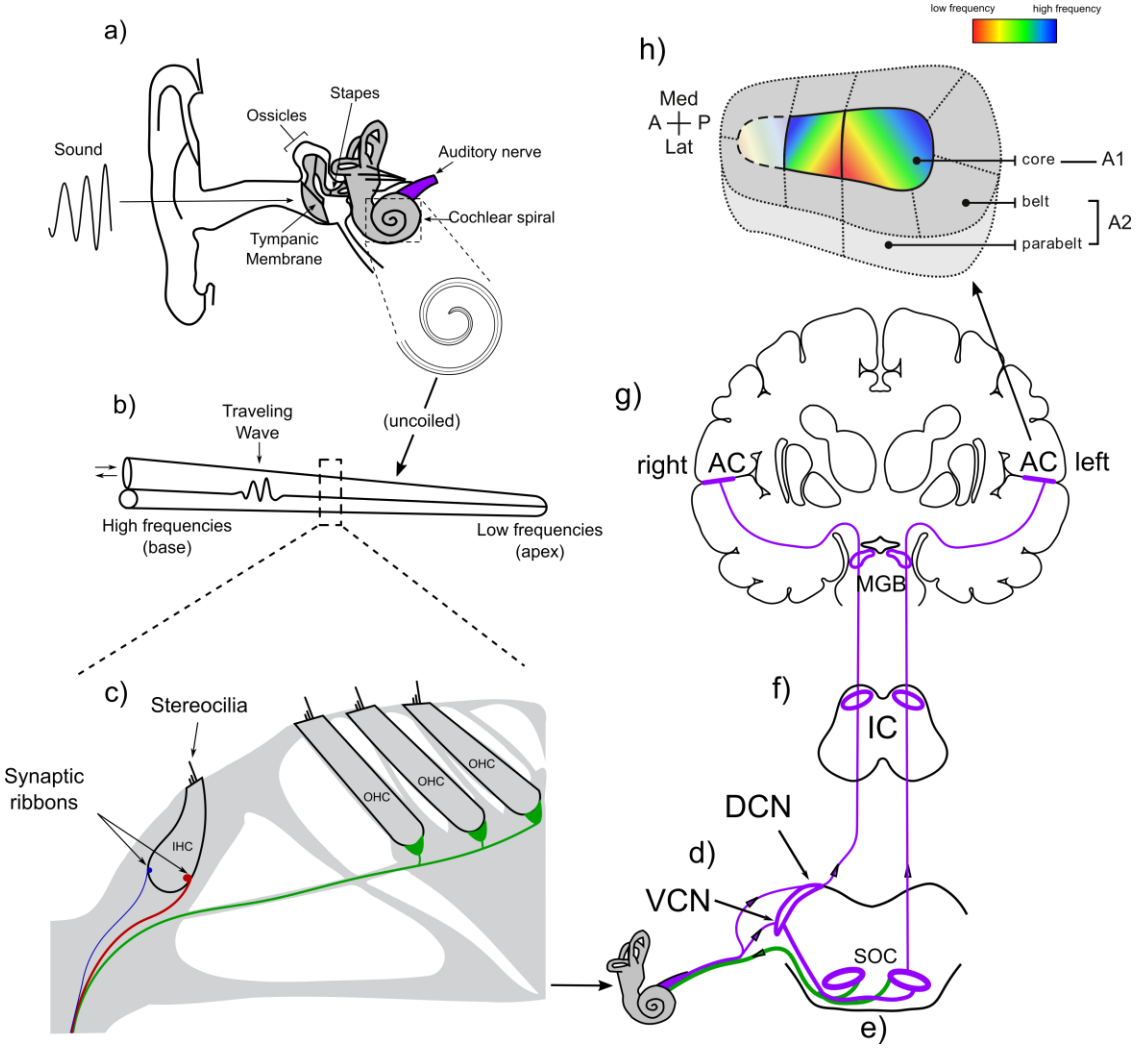

Figure 1.1. The auditory pathway. Complex sounds entering the ear in (a) mechanically vibrate structures of the middle and inner ear. Basilar membrane motion (b) deflects stereocilia of inner hair cells (IHCs) of the organ of Corti, and IHC depolarization is read out as neural impulses on auditory nerve synapses (red and blue) in (c). Active mechanisms of outer hair cells (OHCs) provide nonlinear compression and enhanced frequency selectivity along the BM which is controlled by descending influence through efferent fibers (green). Auditory information encoded in afferent auditory fibers enters the central auditory system and is sent to ventral and dorsal portions of the cochlear nucleus (VCN/DCN) in (d), whose outputs arise to the superior olivary complex (SOC) in (e) and inferior colliculus (IC) in (f). IC outputs ascend into the medial geniculate body (MGB) of the thalamus which then enters the auditory cortex (AC) in (g). In (h) auditory cortex consists of a primary core region (A1) and the surrounding A2 comprised of the belt and parabelt regions. A1exhibits two tonotopic maps sharing a low

frequency border situated laterally (red) and high frequencies radiating medially (blue). Anterior (A) and posterior (P) axes are given. The figure outlines in (a), (c), and (g) are adapted and used in accordance with the Creative Commons Attribution 2.5 Generic license from Wikimedia Commons. (h) is adapted from Saenz and Langers (2013) and is used here with permission from the publisher.

1.2.1 The auditory periphery

In (a), sound waves consisting of compressed and rarefied air pressure induce motion in the tympanic membrane, which is transferred by the bones of the middle ear (ossicles) to the oval window of the cochlea. Movement of the ossicles compresses and decompresses the fluid-filled spiraling cochlea. The fluid motion creates a traveling wave of energy shown in (b) that starts at the base of the cochlea and travels toward the apex, displacing the basilar membrane (BM) located along the cochlear partition. The stiffness of the BM changes along the length such that the natural resonant frequency of vibration changes as a function of position. Stiffness is greatest at the base and least at the apex, meaning that higher frequencies (at most 20 kHz) are naturally coded at the base and lower frequencies (at least 20 Hz) at the apex. Thus, the BM decomposes complex sound signals into constituent frequencies at specific locations along its length, referred to as a “cochleotopic” organization that gives rise to tonotopic maps seen in many structures of the ascending auditory pathway to the auditory cortex. Stimuli of higher intensities displace the BM more than stimuli of lower intensities, and the pattern of displacement with higher intensities is broader along the partition.

The vibration of the BM generates a shearing motion with respect to the tectorial membrane of the organ of Corti (shown in (c) with exception to the tectorial membrane), which is situated above the mechanosensory inner and outer hair cells. The shearing motion deflects stereocilia on top of the hair cells, and connections (called tip links) among stereocilia are thought to open ion channels and depolarize the cell. On inner hair cells (IHCs), which are responsible for transmitting frequency, intensity, and timing information of the BM motion into electrochemical signals, depolarization signals vesicular release of the excitatory neurotransmitter glutamate at the base of each cell in large synaptic zones called ribbon synapses. Glutamatergic receptors on the post-synaptic element are dendritic processes of afferent auditory nerve fibers, and a single nerve fiber contacts each ribbon synapse. These nerve fibers are elements of spiral ganglion cells that together comprise parts of the cochlear nerve, and provide the majority of the afferent input into the central nervous system. Each fiber responds to a range of sound frequencies, but single fibers are maximally responsive (i.e., "tuned") to a frequency corresponding to its location along the BM. This frequency is referred to as its characteristic frequency (CF), and it is common that neurons throughout the auditory system are tuned to specific CFs.

IHCs are contacted by 10-30 afferent fibers (Maison et al., 2013). Around 60% of these nerve fibers have high spontaneous (non-evoked) rates of firing and low stimulus-evoked firing thresholds. Evoked firing rates of these fibers saturate (i.e., do not increase further with increasing stimulus level) at around 40 dB SPL

(Liberman, 1978). Herein these fiber types are called high-SR fibers, and are shown in (c) as red and are concentrated on the pillar side of IHCs (i.e., closer to the lateral portion of the cochlea). In contrast, 40% of fibers have lower spontaneous discharge rates, higher firing thresholds (~35–40 dB SPL), and larger dynamic ranges to sound level than high-SR fibers (Liberman, 1978). These fibers are referred to here as low-SR fibers (shown in (c) as blue) and are concentrated on the modiolar side of IHCs (i.e., toward the middle of the cochlea). In the presence of background noise, evoked responses of high-SR fibers are severely degraded but low-SR fibers are fairly robust (Costaloupes et al., 1984; Young & Barta, 1986). In short, high-SR fibers best code for low-level sounds in quiet and low-SR fibers best code for higher-level stimuli especially in noisy environments (Bharadwaj et al., 2014).

When stereocilia on OHCs are sheared by BM motion, transduction currents lead to active changes in the length of OHCs. At small displacements, OHC lengthening amplifies BM displacement to increase frequency specificity of stimulus encoding along the BM. At larger displacements, OHCs actively compress to maintain frequency selectivity under broad patterns of displacement, which also protects sensitive structures of the cochlea from intense sounds (Ciuman, 2010). OHCs receive predominantly efferent (descending) input shown in green in (c) from the medial olivocochlear (MOC) system, which originates upstream in medial portions of the superior olivary complex (SOC). Modulation through MOC feedback diminishes auditory nerve firing in the presence of

continual stimulation by inhibiting OHCs, which may increase the signal to noise ratio and assist with listening in background noise (Guinan, 2006; Ciuman, 2010). In short, the nonlinear process of OHCs maintains frequency and timing acuity across a large dynamic range of stimulus levels, and OHC efferent feedback boosts stimulus coding in noise.

1.2.2 The central auditory system

As afferent auditory nerve fibers bundle and exit the cochlea, they pass into the central nervous system, divide into two branches, and as shown in (d), innervate neurons in both the dorsal and ventral portions of the cochlear nucleus (DCN and VCN, respectively). Regions of the CN maintain tonotopy, but further refinement of acoustic signals comes by way of inhibitory cells that shape cellular tuning. Some outputs from VCN target neurons in SOC shown in (e), whose primary role is to localize sound sources along the horizontal plane using cues of stimulus timing and stimulus level compared between ears. Originating in medial parts of the SOC are efferents of the MOC which descend to ipsilateral and contralateral OHCs in the cochlea.

Other outputs of both DCN and VCN ascend to the inferior colliculus (IC) in the auditory midbrain (f), a large and complex structure known to be essential in modulating both ascending and descending information, filtering and refinement of acoustic signals, binaural processing, and multimodal integration (Winer & Schreiner, 2005). Outputs from the IC (but also those directly from DCN) innervate neurons in the medial geniculate body (MGB) of the thalamus

shown in (g), which functions to filter and modify sound features encoded in upcoming neural activity before they are relayed to the cortex (Bartlett, 2013).

The auditory cortex (AC), located in Heschl's gyrus, receives input from the thalamus as shown in (g). The auditory cortex consists of a primary region, or the core (A1) shown in (h), which is surrounded by secondary or nonprimary regions called the belt and parabelt, herein referred to as A2. In humans, A1 consists of two tonotopic gradients forming a V-shaped map shown in (h), with lower frequencies situated laterally and the two "arms" of the V comprising the progressively higher frequencies moving medially (Saenz & Langers, 2014). Like most sensory cortical regions, A1 has a laminar (i.e., layered) structure. MGB sends projections directly to neurons in layer 4 and in lower layers 5 and 6, whose output is sent to higher layers 2/3. Thalamic innervation however accounts for around 10% of input to cortical neurons, whereas the dominant number of cortical connections consists of other excitatory and inhibitory cortical cells which may function to maintain sharp spectrotemporal tuning (Abeles, 1991; Schreiner & Winer, 2007). Output from the core extends into A2, which exhibits a weaker tonotopy, contains more diffuse intracortical connections in comparison to A1, and receives diverse multisensory inputs (Tardif & Clarke, 2001; Kaas & Hackett, 2002; Schreiner & Winer, 2007).

1.3 Causes of peripheral deafferentation and factors related to tinnitus

1.3.1 Causes of deafferentation

Noise overexposure and aging are two of the most common causes of damage to hair cells and their synaptic contacts to auditory nerve fibers (Kujawa & Liberman, 2015), and may be leading causal factors behind tinnitus. Standard methods for detecting cochlear damage focus on measurements at threshold, including the aforementioned behavioural audiogram. Thresholds of neural firing in the periphery can also be assessed by the auditory brainstem response (ABR), a potential evoked by tones or clicks that is averaged to reveal five waveforms representing the output of structures in the ascending auditory pathway (Melcher & Kiang, 1996). The first of these waves (ABR wave I) represents the potential generated by the synchronous firing of the auditory nerve.

OHC damage will result in a loss of cochlear amplification, frequency selectivity, and compression (Cody & Russell, 1985). Thresholds will elevate (Borg, 1987), and the presence of background noise will more strongly mask signals (Davis et al., 1989). Because they are an active process, the activity of OHCs produce sounds known as otoacoustic emissions (OAEs) that are detectable by a microphone placed in the external auditory canal (reviewed in Brownell, 1990). Spontaneous OAEs can be recorded, but OAEs are more commonly evoked by the distortion product created on the basilar membrane by two simultaneously presented brief tones (cubic difference tone, $2f_1-f_2$) at different sound pressure levels. These are known as distortion product OAEs (DPOAEs).

Loss of OHCs will diminish both spontaneous OAEs and DPOAEs. Exclusive IHC loss without OHC loss is difficult (if not impossible) to obtain with methods of noise exposure. Alternatively, studies have used carboplatin, a drug commonly used to treat cancer, which selectively kills IHCs while leaving OHCs intact (Wake et al., 1994). Using this method, Lobarinas et al. (2013) demonstrated that audiometric thresholds do not significantly elevate until about 80% of IHCs are lost, speaking to the idea that few of these cells (and thus nerve fibers) are needed to produce a normal audiogram.

Noise exposure can either lead to threshold shifts that do not recover (permanent shifts), or temporary threshold shifts that return to normal after a few days or weeks. Temporary shifts were originally thought to occur without long-term damage (Puel et al., 1998); however, Kujawa and Liberman (2009) found that moderate noise exposure leads to a permanent degradation in suprathreshold ABR wave I responses in high-frequency hearing regions, although wave I threshold shifts were only temporary. Suprathreshold deficits were attributable to an irreversible loss of ribbon synapses contacting low-SR fibers (see blue fibers in Figure 1.1c), while high-SR fibers were less affected (Furman et al., 2013). Several months after exposure, nerve fibers once contacting these synapses degenerated significantly (Kujawa & Liberman, 2009). Loss of peripheral synapses or nerve fibers that is undetected by threshold tests such as the audiogram is now commonly referred to as “hidden” hearing loss (Schaette & McAlpine, 2011).

Because low-SR fibers strongly phase lock to temporal modulations of the sound envelope, such as amplitude modulation (AM), Bharadwaj et al. (2014) reasoned that deficits in AM encoding might indicate hidden low-SR fiber loss. AM encoding can be measured behaviourally in psychophysical tasks or electrophysiologically with the "envelope following response" (EFR), an evoked potential phase locked to an AM sound that arises from the auditory midbrain (Herdman et al., 2002). Bharadwaj et al. (2015) found that individuals with normal audiograms and DPOAEs exhibited a wide range of ability to detect AM in a high-frequency, 4 kHz narrowband noise where hidden hearing damage might be expected. Behavioural AM detection significantly correlated with the EFR such that those with poor AM sensitivity had diminished EFRs evoked by sounds with shallow amplitude modulations. These individuals were also likely to have more self-reported noise exposure. It was suggested that those with AM encoding deficits had more low-SR fiber loss than individuals with good AM sensitivity and robust EFRs. Using an animal model, Shaheen et al. (2015) found that EFRs after noise exposure were likewise reduced, matching predictions of predominantly low-SR fiber loss.

Age-related hearing loss is typically associated with loss of cochlear hair cells and decline of spiral ganglion cells (Makary et al., 2011). Sergeyenko et al. (2013) however recently found that mice unexposed to noise exhibited synaptic ribbon loss over time that preceded spiral ganglion decline and OHC loss. Moreover, acute noise damage has been shown to accelerate this age-related

synaptic decline (Fernandez et al., 2015). In sum, synaptic connections between IHCs and auditory nerve fibers are likely the most sensitive components of cochlear transduction to effects of noise and aging, and are undetected by threshold measurements unless loss exceeds levels around 80%.

1.3.2 Peripheral damage in tinnitus

Despite the assertion that hearing damage following noise exposure and aging can trigger the development of tinnitus, audiometric hearing loss and tinnitus are not always concurrent. Many individuals with audiometric threshold shifts in the high frequency hearing region do not have tinnitus, a circumstance four times more common than the presence of both threshold shifts and tinnitus (Lockwood et al., 2002). In contrast, 15% of individuals without audiometric threshold shifts to 8 kHz do not have tinnitus (Henry et al., 2005). Despite the fact that the audiogram may not be detecting hidden synaptic or nerve fiber loss in these cases, when audiograms between tinnitus and non-tinnitus individuals are compared, differences are still evident that suggest peripheral damage. Roberts et al. (2008) compared audiograms of a large sample of tinnitus subjects closely matched in age to non-tinnitus subjects in order to control for age-related cochlear damage. Subjects in both groups below the age of 50 expectedly had normal hearing thresholds to 10 kHz, whereas subjects over 50 years exhibited elevations of thresholds commencing at 2 kHz which further declined with increasing frequency consistent with age-related hearing loss. However, tinnitus subjects in both age cohorts tended to have thresholds ~11 dB hearing level (HL) higher in

the 2-8 kHz region compared to controls, which may indicate the presence of further cochlear damage.

Assessments of IHC and OHC function however have not been able to distinguish the presence of tinnitus. Some studies have reported reduced DPOAEs in tinnitus subjects within the region of audiometric threshold shift suggesting OHC impairment (e.g., Zhou et al., 2011) but others examining tinnitus sufferers with normal audiograms report that not all individuals show DPOAE deficits (Serra et al., 2015). Tan et al. (2013) inferred OHC integrity by taking psychophysical measurements of frequency selectivity and compression in individuals with threshold shifts that did and did not have tinnitus. Tinnitus subjects were in fact found to have better compression and frequency selectivity than controls, discounting OHC dysfunction in tinnitus and rather suggesting the presence of IHC damage. In agreement Weisz et al. (2006) found evidence for high-frequency IHC loss in tinnitus individuals with normal audiograms using a psychophysical task that tests for cochlear "dead regions," but a more recent report found that only just above half of individuals with tinnitus and high-frequency audiometric loss have the same evidence of IHC loss (Kiani et al., 2013). Another report found no evidence of cochlear dead regions in tinnitus suffers with and without high-frequency threshold shifts (Gilles et al., 2013).

Two recent reports have instead pointed to the presence of synaptic loss in tinnitus individuals (Schaette & McAlpine, 2011; Gu et al., 2012). These studies found that the amplitude of ABR wave I evoked by suprathreshold click stimuli

was smaller in tinnitus subjects with normal audiograms compared to controls with closely-matched audiograms. Diminished auditory nerve responses that would produce these results were attributable to loss of low-SR auditory nerve fibers as found in animals (Kujawa & Liberman, 2009; Furman et al., 2013), since behavioural audiograms were normal (Schaette & McAlpine, 2011). This interpretation however is weakened by recent animal evidence showing that low-SR fibers do not strongly contribute to ABR wave I (Bourien et al., 2014) meaning that the precise pattern of synaptic or nerve fiber loss explaining ABR wave I deficits in tinnitus has not yet been determined. The experiment described in Chapter 2 is aimed to more directly test for low-SR fiber loss in tinnitus subjects with normal audiograms by measuring AM sensitivity and EFRs, similar to approaches taken by Bharadwaj et al. (2015).

1.4 Central changes related to tinnitus

1.4.1 General consequences of deafferentation in central auditory structures

In response to chronic under- or overstimulation, neurons have self-regulating negative feedback processes that keep their firing rates operating within a prescribed range, which are collectively known as mechanisms of homeostatic plasticity (Turrigiano & Nelson, 2004). For example, neurons that are persistently deprived of stimulation below their baseline level of input will change their intrinsic gain by upregulating excitatory properties and downregulating inhibitory properties to maintain a set level of firing activity over the long term (Turrigiano, 2011). It has been widely demonstrated that deafferented central auditory neurons

express hyperactivity consistent with homeostatic plasticity, such as increases of spontaneous firing rates (SFRs) and evoked firing rates, burst firing, and correlated or synchronous firing between pairs of auditory neurons (Noreña & Farley, 2013). These findings led to the hypothesis that tinnitus-related hyperactivity results from homeostatic changes in central gain (Schaette & Kempter, 2006; Noreña & Farley, 2013). A role for non-homeostatic activity-related synaptic plasticity in the formation of tinnitus is also likely, such as spike-timing dependent plasticity (STDP) in which the timing relationship of pre- and post-synaptic firing (spiking) can increase or decrease the strength of the synapse (Koehler & Shore, 2013; Basura et al., 2015). Because this type of plasticity is dependent on finely-regulated timing, STDP is believed to foster synchronous activity in neural networks (Nowotny, 2003).

Aside from tinnitus, a separate condition hypothesized to arise from increased central gain after noise exposure is called *hyperacusis* (Sun et al., 2012), defined as hypersensitivity to loud sounds. Tinnitus sufferers with normal audiometric thresholds are known to likewise have abnormal loudness discomfort levels (evidence of hyperacusis), suggesting enhanced central gain that could have resulted from undetected peripheral synaptic loss (Hébert et al., 2013). The notion that tinnitus and hyperacusis are consequences of similar etiologies and mechanisms is a topic of extensive discussion (Auerbach et al., 2014). However, the review here focuses solely on evidence directly relating to tinnitus.

1.4.2 Models and limitations in animal research

Animal research has significantly advanced the tinnitus research field by uncovering the cellular and molecular consequences of noise exposure on central auditory processing. However animals, unlike humans, cannot simply report that they have tinnitus, meaning that the presence of tinnitus must be inferred from their behaviour (Hayes et al., 2014). Some procedures (e.g., Jastreboff et al., 1988) condition or train animals to only respond (such as lick water or press a lever) in the presence of sound. If after hearing damage animals continue to perform these behaviours in the absence of sound, it is presumed they hear tinnitus. Other more objective methods have inferred the presence of tinnitus by testing the startle reflex of an animal. Sounds preceding a startle stimulus known to inhibit the startle reflex in healthy animals do not inhibit reflexes in tinnitus animals because the presence of tinnitus is assumed to mask the pre-startle sound (Turner et al., 2006). Gap-startle paradigms are not without controversy in that a parallel explanation for why an animal cannot detect a pre-startle pulse may be the presence of undetected hearing loss or impaired temporal processing (Galazyuk & Hébert, 2015). Some studies have found an opposite effect where tinnitus-inducing noise increases the startle response, which may indicate the presence of hyperacusis (Salloum et al., 2016). Although conditioning paradigm and gap-startle animal models of tinnitus are collectively referred to as "animals with behavioural tinnitus" herein, a cautionary note moving forward is that these confounding factors limit the generalizability of animal findings to human tinnitus research.

Aside from noise exposure and aging, tinnitus can be induced by ototoxic drugs such as salicylate, the active ingredient of aspirin (Cazals, 2000) which is a commonly used tinnitus induction method in animal models (Stolzberg et al., 2012). However salicylate may operate differently from noise exposure in that it directly influences central hyperactivity, and at doses necessary to induce tinnitus salicylate does not affect cochlear function (Stolzberg et al., 2012). For this reason discussion of animal research herein focuses on tinnitus following noise exposure. An additional note is that noise exposure across animal studies produces vastly different patterns of cochlear damage, with some protocols producing only temporary threshold shifts while others show permanent shifts. Based on the notion that thresholds only partly reflect cochlear damage due to noise (see Section 1.3), degrees of threshold shift are not distinguished in the discussion below. A final limitation is that many animal studies which may be important to understand the effects of central hyperactivity after hearing loss commonly do not directly measure tinnitus-related behaviours. Such studies and their complement to tinnitus are discussed in Eggermont (2015) and Berger and Coomber (2015), but here focus will be placed on reports that measured for evidence of tinnitus.

1.4.3 Central auditory changes in animal models of tinnitus

Tinnitus research using animal models has focused on measuring three primary electrophysiological correlates of neurons in the central auditory system: increases in SFRs, burst firing, and synchronous firing between pairs of neurons. It has not been determined which correlate or configuration of them is the direct neural

generator of tinnitus, but synchronous activity is a strong candidate since it is likely involved in the formation of auditory objects that may emerge into conscious perception (Brette, 2012). SFR increases are one of the most widely reported correlates of tinnitus-related behaviour in animals following noise exposure, appearing in DCN (Brozoski et al., 2002; Kaltenbach et al., 2004; Dehmel et al., 2012; Wu et al., 2016), IC (Bauer et al., 2008; Longenecker & Galazyuk, 2011), and A1 (Engineer et al., 2011; Basura et al., 2015; Ahlf et al., 2012). Increases in rhythmic burst firing have likewise been found in DCN (Wu et al., 2016), IC (Bauer et al., 2008), and MGB (Kalappa et al., 2014), and increased synchrony has been noted in DCN (Wu et al., 2016), IC (Bauer et al., 2008), and A1 (Engineer et al., 2011).

Hyperactivity in the central auditory pathway of tinnitus animals may relate to homeostatic modifications of neurotransmission (Noreña & Farley, 2013). However, findings implicating the downregulation of inhibitory GABA, which is typically associated with homeostatic changes resulting from reduced input (Turrigiano, 2011), have been less consistent in animals with behavioural tinnitus. GABA has been shown to both decrease (Middleton et al., 2011), and increase in DCN (Brozoski et al., 2012), as well as decrease (Brozoski et al., 2012) and increase (Sametsky et al., 2015) in MGB. Brozoski et al. (2012) interestingly found no difference in excitatory and inhibitory neurotransmission in IC between noise-exposed tinnitus and non-tinnitus animals. A1 also shows evidence for decreased GABA (Llano et al., 2012). DCN neurons in animals with

behavioural evidence of tinnitus have also been associated with reduction of potassium channels (Li et al., 2013; Li et al., 2015) and glycinergic (inhibitory) synapses (Wang et al., 2009) , resulting in increased neuronal excitability.

Changes in the timing rules of STDP are also associated with tinnitus. The trigeminal cranial nerve (responsible for facial movements) supplies inputs into DCN which upregulate after cochlear deafening or noise exposure (Zeng et al., 2012; Dehmel et al., 2012). In normal animals, the strength of synaptic contacts between auditory and somatosensory inputs is known to change through STDP (Koehler et al., 2010). In noise-exposed animals with tinnitus, the timing rules governing STDP between these inputs reverse such that the auditory-somatosensory spike pairing that normally decreases SFRs in non-tinnitus animals conversely increases SFRs in tinnitus animals. The net effect of the STDP change in this manner could increase upstream excitatory drive and promote neural synchrony (Koehler & Shore, 2013). The same inversion of STDP rules has been found in A1 of animals with behavioural evidence of tinnitus (Basura et al., 2015).

An open question is whether or not hyperactivity in each structure of the auditory pathway is necessary for tinnitus. Ropp et al. (2014) found that SFR increases in tinnitus animals four months after exposure only occurred in IC neurons targeted by VCN projections and not DCN. Since DCN ablation a few weeks after exposure abolishes tinnitus (Brozoski et al., 2011) but ablation after 3-5 months does not (Bauer & Brozoski, 2005), the authors suggest a role of the

VCN in maintaining heightened SFRs related to tinnitus at least up to the level of IC, but initial hyperactivity may be primarily driven by DCN. This notion is further supported by the finding that tinnitus animals did not show changes in the content of inhibitory or excitatory neurotransmitters in IC (Brozoski et al., 2012), overall suggesting that the IC changes are driven by tinnitus-related hyperactivity in CN.

A final putative correlate of tinnitus after deafferentation is tonotopic map reorganization, in which A1 neurons in the hearing loss frequency region tend to shift their tuning properties toward neurons that retain their afferent input at the edge of audiometric loss (i.e., before commencement of threshold shift; Noreña et al., 2003) such that the edge frequencies become overrepresented. The view is that changes in the balance of excitation and inhibition in the edge region may be responsible for maintaining the tinnitus percept (Eggermont, 2006). Other studies have found that map plasticity in animals with behavioural tinnitus was only transient (Ahlf et al., 2012), and human neuroimaging evidence failed to find a significant difference in the tonotopic maps of individuals with and without tinnitus matched in audiometric thresholds (Langers et al., 2012) although spatial resolution in this latter study may be insufficient to detect microscopic changes. It is notable that the tinnitus spectrum typically peaks well within the region of threshold shifts and not at the audiometric edge (Roberts et al., 2008). Tonotopic map reorganization may be important to understand more general effects of

deafferentation in auditory cortex, but it is presently unclear if map reorganization directly contributes to the tinnitus percept.

1.4.6 Agreement with human findings

Matching animal findings, evidence of hyperactivity is present in the subcortical pathway of humans with tinnitus. Gu et al. (2012) found that individuals with tinnitus and normal thresholds had reduced ABR wave I to suprathreshold clicks (indicative of synaptic loss) but enhanced wave III (generated in VCN; Melcher & Kiang, 1996) compared to matched controls, suggesting a gain increase in this structure. Wave V (lemniscal input to IC and IC itself) was also increased, agreeing with normalized Wave V amplitudes reported by Schaette and McAlpine (2011). Using functional magnetic resonance imaging (fMRI), increased evoked metabolic responses have been found in IC of tinnitus subjects (Lanting et al., 2008; Melcher et al., 2009) consistent with effects of hyperactivity, although Gu et al. (2010) suggested these results may relate more to the presence of hyperacusis rather than tinnitus.

In auditory cortex, both A1 and A2 regions show increased resting state metabolic activity in tinnitus sufferers (Arnold et al., 1996; Issa et al., 2016) suggestive of ongoing hyperactivity. Resting state recordings of the electroencephalogram (EEG) have shown greater power of spontaneous rhythmic gamma oscillations (neural potentials oscillating at rates > 30 Hz) compared to controls suggesting increases in synchronous neural activity, although some of these findings may not be specifically generated in auditory cortex (Weisz et al.,

2007; Lorenz et al., 2009; Sedley et al., 2012). Decreases in inhibitory-related alpha activity (9-12 Hz) in the resting state EEG have also been found (Weisz et al., 2005; Lorenz et al., 2009). Using magnetic resonance spectroscopy to measure neurochemical content, Sedley et al. (2015) reported a reduction in the presence of GABA in the auditory cortex of tinnitus individuals compared to controls, suggesting diminished inhibition which directly agrees with animal findings (Llano et al., 2012). Evoked activity in auditory cortex is also generally found to be elevated in humans with tinnitus suggesting hyperactivity and reduced inhibition (e.g., Gu et al., 2010; Diesch et al., 2010; Weinbruch et al., 2006) but findings are more inconsistent, depend on stimulus frequency, and are sensitive to task designs (see below).

1.5 Tinnitus and attention

A wealth of evidence suggests that non-auditory brain regions and networks, such as those involved in attention, arousal, distress, and consciousness, are implicated in the perception of tinnitus (Jastreboff, 1990; Roberts et al., 2013; Adjamian et al., 2009; Adjamian et al., 2014; Husain, 2016). The involvement of these networks is unsurprising since tinnitus is frequently associated with depression, pain, and anxiety (Joos et al., 2012; Rauschecker et al., 2015; Landgrebe & Langguth, 2012). Although many human studies have reported inconsistent and contradictory evidence regarding the anatomical (Adjamian et al., 2014) or functional (Adjamian et al., 2009; Husain, 2016) significance of auditory and non-auditory interactions in tinnitus, partly due to methodological or inter-subject

factors, it is clear that the influence of tinnitus on broader cognitive function cannot be ignored.

1.5.1 Overview of attention

One factor relevant to tinnitus may be attention (Roberts et al., 2013). Attention here broadly refers to the direction of consciousness toward a stimulus or stimulus feature. Many frameworks have organized attention into "top-down" and "bottom-up" forms (e.g., Corbetta & Schulman, 2002), with the former referring to voluntary goal-directed selection of desired stimulus features, and the latter a result of detection and involuntary reorienting toward unexpected or incorrectly predicted events or sensory events with high saliency. Some have argued that top-down and bottom-up forms of attention are independent and emerge from distinct neural processes (Pinto et al., 2013), while others have reasoned that the top-down and bottom-up dichotomy is inadequate and rather suggest an integrated attention system based on behavioural priorities (Awh et al., 2012). Although appreciable, this debate cannot be presently resolved and the discussion here and in future chapters revolves around top-down and bottom-up forms of attention.

It is well established that attention influences neural processing in the auditory system in a manner that enhances or facilitates perception (Fritz et al., 2007; Lee et al., 2007). A common manipulation of top-down attention in such studies requires participants to actively attend to stimulus features and detect or categorize differences among them (active listening) which is then compared to a condition prompting subjects to not respond to stimuli and passively listen. The

amplitude of two evoked auditory cortical responses known to be larger during active listening in this manner are the 40 Hz auditory steady state response (ASSR), which is phase-locked to the envelope of a 40 Hz AM tone, and the N1 transient response which reaches a maximum amplitude ~80–120 ms after stimulus onset (Gander et al., 2010a/b). The 40 Hz ASSR is known to be generated in A1 (Gander et al., 2010a/b, Bidet-Caulet et al., 2007), and when evoked by tones of different frequencies the ASSR sources exhibit a frequency organization that parallels the A1 tonotopic map (Wienbruch et al., 2006; Gander et al 2010a). In contrast, the N1 response localizes to generators in regions of A2 (Godey et al., 2001) which are weakly or non-tonotopic (Moerel et al., 2014). Studies reporting increased metabolic activity in A1 and A2 under conditions of active listening (Grady et al., 1997; Paltoglou et al., 2009) agree with attentional effects on the ASSR and N1.

Neurophysiological mechanisms of attention that would enhance A1 and A2 responses are not fully understood, but could implicate neuromodulatory systems that appear to perform attention-like functions in sensory brain regions (Roberts et al., 2013). A likely candidate is the basal forebrain (BF) cholinergic system, wherein cholinergic projections originating from the nucleus basalis innervate sensory cortical neurons and sensitize them to their thalamocortical inputs depending on task requirements (Sarter et al., 2005; Fritz et al., 2007). The BF system can be activated from the bottom-up by salient events in order for neural networks to encode and represent new and relevant stimulus features, but

descending influence from the prefrontal cortex onto the BF system may produce top-down effects (Sarter et al., 2005). The cholinergic influence of the BF system is thought to modulate training-related plasticity of auditory cortical neurons in tasks that require the subject to quickly learn and attend to relevant stimulus features (Weinberger, 2004). BF-directed plasticity may underlie the observation that physiological properties of A1 neurons can be rapidly tuned to best represent stimulus features that align with behaviourally-relevant goals (Fritz et al., 2007). One recent study found that blocking cholinergic reuptake in rat auditory cortex enhanced the 40 Hz ASSR amplitude whereas cholinergic antagonists diminished ASSR amplitude (Zhang et al., 2016), agreeing with observed effects of top-down attention on the ASSR in humans.

1.5.2 Attentional deficits in tinnitus

Roberts et al. (2013) proposed a model suggesting that attention is a contributing or generative factor in the development of tinnitus following deafferentation. In this model it was assumed that a key function of the auditory cortex was to predict its sensory state (i.e., information in the auditory environment) by comparing neural activity representing the sensory environment in auditory cortex to information ascending from subcortical structures, in line with "predictive coding" models that are proposed to be a ubiquitous principle in sensory cortical areas (Friston & Kiebel, 2009). One way this could be achieved in A1 is shown in Figure 1.2a, wherein ascending thalamic (MGB) input provides excitatory drive (black) onto cortical layers 3 and 4 of A1 and feedforward inhibitory drive

(orange) onto deeper layers 5 and 6. Predictions held in upper cortical layers 2 and 3 which maintain the central representation of sound are integrated with upcoming excitatory drive and are combined with information stored in auditory memory and association areas (not shown). Comparisons are made by separate neurons that integrate the excitatory drive from predicted content and the inhibitory drive from deeper layers. If these two inputs are the same, they cancel, and the auditory cortex continues to generate predictions and inform behaviour in line with its current state.

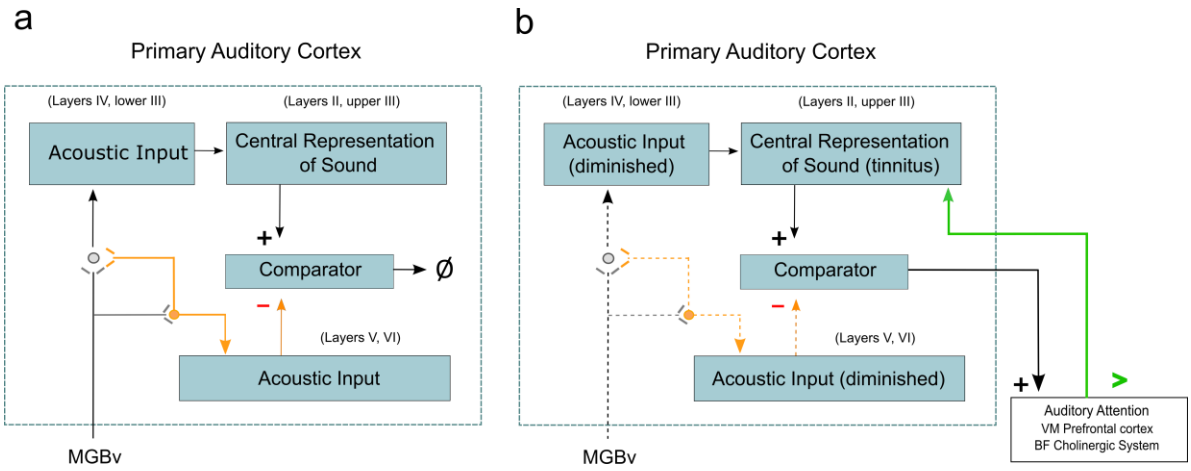

Figure 1.2 Attention model of Roberts et al. (2013). Adapted and used here with permission from the publisher. Black lines are excitatory and orange lines are inhibitory. Green lines in (b) are modulatory.

In the circumstance of tinnitus shown in Figure 1.2b, the presence of tinnitus-related hyperactivity maintained in cortex (reinforced further by its presence in auditory memory) does not match the diminished input coming in from the auditory periphery. The prediction error calls attention in a bottom-up fashion (here represented by the BF cholinergic system and directed by prefrontal

cortex) to resolve the disparity. Since the disparity cannot be resolved, the cholinergic system is chronically engaged in A1, sensitizing the already hyperactive neurons in the TFR and fostering plasticity in a manner that strengthens the connections between them. It should be noted that a more recent model of tinnitus also suggest failures of predictive coding (De Ridder et al., 2015) but does not presume deafferentation is necessary for tinnitus.

The summary of the model in Figure 1.2 is that an attention system is persistently engaged which may maintain the neural activity that is experienced as the tinnitus sound. Behavioural and neuroimaging studies can be interpreted to support this view. Individuals with tinnitus are known to perform poorly on auditory (Cuny et al., 2004) and non-auditory tasks measuring working memory, divided attention, and reaction times (Rossiter et al., 2006; Stevens et al., 2007; Heeren et al., 2014; Donhoffer et al., 2006), suggesting that attention directed to the presence of tinnitus may detract from shared cognitive resources. Resting state neuroimaging studies have found a significant engagement between attention networks and auditory regions in tinnitus subjects (Mirz et al., 2000; Maudoux et al., 2012; Burton et al., 2012), and increased evoked metabolic responses in A1 and A2 observed under passive listening conditions in tinnitus subjects relative to controls (Gu et al., 2010; Langers et al., 2012) may likewise suggest an ongoing expression of attention in these areas, notwithstanding enhancements attributable to central gain (Roberts et al., 2013).

Bottom-up forms of auditory attention likely function to assist cortical areas in rebuilding an accurate representation an acoustic scene after a novel or unexpected input, meaning that bottom-up sensitization of auditory neurons should be broadly deployed across the frequency range of hearing in order to best capture unpredicted information (Roberts et al., 2013). Under the attention model in Figure 1.2 this would mean that evidence for persistent attention should also occur in frequency regions outside the TFR that are not affected by cochlear damage. In agreement, tinnitus subjects in Roberts et al. (2015) were reported to have larger ASSR and N1 responses evoked at 500 Hz (a frequency well below the TFR) recorded in conditions where subjects did not respond and passively listened than those observed in matched control subjects, suggesting a possible attentional enhancement for frequency regions not affected by deafferentation in both A1 and A2. N1 responses evoked at 5 kHz in this study were also larger in tinnitus subjects compared to controls suggesting attentional facilitation, but the ASSR at 5 kHz was smaller likely owing to the presence of hypersynchrony that disrupted the ability of A1 neurons in this region to phase lock to the AM envelope of the stimulus (i.e., a "busy-line" effect). ASSR responses below 2 kHz reported in by Wienbruch et al. (2006) were likewise larger in tinnitus subjects, although responses above 2 kHz were not statistically different from controls. Diesch et al. (2012) however failed to find N1 enhancements in tinnitus subjects compared to controls although ASSR enhancements in tinnitus were significant, which was interpreted to mean that attention was not persistently engaged in

tinnitus subjects. Further inconsistent N1 findings in tinnitus were reviewed by Roberts et al. (2013), who discussed several factors that may affect whether transient N1 responses are sensitive to attentional modulation in tinnitus, similarly paint an uncertain picture for attentional enhancement at least in the area of A2.

In a more direct approach, Roberts et al. (2012) found that the ASSR and N1 amplitude evoked by a 5 kHz tone (a frequency within the TFR) was not enhanced in a top-down active listening condition compared to a passive listening condition, but attentional enhancement of these responses was normal in age-matched controls with similar audiograms. Consistent with the model in Figure 1.2, a first explanation for these results could be that the top-down attention system was already engaged in tinnitus subjects in the passive condition, limiting any further facilitation during the active listening task. Subjects in Roberts et al. (2012) went on to train on the auditory task for several sessions, which resulted in the emergence of a significant attentional modulation of the 5 kHz ASSR amplitude after only a few sessions that was not observed for N1. Training-related effects were notably not found for control subjects for either evoked response. These findings invite a second explanation that top-down attention operates normally in tinnitus but could not initially be expressed within the deafferented region of A1 (and perhaps A2) because neural networks in this region were occupied with coding the tinnitus percept. The resistance of A1 neurons to top-down attention might have been reduced by active training, since the increased amplitude of the ASSR observed with training suggested that new neurons had

been recruited to represent the 5 kHz training sound. Without evidence further supporting one of these alternatives, the role attention plays in tinnitus under the deafferentation model remains uncertain.

Empirical work in Chapter 3 tested these alternatives by replicating the experimental procedure used in Roberts et al. (2012) using tinnitus and control subjects matched in age and hearing thresholds, but instead used a 500 Hz AM tone stimulus which is well below the TFR. If attentional modulation of the ASSR and N1 at 500 Hz is absent it could suggest that, similar to 5 kHz, top-down attention was already widely deployed in a frequency nonspecific manner (Gander et al., 2010a) and could not offer further enhancement under active listening conditions. If attentional modulation of these low-frequency responses is observed in tinnitus, it would suggest that the top-down attention system operates normally in tinnitus, but persistent tinnitus-related neural activity may impede its expression in stimulus-evoked transient responses. Results in Chapter 3 favoured the second hypothesis.

1.5.3 Bottom-up factors interacting with top-down attention in tinnitus

The suggestion that neural populations in A1 are resistant to modulation by top-down attention may in principle result from the fact that neurons in the TFR are generally insensitive to their inputs, provided that they are otherwise occupied with coding tinnitus-related activity. A different test to evaluate this notion is to briefly disrupt stimulus encoding from the bottom-up and evaluate if A1 neurons in the TFR are sensitive to the change. Bottom-up factors in this sense refer to the

ability of cortical neural networks to index small changes in ongoing stimuli and not specifically refer to mechanisms of bottom-up attention, although it should be noted that these two instances likely operate cooperatively in normal information processing.

One way to test the sensitivity of auditory cortical networks to bottom-up input is to use the ASSR reset response (Ross & Pantev, 2004), shown in Figure 1.3. By inserting a brief silent gap in the AM sound, the ASSR phase desynchronizes from the AM rhythm and the ASSR amplitude rapidly decreases. After stimulation resumes, the ASSR recovers its phase and amplitude over the period of about 200 ms. The 200 ms recovery window can be considered a snapshot into how A1 neurons rebuild the AM envelope from the bottom up. This stimulus design has a similar potential to evaluate the role of attention in tinnitus, specifically by testing if an affected population of A1 neurons can rebuild the phase and amplitude of the AM representation more quickly (or perhaps slowly) than controls, speaking to the prior engagement of attention-related facilitation.

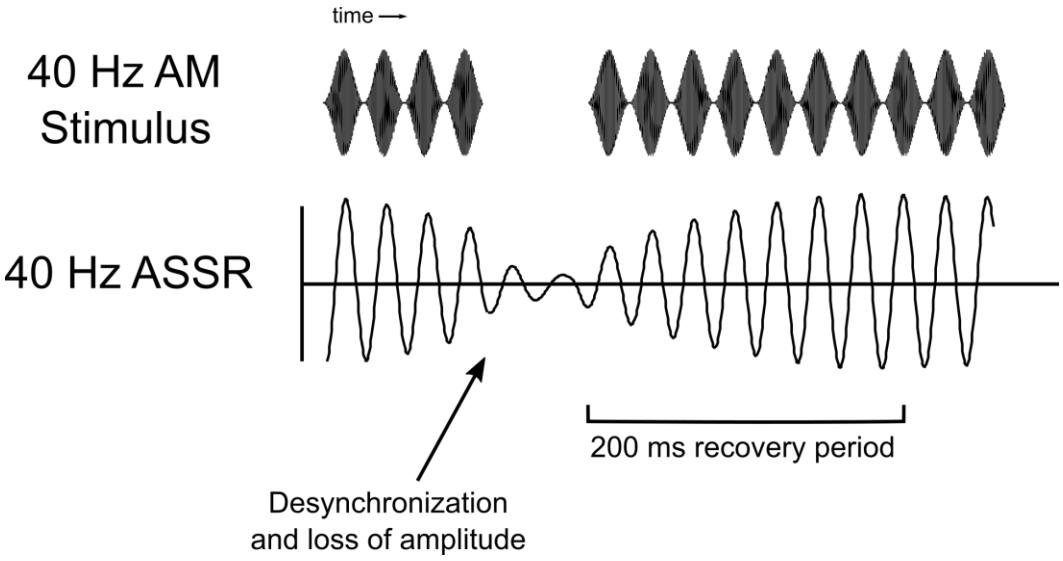

Figure 1.3. ASSR reset response. A silent gap uncouples the AM stimulus and the ASSR. After stimulation restarts, the phase and amplitude of the ASSR recovers within 200 ms.

Despite this conception, there is no previous research that determines if the phase or amplitude of the ASSR reset is sensitive to top-down attention. Empirical work in Chapter 4 tests this hypothesis for ASSR resets evoked by 400 Hz and 5 kHz carriers using young non-tinnitus subjects in order to establish a baseline set of results. The findings were that spectral estimates of phase shifts during the 200 ms recovery period were sensitive to the manipulation of top-down attention for both high and low frequency probes, but amplitude was not. With this finding, future studies can bring the ASSR reset paradigm into use with tinnitus subjects to evaluate nuanced effects of top-down attention and sensitivity of neurons in the TFR to bottom-up input.

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Chapter 2: Amplitude modulation encoding deficits suggest hidden hearing loss in individuals with tinnitus and normal audiograms

Paul, B. T., Bruce, I.C., Roberts, L.E. (Submitted). Amplitude modulation encoding deficits suggest hidden hearing loss in individuals with normal audiograms.

2.1 Preface

The observation that audiometric threshold shifts and tinnitus are not always coincident has challenged the view that hearing loss is necessary for tinnitus (Adjamian et al., 2009), which may be considered one of the biggest challenges to the deafferentation model. However, hearing thresholds are not sensitive to detect loss of peripheral nerve fibers and synapses which can occur after noise damage (Kujawa & Liberman, 2009; Furman et al., 2013) and aging (Sergeyenko et al., 2013). Thus, it is possible that tinnitus sufferers with normal audiograms have such "hidden" loss of peripheral nerve fibers or synapses that remains undetected by the audiogram.

This idea was first supported in humans by studies showing that Wave I of the click-evoked ABR (representing auditory nerve output) was smaller in tinnitus subjects with normal audiograms compared to controls (Schaette & McAlpine, 2011; Gu et al., 2012). The computational model of Schaette and McAlpine (2011) suggested that low-SR fiber loss was responsible for this result, but the small contribution of these fiber types to the ABR (Bourien et al., 2014) casts

doubt on this assumption. A separate way to assess the function of peripheral nerve fibers is by the envelope following response (EFR; Bharadwaj et al., 2014; Shaheen et al., 2015) which had previously been used in non-tinnitus subjects to reveal suprathreshold deficits consistent with low-SR fiber loss (Bharadwaj et al., 2015).

Using similar approaches, the empirical study presented in this chapter tested the hypothesis that tinnitus sufferers with normal audiograms have evidence for loss of auditory nerve fibers. We measured EFRs evoked by an 85 Hz AM tone with a 5 kHz carrier frequency (in the TFR). EFRs were evoked in conditions of quiet and background noise at a level sufficient to strongly saturate high-SR fibers so that contributions of low- and high-SR fibers could be separately assessed. Additionally we measured the detection threshold for an AM tone in background noise, which was compared to EFR measures. Finally, we used a model of the auditory periphery (Zilany et al., 2014) to simulate auditory nerve responses under our stimulus conditions that could be compared to EFR observations. Evidence of "hidden" hearing loss in tinnitus subjects would support the notion that their tinnitus was triggered by deafferentation.

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2.2 Abstract

Most cases of tinnitus (persistent ringing of the ears) are related to audiometric hearing loss, but some individuals with tinnitus do not have threshold shifts measured by the clinical audiogram. We tested whether these individuals may have hidden synaptic losses on auditory nerve fibers with low spontaneous rates of firing (low-SR fibers) that are important for coding suprathreshold sounds in noise while high-SR fibers determining threshold responses in quiet remain relatively unaffected. Tinnitus and control subjects with clinically normal thresholds were required to detect the presence of amplitude modulation (AM) in a 5 kHz, suprathreshold tone when the AM tone was embedded within background noise intended to degrade the contribution of high-SR fibers such that AM coding was preferentially reliant on low-SR fibers. We also recorded by EEG the "envelope following response" (EFR, generated in the auditory midbrain) to a 5 kHz, 85 Hz AM tone in conditions of noise and no noise. Control subjects with EFRs that were comparatively resistant to the addition of background noise displayed better AM detection than controls whose EFRs were more affected by noise. Simulated auditory nerve responses to our stimulus conditions using a well-established peripheral model suggested that low-SR fibers were better preserved in the former cases. Tinnitus subjects had worse AM detection thresholds and reduced EFRs overall compared to controls. Simulated auditory nerve responses found that in addition to severe low-SR fiber loss, a significant degree of high-SR fiber loss that would not be expected to affect audiometric thresholds was needed to explain the results in tinnitus subjects. The results

indicate that hidden hearing loss could be sufficient to account for impaired temporal coding in subjects with normal audiograms as well as for cases of tinnitus without audiometric hearing loss, but do not preclude a role for central factors in these cases.

2.3 Introduction

Most cases of chronic tinnitus are believed to arise from neuroplastic changes that occur in central auditory pathways following hearing loss caused by noise overexposure or aging (Shore et al., 2016; Kaltenbach, 2011). A majority of individuals with tinnitus exhibit hearing loss detectable by the audiogram, a standard clinical test that measures thresholds of detection for tones in quiet. The sound frequencies judged by tinnitus sufferers to resemble their tinnitus commence at the edge of hearing loss and span the region of threshold shift, typically peaking near or above 5 kHz (Roberts et al., 2008), such that threshold shifts and tinnitus frequencies overlap. However, around 15% of individuals with tinnitus do not have audiometric threshold shifts (Henry et al., 2005). While some studies have focused on central auditory factors to explain these cases (Vanneste and De Ridder, 2015; Rauschecker et al., 2010), we investigated whether such cases might reflect peripheral damage that is undetected by the audiogram.

Animal studies have demonstrated that moderate noise exposure that only temporarily raises thresholds can permanently degrade auditory responses to suprathreshold sounds (Kujawa and Liberman, 2009). Such damage is observed predominantly in synaptic terminals of inner hair cells (IHCs) innervated by

auditory nerve fibers (ANFs) with low spontaneous rates of firing and high firing thresholds (low-SR ANFs; Furman et al., 2013), while ANFs with high spontaneous rates and low firing thresholds (high-SR ANFs) are less affected. Because low-SR fiber loss without accompanying high-SR fiber loss will not affect auditory thresholds in quiet, this pattern of damage has been termed "hidden hearing loss" (Schaette and McAlpine, 2011). Previous studies found that tinnitus subjects with normal thresholds had smaller amplitudes of the auditory brainstem response (ABR) wave I (reflecting synchronous firing of ANFs) to suprathreshold clicks compared to controls, suggesting the presence of hidden hearing loss (Schaette and McAlpine, 2011; Gu et al., 2012). However, because low-SR fibers appear to make only a minor contribution to ABR Wave I (Bourien et al., 2014), synaptopathy in tinnitus remains to be characterized.

Low-SR fibers typically respond at sound levels above 40 dB SPL (the level where the discharge rate of high-SR fibers is strongly saturated; Yates et al., 1990) and are robust to masking by background noise (Costaloupes et al., 1985; Young and Barta, 1986). Low-SR fiber loss is thought to degrade auditory sensitivity to temporal modulations of sound that are important for listening in noisy environments, such that individual differences in listening under these conditions may reflect hidden low-SR fiber synaptopathy. Bharadwaj et al. (2015) found large individual differences in the ability of subjects with normal audiograms (and without tinnitus) to detect the presence of amplitude modulation (AM) in a sound presented in narrowband noise. Poor AM detection was further

associated with impaired performance on other temporal coding measures, including a reduction in the magnitude of the scalp-recorded "envelope following response" (EFR) which is evoked from the auditory midbrain when sounds are AM at rates exceeding ~80 Hz (Herdman et al., 2002). Bharadwaj et al. (2015) suggested that poor AM sensitivity and reduced EFR magnitudes may have reflected low-SR fiber loss that was hidden to the audiogram in their otherwise normal hearing subjects. Subjects exhibiting comparatively poor temporal coding abilities were also more likely to report a history of noise exposure, which may have induced hidden low-SR fiber loss in these subjects (Furman et al. 2013).

Given the role of noise exposure in tinnitus (Axelsson and Barrenas, 1992; Penner and Bilger, 1995), we investigated whether hidden hearing loss may be present in young adults experiencing persistent tinnitus with normal audiograms. To assess the contribution of low-SR fibers to AM coding, we measured AM detection thresholds when the contribution of high-SR fibers was degraded by background noise, and EFRs evoked at different AM depths in the same background noise. EFRs were also evoked by a fully modulated tone without background noise, where both high- and low-SR fibers were expected to contribute. Control subjects were included that were matched to the tinnitus subjects for normal hearing thresholds at the tested frequencies but had no history of tinnitus. We then used a model of the auditory periphery (Zilany et al., 2014) to simulate ANF responses to our stimuli with different mixtures of high- and low-SR fiber loss. We found that control subjects with EFRs comparatively resistant to

the addition of background noise displayed better AM detection in background noise than controls whose EFRs were more affected by noise. Simulated auditory nerve responses to our stimulus conditions suggested that low-SR fibers were better preserved in these former cases. We also found that tinnitus subjects had smaller EFRs and poorer AM detection than controls for tones in the frequency range where hidden hearing loss was expected to be present. Cochlear modeling suggested that in addition to severe low-SR fiber loss, a significant loss of high-SR fibers that would not affect the audiogram was needed to explain the EFRs of the tinnitus sufferers.

2.4 Materials and Methods

2.4.1Participants

A total of forty-five subjects were recruited from the McMaster University undergraduate psychology program. Thirty subjects without chronic tinnitus were initially recruited, followed by fourteen individuals that reported chronic tinnitus in both ears. Because chronic tinnitus is less prevalent in young individuals than in older adults (Henry et al., 2005), it was expected that fewer subjects would be found for the tinnitus group. One control subject was excluded as a result of cerumen that occluded the right insert earphone. Three additional control subjects were excluded because of technical failure during measurement of the EFR. One tinnitus subject and two control subjects did not reach our statistical criterion for the presence of an EFR (see below). The remaining 24 control subjects and 13 tinnitus subjects completed all portions of the study, and their demographic

information is portrayed in Table 2.1. One additional tinnitus subject was tested who reported hearing tinnitus at the time of each experimental session but indicated he experienced tinnitus only intermittently at other times. The data of this subject (referred to herein as intermittent tinnitus, IT) will be reported separately from the analysis of group data which was based on the remaining 13 tinnitus subjects all of whom reported experiencing constant tinnitus. No subjects reported a history of head trauma or reported the use of medication during the time of the study. All procedures were approved by the Research Ethics board at McMaster University. Subjects provided informed consent and received monetary compensation (CAD 10.00 / hour) or course credit for participation.

2.4.2 Initial Session (Tinnitus group only)

The initial session began with a questionnaire gathering a detailed personal history of each subject's tinnitus, including how long tinnitus had been experienced, perceived location of tinnitus (e.g., in ears, in head), how often during the day tinnitus is heard, how bothersome it is, and other non-tinnitus factors such as sensitivity to loud sounds and daily hearing difficulties. Each subject then completed the Tinnitus Handicap Questionnaire (THQ, Kuk et al., 1990) to assess the impact of tinnitus on quality of life. After, we obtained hearing thresholds from 125 Hz to 16 kHz (Telephonics TD-39 296 D200 headphones; 8 to 16 kHz through Sennheiser HD250 headphones) in 5 dB steps using the pulsed-tone method for left and right ears separately. We took additional diotic measurements (left and right ear together) at 500 Hz and 5 kHz in 2 dB steps also

using the pulsed-tone method. Subjects then used computerized tools developed by Roberts et al. (2008) to indicate the properties of their tinnitus. The ear of tinnitus (left, right, bilateral) and the quality of tinnitus (tonal, ringing, hissing) were first obtained, followed by a rating of tinnitus loudness on the Borg CR100 scale (Borg and Borg, 2001). Thereafter, subjects adjusted the loudness of 11 pure tones (randomly ordered) ranging from 0.5 to 12 kHz to match the loudness of their tinnitus. Subjects then rated the likeness of each of the same 11 pure tones to their tinnitus on a 100 point scale. The preliminary session concluded with a brief test for residual inhibition (Roberts et al. 2008).

	Tinnitus (N = 13)	Control (N = 24)
Age	M = 23.2 (SD = 6.15; Range = 18–39)	M = 19.5 (SD = 2.82; Range = 18–28)
Sex	9Females; 4 Males	20 Females; 5 Males
500 Hz pure-tone threshold (dB HL, diotic)	M = 0.23 (SD = 4.82; Range = −6–7.5)	M = 1.63 (SD = 3.55; Range = −4–10)
5 kHz pure-tone threshold (dB HL, diotic)	M = 1.65 (SD = 5.63; Range = −8–11)	M = 0.40 (SD = 3.46; Range = −5–8)
Tinnitus Qualities		
Tinnitus Ear	Bilateral = 13	-
Tinnitus Sound	Tonal = 9; Ringing = 4;	-
Tinnitus Duration	Range: 25 yr. to 3 mo.; 1 unsure	

THQ	M = 17.6 (SD = 9.9; Range = 4–40.4)	-
CR100 loudness rating	M = 32.5 (SD = 20.8; Range = 10–65)	-
Loudness match across .5. 1, and 5 kHz (dB SL)	M = 8.20 (SD = 8.55; Range = −4.1–15.5)	-
Likeness match at 5 kHz (1–100 scale)	M = 42.5 (SD = 17.1; Range = 13–79)	-

Table 2.1 Demographic information for tinnitus and control groups, and summary of tinnitus characteristics. M refers to the average and SD refers to one standard deviation.

Audiometric thresholds for the 13 subjects with chronic tinnitus were in the normal range up to 8 kHz (< 25 dB HL; see Figure 2.1a). Above 8 kHz three subjects exceeded 25 dB HL to a maximum of 45 dB HL. Tinnitus likeness spectra of these subjects are shown in Figure 2.1b. Likeness ratings tended to increase with increasing tone frequency, agreeing with tinnitus spectra of younger subjects with normal thresholds reported previously (Roberts et al., 2008). Chronic tinnitus subjects on average reported experiencing their tinnitus for 10.7 years (SD = 9.90); one subject reported uncertainty as to when they began experiencing tinnitus. Tinnitus loudness matches (reported in Table 2.1) averaged 8.20 dB SL (SD = 8.55 dB) for the frequencies 500 Hz, 1 kHz, and 5 kHz, and 4.13 dB SL at 1 kHz (the standard reported by Roberts et al., 2008). THQ scores

averaged 17.6 (range 4 – 40.4), indicating that most subjects experienced a mild tinnitus that for most was not highly bothersome.

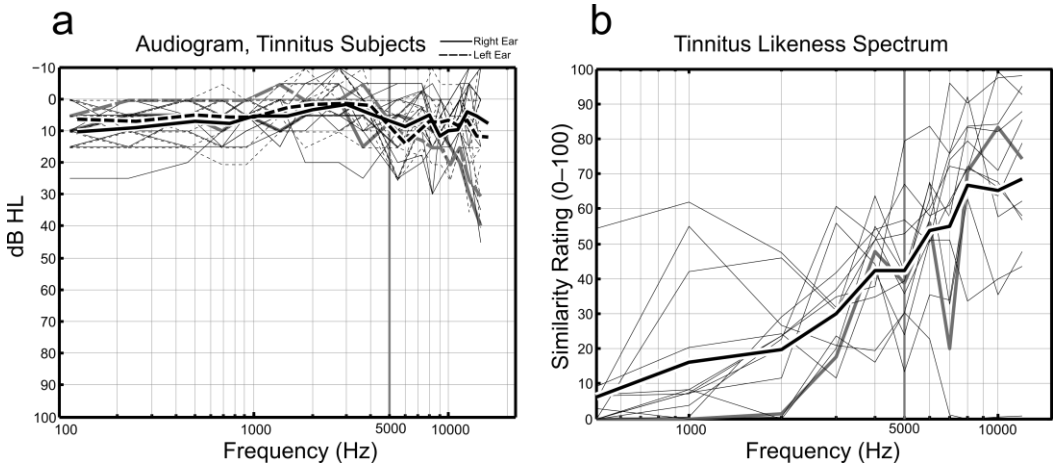

Figure 2.1. Characteristics of the tinnitus group. a) Audiometric thresholds for all individuals (thin lines) from 125 Hz to 16 kHz. Group means for left (dashed line) and right (solid line) ears are plotted as thick black lines. A vertical line is plotted at 5 kHz, where suprathreshold hearing function was measured. Audiometric thresholds for the intermittent tinnitus case are represented by thick grey lines. b) The tinnitus likeness spectrum in which tinnitus subjects rate the similarity of their tinnitus to pure tone frequencies. Thin lines represent individual spectra, which were measured three times. The median of these three values was taken as the spectrum. The thick line is the group mean. The intermittent tinnitus subject is represented by the thick grey line.

2.4.3 Main session (tinnitus and control subjects)

The main session began by administering to control subjects the same questionnaire administered to the tinnitus group in their initial session, excluding questions related to tinnitus. Hearing thresholds were then measured at 500 Hz and 5 kHz in all subjects diotically (left and right ears together) in 2 dB steps using the pulsed-tone method. After, participants were tested on AM detection

and measurement of the EFR to AM sounds. During the experiment participants sat in a chair distanced 1.4 m from a computer monitor in a sound-attenuated (ambient noise level 16 dBA SPL) and electrically-shielded booth. All stimuli were generated by a Tucker-Davis RP2.1 digital signal real-time processor and presented through EARtone 3A transducers inserted in both ears.

Amplitude modulation detection task. AM sensitivity was first behaviorally assessed in each subject by obtaining their threshold for AM detection in the presence of a narrowband background noise (NBN). Stimuli were 5 kHz tones that were sinusoidally amplitude modulated at a rate of 19 Hz and presented at 75 dB SPL. Modulation depth (m), where m = 1 corresponds to 100 % modulation, during the task was adaptively varied and herein will be expressed in dB ($20 \log_{10} m$). The AM tone was embedded in NBN centered at 5 kHz with spectrum level set to 40 dB, a level sufficient to degrade high-SR fiber responses (Costaloupes et al., 1985) leaving temporal envelope encoding in the auditory nerve preferentially reliant on low-SR fibers (Bharadwaj et al., 2014). The bandwidth of the NBN was set at 1/3 of an octave. This bandwidth was determined by observing the spread of excitation across the range of auditory nerve fibers with characteristic frequencies (CFs) responding to the 75 dB SPL, 5 kHz AM probe in the Zilany et al. (2014) auditory periphery model, such that the 40 dB spectrum level NBN adequately covered this range.

In a 3-alternative forced choice task, subjects listened to three tones embedded in NBN. One tone was AM, while the other two were unmodulated. After all three tones were presented, subjects indicated by keypress which tone they perceived was amplitude modulated (the target). Each tone was presented for 1 s, with background NBN commencing 500 ms before the first tone and played continuously until 500 ms after the third tone. The spacing between each tone was fixed at 1 s. The position of the target tone on each trial was random, and a target tone was present on each trial. AM depth of the target started at −6 dB (50% AM) and was adjusted adaptively by the method of parameter estimation through sequential testing (PEST; Taylor and Creelman, 1967) until a final modulation step size of 0.45 dB was achieved. The average AM depth of the two final steps was taken as the threshold. It should be noted that because AM detection thresholds are represented with respect to 100% AM depth in dB ($20 \log_{10} m$), large negative values in this metric signify low (good) AM detection thresholds.

Each subject also completed the task for a 500 Hz tone amplitude modulated by 19 Hz, to compare results at 5 kHz to a frequency region where hidden hearing loss was unlikely to be present in these groups, and where tinnitus is unlikely to be experienced in the tinnitus cohort. The 500 Hz tone was presented at 75 dB SPL and the 40 dB spectrum level background NBN was centered at 500 Hz with a 2/3 octave bandwidth.

EFR measurement. Following the AM detection task, we recorded the 32-channel EEG and evoked the EFR in five conditions depicted in Figure 2.2. All tones evoking the EFR were at 5 kHz, presented at 75 dB SPL, and were amplitude modulated by 85 Hz, a rate consistent with anatomical generators in the auditory midbrain (Herdman et al., 2002). The first condition was an amplitude modulated tone with an AM depth of 0 dB (100% modulation) presented without background NBN, intended to evoke contributions from both low- and high-SR fibers to AM coding. The second through fourth conditions embedded the AM tones in background NBN (identical to that used in the AM detection task) aimed at saturating high-SR fibers, leaving primarily low-SR fibers available to code AM. Low-SR fibers were further tested in these conditions by reducing AM depths from 0 dB (100% AM depth), to −2.5 dB (75% depth), and −6 dB (50% depth). Finally, a no-tone NBN-only condition was included as a control. For each condition the stimulus was presented continuously for three minutes. After each condition there were three seconds of silence before the next condition began. The condition order was randomly selected for each participant. During EFR recording participants were instructed to ignore sound stimuli and watch a subtitled silent film of their choice.

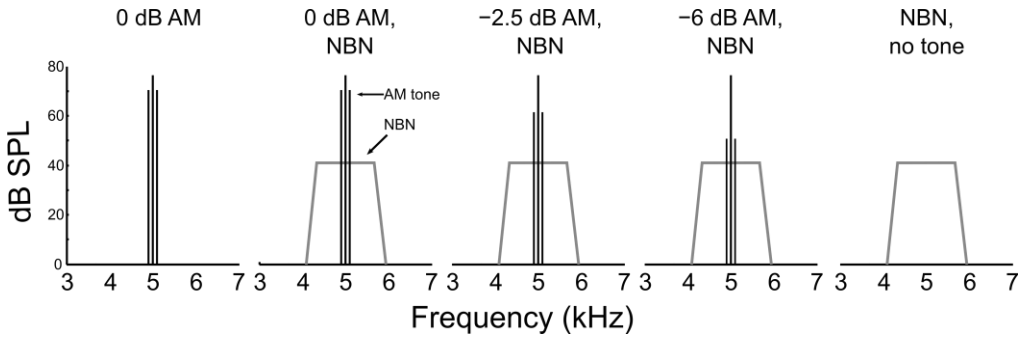

Figure 2.2. Frequency spectrum representations of AM stimuli that evoked the EFR. Solid black lines on the spectrum represent the 5 kHz amplitude modulated (AM) tone. Gray lines depict the bandwidth and level of the background narrowband noise (NBN). Stimulus AM depth is expressed in dB relative to 100% modulation depth: 0 dB = 100% AM depth, –2.5 dB = 75% AM depth, and –6 = 50% AM depth.

EEG recording and analysis. The EEG was sampled at 2048 Hz by a BioSemi ActiveTwo amplifier (Cortech Solutions, Wilmington, NC) from 0 to 417 Hz and stored as continuous data files. Using custom offline routines in MATLAB (The Mathworks, Natick, MA) the EEG was first rereferenced to the scalp average, high-pass filtered at 70 Hz, and downsampled to 850 Hz. For each condition data were epoched into 1000 ms periods, creating 180 total trials per condition per person. Epochs that exceeded ± 50 μV in any channel were rejected. If a channel contributed to more than 1/3 of trials being rejected, the channel was removed. With these criteria an average of 156.2 trials were retained per subject per condition. To extract EFR power at 85 Hz, we used a multi-channel complex principal components analysis approach (Bharadwaj and Shinn-Cunningham, 2014) that was likewise used for EFR analysis in Bharadwaj et al. (2015). This technique adjusts for phase disparities of the EFR present in each channel and

combines them across recording sites, providing a more reliable EFR measurement and robustness to noise compared to single-channel recordings.

The presence of an EFR was assessed statistically for each subject and each condition by comparing the phase locking value (PLV) of the response at 85 Hz to 20 adjacent frequency bins (71-81 Hz; 89-99 Hz in 1 Hz steps). We subtracted the mean PLV of the adjacent bins from the 85 Hz PLV and divided this value by the standard deviation across the adjacent bins, creating a standardized score. Following Kuwada et al. (2002), if this score was greater than 3 (corresponding to the 99^{th} percentile of the normal distribution) the EFR was deemed statistically present. Two control subjects and one tinnitus subject of the original recruited sample did not meet this criterion in one or more conditions, and were excluded. EFR power at 85 Hz herein is expressed in dB as a signal to noise ratio (SNR) relative to mean of the same 20 frequency bins used in the PLV analysis.

2.4.4 Peripheral auditory modeling

To determine if our EFR results could be interpreted by loss to populations of ANFs, we used a model of the auditory periphery (Zilany et al., 2014) to simulate levels of damage to ANF types that could modify the response of the auditory nerve to our stimulus conditions. Stimuli from each EFR condition were shortened to 400 ms and passed into the model. After the signal passes through a series of components simulating realistically the filtering and compression properties of the middle ear and cochlear structures, the model generates a

neurogram depicting the spiking response of a set of ANFs to the stimulus. For our simulations we used 128 sets of ANFs with characteristic frequencies (CFs) ranging from 170 Hz to 7 kHz. For each CF there were 150 ANFs, reflecting the number of fibers covering this range in the human cochlea as determined by the Greenwood function (Greenwood, 1961). The fiber types were distributed in a 3:1:1 ratio for high-, medium-, and low-SR fiber types respectively, roughly consistent with the distribution found in the cochlea (Liberman, 1978). In modeling simulations that involved removal of ANFs, medium and low-SR fibers were always removed together. Herein loss of both fiber types is simply referred to as low-SR fiber loss.

In order to calculate the modulation response of the auditory nerve at 85 Hz, we collapsed the neurogram into an average of 4 AM periods and then passed this average through a spectro-temporal modulation filter bank (modified from Elhilali et al., 2003) to obtain a modulation power spectrum for modulation rates ranging from 0 to 105 Hz. We then averaged the response of CFs from 2.8 to 7 kHz, which covered the range over which spiking was observed in the model neurograms to our stimuli, and averaged again over all spectral modulation rates and over time. The absolute value of the averaged modulation magnitude for the filters with best modulation frequencies neighboring 85 Hz was taken and referred to herein as the "modulation response magnitude." Similar to the EFR, the model's modulation response magnitude was expressed as dB SNR relative to the mean 85-Hz modulation response of CFs averaged from 1 to 2.6 kHz where no

signal energy was present in the stimuli. All simulations reported below were repeated 8 times to obtain a mean and variance.

2.4.5 Statistics

Unless otherwise noted, independent samples *t* tests and Pearson's *r* were two-tailed, and the alpha criterion for all tests was set at 0.05. Statistics were performed using the MATLAB statistics toolbox.

2.5 Results

2.5.1 Audiometric thresholds and behavioural AM detection

Diotic audiometric thresholds measured at 500 Hz and 5 kHz and AM detection thresholds measured in NBN are reported for the control group and the 13 subjects with chronic tinnitus in Figure 2.3. Audiometric thresholds (Figure 2.3a) did not exceed 11 dB HL for any subject (Table 2.1). A 2x2 mixed-model analysis of variance (ANOVA) evaluating the between-subjects factor of group (tinnitus versus control) and within-subjects factor of audiometric frequency (5 kHz versus 500 Hz) on audiometric thresholds found no main effects or interactions (ps > 0.17). AM detection thresholds in NBN for both groups shown in Figure 2.3b were similarly examined in a 2x2 ANOVA with the between-subjects factor as group (tinnitus versus control) and stimulus condition (500 Hz vs 5 kHz) as a within-subjects factor. A main effect of stimulus condition was found ($F(1,70) = 376.78$, $p < 0.0001$) suggesting that AM detection for the 5 kHz probe was higher (i.e., worse) than the 500 Hz probe. A main effect of subject group was not present ($p = 0.177$) but a significant interaction was found between subject group

and stimulus condition ($F(1,70) = 6.44$, $p = 0.013$). Follow-up tests indicated that tinnitus subjects had worse AM detection thresholds for the 5 kHz probe ($p = 0.016$, Fisher's Least Significant Difference (LSD) test), but AM detection thresholds for 500 Hz did not significantly differ ($p = 0.48$, LSD test).These results indicate that the tinnitus group had poorer suprathreshold AM sensitivity in background noise for the 5 kHz tone, but had normal AM sensitivity at 500 Hz.

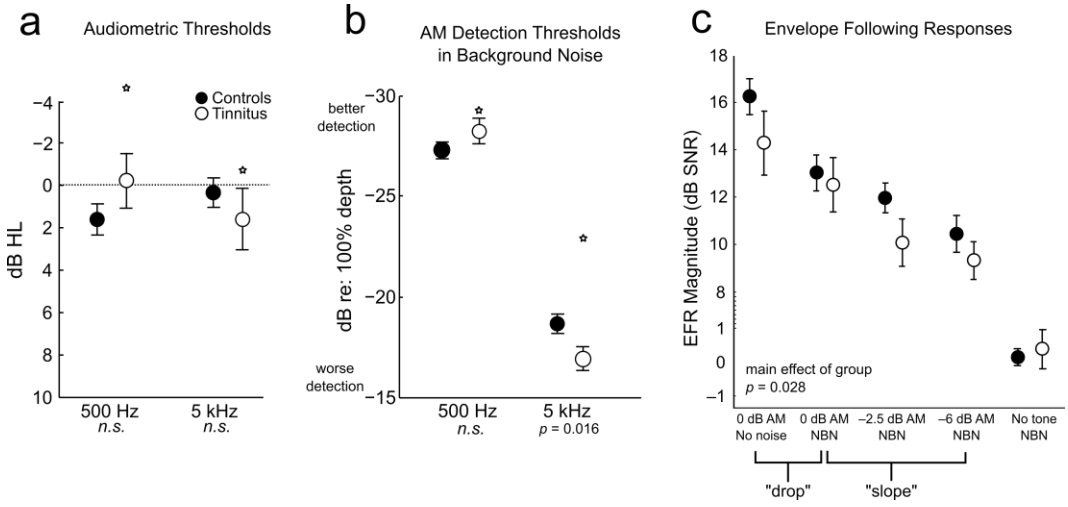

Figure 2.3. Behavioral and electrophysiological measurements for tinnitus and control groups. a) Diotic audiometric thresholds for both groups at 500 Hz and 5 kHz. The intermittent tinnitus case is represented by the white stars. b) Amplitude modulation detection thresholds for 500 Hz and 5 kHz AM tones embedded in background narrowband noise. AM thresholds for the intermittent tinnitus case are depicted as white stars. c) EFR magnitudes (dB SNR) for tinnitus and control groups for the five stimulus conditions in which the EFR was evoked. Error bars for all panels are 1 standard error of the mean (SEM).

2.5.2 Envelope Following Responses

EFR power values for each stimulus condition and subject group are plotted in Figure 2.3c. For both groups, power decreased significantly with the addition of

NBN to the AM tone. During the three conditions in which AM depth decreased while the tone was in NBN, EFR power further declined. Group differences were examined using a two-way mixed-model ANOVA with factors of group (tinnitus versus control) and condition (all conditions in Figure 2.2 excluding the last NBN-only condition). A main effect of group was found ($F(1,140) = 4.9$, $p = 0.028$) indicating that individuals with tinnitus had overall smaller EFRs suggesting poorer subcortical AM coding. A main effect of stimulus condition was also found ($F(3,140) = 14.55$, $p < 0.0001$) indicating that EFR power depended on stimulus condition. The interaction of group with condition was not significant. Post-hoc tests of the group main effect did not reveal significant contrasts between tinnitus and control groups for any individual stimulus condition, although the 0 dB condition ($p = 0.16$) and the –2.5 dB AM, NBN condition ($p = 0.10$, LSD tests) appeared to have contributed most to the overall group effect. Post-hoc LSD tests of the condition main effect found that in controls, the no-noise, 0 dB AM (fully modulated) condition in quiet was larger than the remaining conditions ($ps < 0.0001$). In tinnitus subjects the EFR in the 0 dB AM condition in quiet was larger than in the –2.5 and –6 dB AM conditions in NBN ($ps < 0.016$), but not the 0 dB AM condition in NBN ($p = 0.310$).

2.5.3 Individual differences in AM detection and subcortical encoding in background noise

Control Subjects. Although the average EFR power of control subjects dropped significantly with the addition of background NBN ("drop" referring herein to the decrease of EFR power from the no-noise, 0 dB fully modulated AM tone to the 0 dB AM tone in NBN; see Figure 2.3c), some control subjects showed large drops whereas others showed small drops. These individual differences significantly correlated with AM detection thresholds in NBN (Figure 2.4a; $r = 0.45$, $p = 0.027$). Thus control subjects with EFRs that were strongly diminished by NBN had worse AM detection thresholds in NBN compared to subjects whose EFR power dropped little in NBN.

One explanation of these results could be that subjects with large EFR drops and worse AM detection in NBN had fewer low-SR fibers available to support AM coding once high-SR fibers were degraded by NBN. To assess this assumption we used a peripheral auditory model (Zilany et al., 2014) to simulate ANF responses to our stimulus conditions. We computed first a set of simulations in which all ANFs were intact (0% loss), and then in subsequent simulations removed low-SR fibers progressively until all low-SR fibers were absent. Similar to EFR power, the addition of NBN to the AM tone severely reduced ANF modulation response magnitude for all conditions, and the size of the response drop increased as more low-SR fibers were removed (see Figure 2.4b). This result is qualitatively consistent with the suggestion that control subjects with large EFR drops had more low-SR fiber loss.

With high-SR fibers predominantly saturated by NBN, we aimed to further assess low-SR fiber function by reducing the AM depth of the stimulus. We fit a straight line to each subject's three EFR power values for the 0 dB, –2.5dB, and –6dB AM depth conditions (Figure 2.2, the three middle stimuli) and calculated the slope of this line ("slope" referring herein to changes in response magnitude across these three conditions). For control subjects, slope did not correlate with AM detection thresholds (r = –0.01, p = 0.96). To better understand if EFR slope related to the effect of adding NBN, we correlated EFR slope with the EFR drop observed when NBN was added to the AM tone. A relationship was found indicating that individuals with smaller EFR drops had steeper EFR slopes (r = 0.47, p = 0.019). In other words, individuals whose EFR was not strongly reduced when NBN was added showed a sensitivity to decreasing AM depths in NBN (steep slopes), perhaps because (in contrast to the remaining individuals) they had enough low-SR fibers to code variations in AM depth. Peripheral model simulations (not shown) supported this interpretation, revealing that when low-SR fiber loss was greater the slope of the ANF response magnitude across the three NBN conditions was shallower. These results suggest that the EFR recorded at varying AM depths in NBN can reveal information about the status of low-SR fibers, but only when a sufficient number of low-SR fibers is available to encode suprathreshold sound.

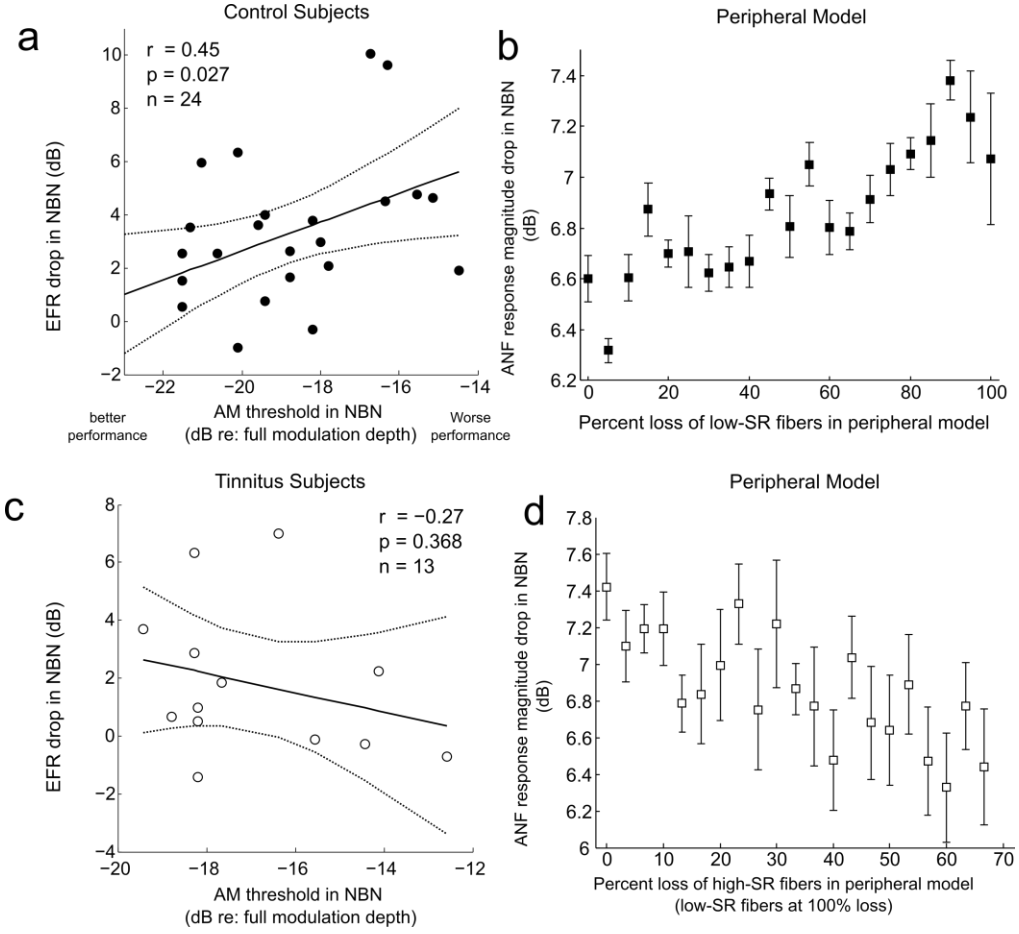

Figure. 2.4. Individual differences in AM detection and EFRs in tinnitus and control groups, and modeling simulations of ERF magnitude. a,c) Behavioral AM detection thresholds in NBN plotted against the size of EFR drop when NBN was added to the AM tone. Dotted lines are 95% confidence intervals around the solid regression line. b,d) Auditory peripheral model simulations which calculate the ANF response magnitude drop when NBN was added to the AM tone (ordinate). Percent loss of either low or high spontaneous rate fibers is plotted along the abscissa.

Tinnitus Subjects. Control subjects with EFRs strongly degraded by NBN had poorer AM detection thresholds (Figure 2.4a), consistent with low-SR fiber loss in the model (Figure 2.4b). In contrast to this finding, tinnitus subjects with worse AM detection thresholds tended to have smaller EFR drops. Although the

correlation relating EFR drop to AM detection thresholds did not reach significance in the tinnitus subjects (r = –0.27, p = 0.37, see Figure 2.4c), its direction was opposite to that seen in controls (contrast Figures 2.4a and 2.4c), and the opposing correlations differed significantly between the two groups (p = 0.047, Fisher's r-to-z transformation). A possible explanation for these results could be that undetected high-SR fiber loss in the tinnitus subjects may have reduced their EFRs in the no-noise condition compared to controls (see Figure 2.3c earlier and 2.5b below), such that smaller EFR drops occurred in tinnitus subjects when NBN was added. To assess this possibility, we progressively removed high-SR fibers in the peripheral model with low-SR fiber loss set at 100%. As shown in Figure 2.4d, the size of ANF response magnitude drop caused by NBN decreased as high-SR fibers were removed. If a pattern of undetected high-SR fiber loss (affecting EFR drops) and substantial low-SR fiber loss (affecting EFR slope) were to approximate the conditions present in our tinnitus subjects, relationships of EFR drops to AM detection and of EFR drops to slope would be expected to differ from those of control subjects where ANF function is likely better preserved for both fiber types. While EFR drops were significantly correlated to AM detection in background noise and EFR drop in controls (r = 0.45, p = 0.027 and r = 0.47, p = 0.019, respectively, see section 2.5.3), neither correlation reached significance in the tinnitus subjects.

2.5.4 Comparison of simulated and observed EFR responses in control and tinnitus subjects

Figure 2.5a presents a summary of model results for all EFR stimulus conditions, including simulations of no ANF loss, 100% low-SR fiber loss, and varying amounts of high-SR fiber loss. Because the amount of high-SR fiber damage that would best describe EFRs of tinnitus subjects is unclear from the observed data, we plotted a range from 0% high-SR fiber loss to 70% high-SR fiber loss, which is the range of loss over which normal audiometric thresholds could still be expected as observed in our tinnitus and control subjects. In Figure 2.5b we present for qualitative comparison to the simulated results the mean EFR of the 13 subjects with chronic tinnitus and the EFRs of the control subjects that were separated into two subgroups based upon a median split of their AM detection thresholds in NBN. It may be noticed that the EFRs of the two control subgroups do not differ in quiet, suggesting little high-SR fiber loss in the poor AM subgroup. However, the EFRs of this subgroup dropped more with the addition of NBN than was the case for controls with good AM detection, in agreement with the correlation of EFR drop to AM detection reported in Figure 2.4a. The EFRs of the poor AM detection subgroup also trended lower across the NBN conditions than was the case for controls with good AM detection, suggesting an impairment of low-SR fibers for AM coding in this subgroup. Comparison of these data with the simulations in Figure 2.5a for no fiber loss (0%) and 100% low-SR fiber loss are qualitatively congruent with this picture. The modeled EFRs of the tinnitus group with 100% low-SR fiber loss and 0–70% fiber loss in Figure 2.5a were

overall lower than in the two control subgroups, consistent with their EFRs which were overall significantly smaller than in controls as were their AM detection thresholds in NBN (*p*s = 0.028 and 0.012 respectively, reported earlier).

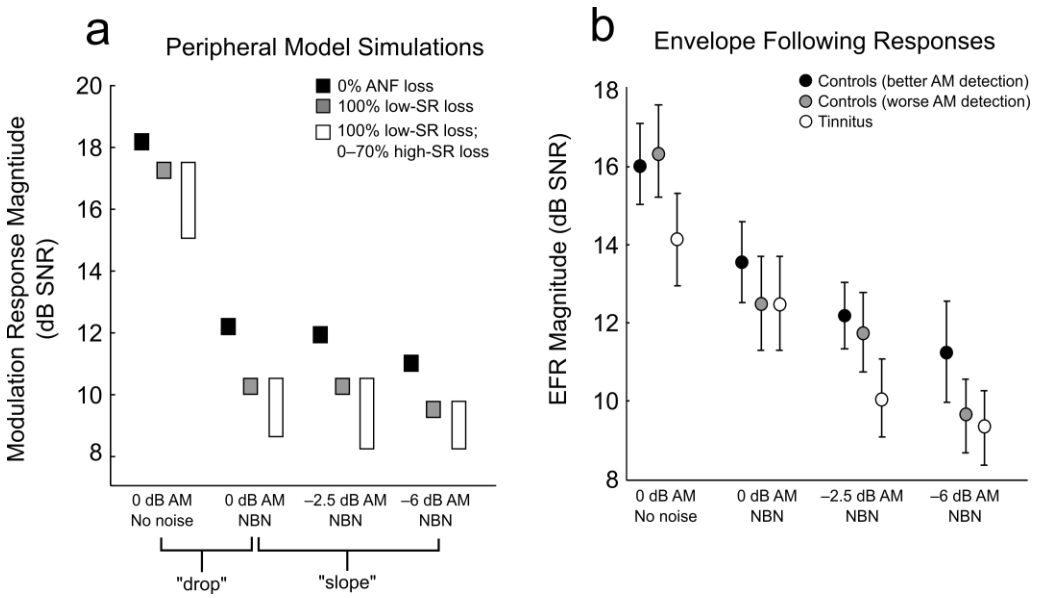

Figure 2.5. Summary of simulations of the auditory nerve and corresponding EFR power of tinnitus and control subjects separated by AM detection thresholds. a) Means of modulation response magnitude in dB SNR of the peripheral auditory model to the five EFR stimulus conditions. The white bars represent the range of means observed for 0–70% high-SR loss. b) EFR magnitudes for tinnitus and control subjects. Control subjects are broken out into two groups based upon the median split of their AM detection thresholds. Error bars are 1 SEM calculated for each condition separately.

2.5.5 Relationship of AM detection and EFR to audiometric thresholds

For both groups, diotic audiometric thresholds at 5 kHz did not correlate with 5 kHz AM detection in NBN (Control: $r = –0.27$, $p = 0.206$; Tinnitus: $r = –0.243$, $p = 0.423$), EFR drop in NBN (Control: $r = 0.07$, $p = 0.732$; Tinnitus: $r = 0.17$, $p = 0.590$) or EFR slope as AM depth decreased (Control: $r = –0.07$, $p = 0.755$;

Tinnitus: $r = –0.05$, $p = 0.882$). AM detection thresholds in NBN at 500Hz also did not correlate with diotic 500 Hz thresholds (Control: $r = 0.14$, $p = 0.522$; Tinnitus: $r = –0.342$, $p = 0.253$). These results indicate that suprathreshold measurements of AM detection and EFR encoding did not relate to audiometric thresholds in either subject group. Hence the synaptic losses suggested by our simulations for controls with poor AM coding and in the tinnitus group appear to have been hidden from the audiogram. This is consistent with animal data showing that synaptopathy consequent destruction of up to 80% of IHCs (and thus loss of drive to ANFs) does not affect audiometric thresholds (Lobarinas et al., 2013).

2.5.6 Intermittent Tinnitus Case

The individual who reported intermittent (IT) episodes of tinnitus was an 18 year old male who experienced tinnitus bilaterally and with a tonal quality for 16 months. His THQ score was 14.4 indicating a mild tinnitus. The audiogram for left and right ears of this subject is plotted in Figure 2.1a as thick grey lines, showing normal thresholds (< 25 dB HL) in both ears to 12.5 kHz, but at 14 and 16 kHz threshold shifts were present in both ears to a maximum of 40 dB HL. His diotic thresholds were –6 dB HL and 1 dB HL for 500 and 5 kHz respectively (Figure 2.3a, white stars), which were lower than but within two standard deviations of the group mean for subjects with chronic tinnitus. The tinnitus likeness spectrum of the IT subject is produced in Figure 2.1b as a thick grey line, showing an increasing tinnitus match to increasing frequency, which did not

appear to qualitatively differ from the chronic tinnitus group. Notably, his tinnitus loudness matches at the time he experienced his tinnitus in the laboratory averaged 55 dB SL across the frequencies of 500 Hz, 1 kHz, and 5 kHz, which was 5.51 SD higher than the chronic tinnitus group mean. AM detection thresholds in NBN for the IT subject (Figure 2.3b, white stars) were –23.6 dB for 500 Hz, close to the chronic tinnitus group mean for this frequency, and surprisingly –23.2 dB for the 5 kHz condition which was 3.4 standard deviations better than the chronic tinnitus group mean. This individual also had an EFR that was 2.54 standard deviations higher than the mean of the chronic tinnitus subjects. Good AM detection thresholds in NBN and large EFRs are consistent with each other but inconsistent with tinnitus-associated low/high-SR fiber loss. Given the many aetiologies of tinnitus (Henry et al., 2005), it is possible that this individual's intermittent tinnitus may have been caused by inflammatory (Yüksel and Karataş, 2016), neuromodulatory (Simoens and Hébert, 2012), or unknown transient factors and not by cochlear injuries. The episodic nature of his tinnitus is also not consistent with ANF damage, although environmental masking of the tinnitus sound cannot be discounted.

2.6 Discussion

2.6.1 Summary

Low-SR fiber loss has been hypothesized to be present in individuals with tinnitus and normal audiograms (Schaette and McAlpine, 2011; Plack et al., 2014; Roberts et al., 2013). To evaluate this hypothesis, we measured detection thresholds for

AM and EFRs evoked by 5 kHz tones at 75 dB SPL in young adults experiencing chronic tinnitus with audiometrically normal hearing and in non-tinnitus control subjects matched for age and hearing level for the test stimuli. Measurements were taken in quiet and again when the contribution of high-SR fibers was suppressed by 40 dB spectrum level background noise such that AM coding relied preferentially on low-SR fibers. EFRs of control subjects with poor AM detection in noise were more strongly reduced by the addition of background noise than was the case for controls with good AM detection, which was consistent with hidden low-SR fiber loss in the poor-performing control subjects estimated by a computational model of the auditory nerve. Tinnitus subjects had overall worse AM detection in noise and smaller EFRs across no-noise and noise conditions compared to controls, which was in agreement with the hypothesized hidden hearing impairment in this group. The EFRs of tinnitus subjects with poor AM detection however were not best approximated by 100% low-SR fiber loss alone, as was suggested for controls with poor AM detection. An additional loss of high-SR fibers gave an improved congruence of the modeled and obtained EFR results for tinnitus subjects, particularly in the no-noise condition where high-SR fibers would have been expected to contribute to AM coding. This extent of high-SR fiber loss would not be expected to elevate audiometric thresholds which remain unaffected with up to ~80% IHC loss (Lobarinas et al., 2013). Correspondingly, EFRs and AM sensitivity did not correlate with audiometric thresholds at 5 kHz in either subject group.

2.6.2 Individual differences in AM sensitivity in control subjects

Our control subjects with normal audiometric thresholds at the test frequencies varied widely in their AM detection thresholds and in the magnitude of their EFRs when these auditory processing attributes were measured in background noise. Comparatively poor performance on these tasks may signify difficulties in coding temporal modulations in sound that can impact spatial listening and speech comprehension in multi-talker environments (Ruggles et al., 2011; Mehraei et al., 2016). Consistent with this supposition, individual differences in the extent to which temporal modulations are coded by the EFR have been shown to correlate positively with the ability of subjects to detect shifting interaural time delays (a skill needed to detect a change in the spatial location of a sound source) and with their ability to direct attention to one of two speech streams presented simultaneously (Bharadwaj et al., 2015). These same investigators also found that EFR slope measured at different modulations depths in notched background noise correlated inversely with behavioral AM detection thresholds, such that subjects with steep slopes were comparatively poorer at AM detection. This relationship was taken to suggest the presence of low-SR synaptopathy in the latter subjects. Although this correlation was not present in our control subjects, it should be noted that Bharadwaj et al. (2015) measured the EFR in background noise containing a notch around the AM tone. This procedure was intended to prevent off-frequency contributions to performance, but in doing so it allowed both high- and low-SR fibers to contribute to AM coding. In contrast, we suppressed the contribution of high-SR fibers to AM coding with unnotched background noise,

and reduced AM depth thereafter to test predominantly low-SR fibers. We found that the slope was significantly steeper in controls for subjects whose EFR drop was smaller when NBN was added to 100% AM depth. Cochlear modeling suggested that low-SR fibers were comparatively well preserved in these subjects, such that the EFR now decreased in noise as AM depth diminished.

2.6.3 Role of high- and low-SR fiber loss in tinnitus

Our findings suggesting hidden hearing loss in our tinnitus subjects invite a re-evaluation of earlier results revealing differences in audiometric thresholds between individuals with and without tinnitus. Roberts et al. (2008) compared audiometric thresholds to 16 kHz between human tinnitus sufferers and controls matched in age while thresholds were allowed to vary. Audiometric thresholds were normal (≤20 dB HL) to 10 kHz in controls and tinnitus subjects aged less than fifty years, whereas those over fifty showed expected high-frequency audiometric hearing loss above 2 kHz. However, audiometric thresholds above 2 kHz were on average 11 dB higher in tinnitus than control subjects in both age cohorts. Similar results are found in studies by Paul et al. (2014) and Wienbruch et al. (2006). If age matching is assumed to control for OHC and IHC losses caused by aging (Sergeyenko et al., 2013), the difference between young tinnitus and control subjects with normal thresholds to 10 kHz could reflect a degree of hair cell damage and or synaptic losses that reflected a vulnerability to the effects of noise exposure insufficient to increase audiometric thresholds into the clinically abnormal range. These losses may have been comparatively greater

among older subjects with tinnitus and threshold shifts compared to their age matched controls owing to the cumulative effects exposure to environmental sounds. Detailed sound exposure histories were not collected in these studies, and we were not able to distinguish our tinnitus and controls subjects on the basis our questionnaire data. However, risky listening habits are highly prevalent among adolescents and young adults (Sanchez et al., 2016) and histories of exposure to loud environmental sounds are prevalent among older adults with tinnitus (Axellson and Barrenas, 1992). In animals, low-SR synaptopathy for ANFs tuned to high frequencies can be induced by a single noise trauma and is known to accelerate synaptic changes that occur with aging (Fernandez et al., 2015).

Other findings point to undetected cochlear pathology as a factor distinguishing tinnitus and control subjects that were (unlike the above) explicitly matched for audiometric thresholds. Tan et al. (2013) found that tinnitus subjects with high-frequency threshold shifts had better frequency selectivity and compression measured psychophysically compared to non-tinnitus subjects matched closely in thresholds. The results suggested that tinnitus was not strongly associated with OHC impairment estimated by the psychophysical tests, and by that inference IHC cell dysfunction and reduced auditory innervation may underlie threshold elevation and the presence of tinnitus. Our modeling results are consistent with this view and add the hypothesis that hidden synaptic loss including a degree of high-SR fiber loss is also present in individuals with chronic tinnitus and hearing thresholds in the clinically normal range. Weisz et al. (2006)

studied a group of young tinnitus subjects (mean age 26.7 years) and age-matched controls with normal hearing thresholds, comparing the groups for evidence of off-frequency listening expected at frequencies where hidden cochlear dead regions may have been present. Compared to controls, tinnitus subjects showed steeper slopes for functions relating perceived pitch to frequency, reflecting a shift of pitch judgments for sounds in the tinnitus spectrum toward lower frequencies and suggesting off-frequency listening in the tinnitus group. Auditory thresholds measured in threshold-equalizing noise (TEN test, Moore et al., 2000) after pitch scaling were higher for sounds in the tinnitus region in 8 of the 11 tinnitus subjects studied (Weisz et al., 2006), giving added confirmation that hidden cochlear injury was present. The findings were taken to suggest damage specifically to inner hair cells in the tinnitus group. Hidden synaptic loss is an alternative and not incompatible interpretation of these findings, but synaptic loss could be a more likely explanation owing to a separate study which attempted to replicate Weisz et al. (2006) but failed to find evidence of off-frequency listening in tinnitus subjects with normal audiograms (Gilles et al., 2013). In this regard, some animal models of tinnitus have reported evidence for high-SR fiber loss after noise exposure while concurrent low-SR fiber loss (although present) was insufficient to produce tinnitus behavior (Bauer et al., 2007; Rüttiger et al., 2013). Knipper et al. (2015) suggested that the protein expression necessary to centrally compensate for peripheral deafferentation could not be achieved once high-SR fibers sustained a significant loss, which may trigger central hyperactivity and

hypersynchrony underlying tinnitus (Rüttiger et al., 2013; Singer et al., 2013; Wu et al., 2016). This notion could also explain why some individuals with suprathreshold temporal processing deficits consistent with low-SR fiber synaptopathy do not develop tinnitus (Bharadwaj et al., 2015; Mehraei et al., 2016; control subjects with poor AM detection in the present data).

A summary model incorporating these results for normal and impaired temporal coding in subjects with and without tinnitus with a normal audiogram is presented in Figure 2.6. IHCs and ANFs tuned to high frequencies covering the tinnitus spectrum are depicted for three cases. Tinnitus is not present in cases (a) and (b) since high-SR fibers are preserved; however, temporal coding dependent on intact low-SR fibers is reduced in case (b) in the presence of background noise. Tinnitus is present in case (c) owing to added partial loss of high-SR fibers but not enough to elevate audiometric thresholds. AM coding in quiet is also reduced in this case, since high-SR fibers would be expected to contribute to temporal processing when not saturated by background sound. Although the subjects we studied did not have high frequency hearing loss, the model can be extended to account for audiometric threshold shifts with and without tinnitus. Here tinnitus *with* threshold shift would correspond to case (c), and threshold shift without tinnitus to case (b), adding OHC loss and/or hair cell stereocilia damage in both cases to elevate audiometric thresholds.

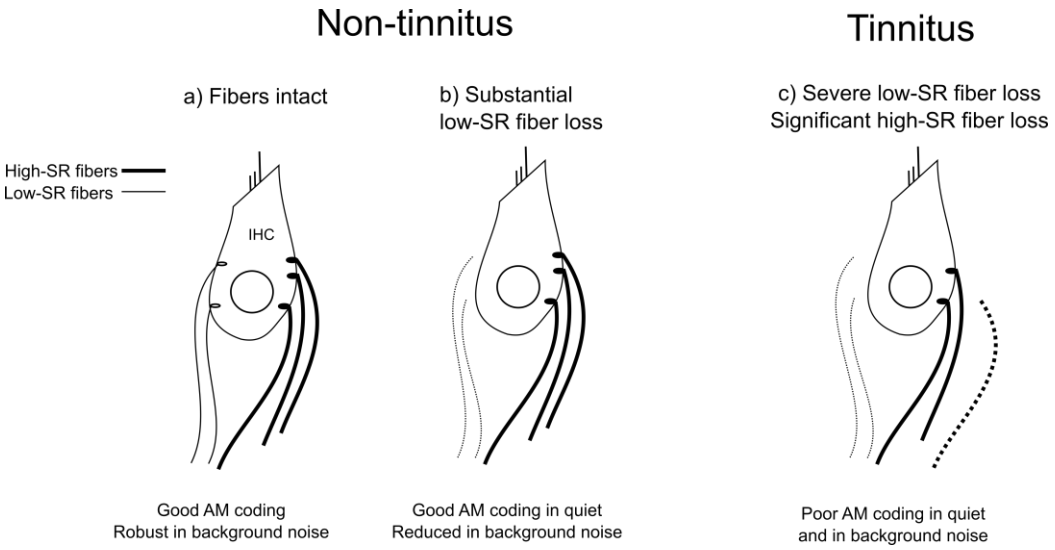

Figure 2.6. Conceptual model of peripheral synaptic loss (broken lines) in tinnitus and non-tinnitus individuals. Noise exposure or other auditory insults damage vulnerable low-SR auditory nerve fibers (thin lines) and less vulnerable high-SR nerve fibers (thick lines). (a) Subjects with normal AM coding, normal audiometric thresholds, and no tinnitus. All synapses are intact. (b) Subjects have low-SR loss that degrades AM coding, but because hair cell mechanical function and a sufficient population of high-SR fibers remain, tinnitus is not present and auditory thresholds are normal. (c) Tinnitus subjects have additional synaptic loss to high-SR fibers sufficient to trigger aberrant hyperactivity in higher auditory structures, but the extent of high-SR fiber loss is not enough to affect auditory thresholds.

2.6.4 Role of central mechanisms in AM coding

The EFR reflects a composite of phase-locked activity from the auditory nerve and subcortical structures (Kuwada et al., 2002; Shaheen et al., 2015) and when evoked at 85 Hz the EFR has sources consistent with generators in the auditory midbrain (Herdman et al., 2002; Kiren et al., 1994). After noise exposure, animals with behavioral evidence of tinnitus typically exhibit increased spontaneous activity throughout the auditory pathway including in the auditory cortex (Basura et al., 2015), the dorsal cochlear nucleus (DCN; Kaltenbach et al., 2004; Demel et al., 2012; Wu et al, 2016), and the inferior colliculus (IC; Bauer et al. 2008).

Increased neural synchrony accompanies these changes in the auditory cortex (Engineer et al., 2011), IC (Bauer et al., 2008), DCN (Wu et al., 2016) and possibly other brain regions (Eggermont and Roberts, 2014). These changes are believed to reflect diminished inhibition and increased central gain in central auditory pathways (Berger and Coomber, 2015; Noreña and Farley, 2013) after deafferentation, with altered spike-timing dependent plasticity playing a role (Dehmel et al., 2012). Any of these effects could disrupt subcortical AM processing (Burger and Pollack, 1998) and thereby alter EFR amplitude in tinnitus, although increased central gain (Gu et al, 2012) might be expected to have the opposite effect. In mice, EFRs recorded at AM rates of <600 Hz appear to be less sensitive to verified synaptic losses on IHCs compared to EFRs recorded at higher AM rates, possibly because EFRs recorded at lower rates reflect activity in the later-occurring ABR waves IV and V which localize to midbrain nuclei and are subject to increased gain after deafferentation (Shaheen et al., 2015) while EFRs recorded at higher AM rates are more closely related to the activity of the cochlear nerve. To the extent that gain changes increase the EFR, the difference we observed between tinnitus and control subjects may understate those that might be observed at higher modulation frequencies, particularly for carrier frequencies close to the peak of individual tinnitus spectra. These considerations underscore that adaptations in central auditory pathways consequent on deafferentation may modulate EFRs recorded from individuals with tinnitus. Our findings indicate, however, that hidden synaptopathy could by

itself be sufficient to account for impaired temporal coding in subjects with normal audiograms, and for cases of tinnitus unrelated to audiometric hearing loss.

While the auditory periphery model was able to qualitatively describe the patterns of EFR responses in the different stimulus conditions for both of our control and tinnitus groups, the range of EFR drops observed in our control subjects exceeded the comparatively narrower range estimated by the model (cf. ordinates, Figure 2.4a and 2.4b). Central AM processing is known to enhance strong but not weak modulations (Joris et al., 2004), which could partly explain the quantitative differences between the obtained and modeled EFR data. In addition, not evaluated in the model was the function of the medial olivocochlear reflex (MOCR), an efferent gain control mechanism that suppresses ANF firing rates under continuous stimulation and may assist listening in noise (Guinan, 2006). There are known individual differences in MOCR strength (De Ceulaer et al., 2001), which may additionally explain the wider range of EFR drops in the control subjects. Central hyperactivity present in tinnitus may also modify the MOCR such that cochlear gain is suppressed in the absence of background noise, but this is unlikely to have occurred for our tinnitus subjects since cochlear gain reduction would have produced audiometric threshold shifts in quiet compared to controls, which we did not observe.

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Chapter 3: Modulation of Electrocortical Brain Activity by Attention in Individuals with and without Tinnitus

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3.1 Preface

One view derived from the results and conceptual model of Chapter 2 is that peripheral loss may be necessary for tinnitus to arise in the absence of audiometric threshold shifts, which may also extend to cases in which threshold shifts are present. Deafferentation would also be expected to have consequences on central auditory and non-auditory factors. The next two chapters investigate how attention is modified in tinnitus. The observation that tinnitus arises in conscious perception and is known to detract from attentional focus formed the basis for a model that positioned a role for an attention-related neuromodulatory system in the development of tinnitus (Roberts et al., 2013). The experiment in the present chapter first tests if the attention system itself might be persistently engaged in tinnitus subjects.

In a previous study, Roberts et al. (2012) evoked ASSR and N1 responses using a 5 kHz tone (well within the TFR) in tinnitus and control subjects that were matched in age and hearing threshold shifts. In one condition, subjects were asked

to either listen and respond to tones evoking these responses or in another condition passively listen and not respond. ASSR and N1 amplitudes increased on active listening trials compared to passive trials in controls, but this effect was absent in tinnitus subjects. The results could either be interpreted as a failure of top-down attention to modulate neurons in A1 and A2 of the TFR because of the presence of tinnitus-related activity, or that attention was persistently engaged and active listening could not increase A1 and A2 responses any further. These alternatives stem from one aspect of the Roberts et al. (2013) attention model suggesting that attention is broadly engaged across auditory cortex in the tinnitus brain as it was trying to resolve the disparity between the tinnitus activity in A1 and the diminished input coming in from the periphery. This view could be supported if attentional modulation failed for tones evoked outside the TFR in tinnitus subjects.

Accordingly, the experiment in Chapter 3 replicated the design used in Roberts et al. (2012) but instead used a 500 Hz tone well below the TFR to evoke the ASSR and N1. These results were combined with the 5 kHz data in a unified analysis comparing the effects of active listening on A1 and A2 responses in attention. Also included was an analysis on the effects of attention on the late-latency N2 and sustained responses in tinnitus and control subjects at both frequencies.

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Research Article

Modulation of Electrocortical Brain Activity by Attention in Individuals with and without Tinnitus

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Age and hearing-level matched tinnitus and control groups were presented with a 40 Hz AM sound using a carrier frequency of either 5 kHz (in the tinnitus frequency region of the tinnitus subjects) or 500 Hz (below this region). On attended blocks subjects pressed a button after each sound indicating whether a single 40 Hz AM pulse of variable increased amplitude (target, probability 0.67) had or had not occurred. On passive blocks subjects rested and ignored the sounds. The amplitude of the 40 Hz auditory steady-state response (ASSR) localizing to primary auditory cortex (A1) increased with attention in control groups probed at 500 Hz and 5 kHz and in the tinnitus group probed at 500 Hz, but not in the tinnitus group probed at 5 kHz (128 channel EEG). N1 amplitude (this response localizing to nonprimary cortex, A2) increased with attention at both sound frequencies in controls but at neither frequency in tinnitus. We suggest that tinnitus-related neural activity occurring in the 5 kHz but not the 500 Hz region of tonotopic A1 disrupted attentional modulation of the 5 kHz ASSR in tinnitus subjects, while tinnitus-related activity in A1 distributing nontonotopically in A2 impaired modulation of N1 at both sound frequencies.

1. Introduction

Forms of neural plasticity are expressed by many neurons in central auditory structures and are believed to sculpt the neural changes that underlie the development of tinnitus and hyperacusis associated with hearing loss [1, 2]. Examples of neural changes attributed to neural plasticity in animal models include upregulation of somatosensory inputs to principal neurons in the dorsal cochlear nucleus (DCN) following section of the cochlear nerve [3] and broadening of the temporal integration window of spike-timing dependent plasticity for neurons in the DCN [4] and auditory cortex [5] in animals exhibiting behavioral evidence of tinnitus. Neural changes taking place after deafferentation may in turn affect how neural activity is modified when auditory training is applied to individuals with tinnitus, as is done by sound therapies intended to treat this condition. Roberts et al. [6] trained individuals with tinnitus and age and hearing-level matched controls to detect an auditory target embedded in a 5 kHz 40 Hz amplitude modulated (AM) sound. The 5 kHz 40 Hz AM sound was in the tinnitus frequency region (TFR) of the tinnitus subjects and evoked the stimulus-driven 40 Hz auditory steady response (ASSR) known to localize to sources in primary auditory cortex (A1) [7–10]. In agreement with earlier results obtained from normal hearing subjects [11, 12], the phase of the ASSR phase (the time delay between the 40 Hz stimulus and response waveforms) decreased progressively over training sessions in the control group, but ASSR phase did not change in the tinnitus group. In contrast, the amplitude of the ASSR (which was known from earlier research to be resistant to change) did not increase with training in controls, but ASSR amplitude increased with training in the tinnitus group, as did online ratings of the loudness of their tinnitus percept. It was suggested that abnormal synchronous neural activity underlying the tinnitus percept may have obstructed changes in ASSR phase in the tinnitus group, whereas reduced inhibition in A1 associated with tinnitus may have permitted an expansion of

the cortical representation for 5 kHz that was prevented by competitive interactions within the tonotopic map of control subjects without tinnitus [6].

These results suggest that the effects of plasticity are modified in tinnitus sufferers by tinnitus-related neural activity occurring in auditory pathways. Further findings of Roberts et al. [6] suggested that effects of attention on neural responses are also modified in the tinnitus brain. In hearing-intact animals, neural plasticity is modulated by subcortical cholinergic and other neuromodulatory systems that receive top-down input from prefrontal cortex and project widely to the neocortex where they perform an attention-like function, making neurons more sensitive to their afferent inputs [13–17]. These mechanisms may account for the observation in normal hearing humans that auditory tasks that require top-down attention increase not only the amplitude of the ASSR localizing to A1, but also the amplitude of the N1 transient response whose cortical sources localize to secondary auditory cortex (A2) in the region of the planum temporale [7, 18]. In agreement with results obtained in normal hearing subjects, control subjects in the study of Roberts et al. [6] showed increased ASSR and N1 amplitude on active trials where detection of targets was required, compared to a passive condition where subjects were told to ignore the sounds and rest until the next active block was presented. However, modulation of ASSR and N1 amplitude by attention was abolished in the tinnitus group for N1 in all sessions of training and for ASSR amplitude on the first session with a weak modulation appearing subsequently as ASSR amplitude increased over trials. The results suggested that, although the top-down auditory attention system may work normally in tinnitus, its expression was obstructed by tinnitus-related neural activity occurring in the TFR of the tinnitus group where the sound to be detected (a 40 Hz AM 5 kHz carrier frequency) was located.

The present experiment evaluated this hypothesis by determining whether deficient modulation of ASSR and N1 amplitude by attention is observed when subjects with tinnitus are required to detect auditory targets embedded in a 40 Hz AM carrier of 500 Hz, which is well below the region where tinnitus-related neural activity is expected to occur. The results were compared in a unified analysis to the 5 kHz groups reported by Roberts et al. [6] which performed the same auditory detection task except for the carrier frequency chosen. In addition, two additional long-latency responses, namely, the N2 transient response (latency ~325 ms) and the auditory sustained response (SR, commencing after N2 and persisting to the end of stimulation), were studied in both groups, to determine whether modulation of these late responses was similarly affected by tinnitus.

2. Methods

2.1. Participants and Design. 60 subjects (30 tinnitus and 30 controls) were recruited via McMaster University faculty and staff by email list servers and from our laboratory archive. One control subject was excluded from analysis due to noise in the electroencephalogram (EEG) that could not meet the requirement for artifact rejection in offline processing. Two further controls withdrew for unrelated medical reasons. Two tinnitus participants withdrew after expressing concern that the procedures might worsen their tinnitus. Of the remaining 55 subjects, 22 completed the 5 kHz study of Roberts et al. [6] and 33 subjects were new recruits assigned to 500 Hz and tested here. No subjects in the total sample reported use of medication during the time of the study; controls reported no history of tinnitus or ear diseases. Participants received an honorarium of $10 CAD per hour as well as reimbursement for parking fees. Subjects provided informed consent using procedures approved by the Research Ethics Board of McMaster University and consistent with the Declaration of Helsinki.

Tinnitus subjects completed a preliminary interview (intake session, about 90 minutes) that collected detailed information on personal history of their tinnitus. The Tinnitus Handicap Questionnaire (THQ) was administered to assess tinnitus attributes and impact on quality of life [19]. Pure-tone audiometric thresholds were measured using a GSI 61 audiometer with Telephonics 296 D200 (0.125–8.0 kHz) and Sennheiser HDA 200 (8.0–16 kHz) headphones using the pulsed-tone method. Properties of tinnitus were measured by computerized tools described by Roberts et al. [12]. Using the tools, subjects first identified the ear of tinnitus (left, right, or both) and tinnitus bandwidth (tonal, ringing, or hissing) following which they rated tinnitus loudness on a Borg CR100 visual analog scale. Next, subjects adjusted the loudness of each of 11 pure tones between 0.5 and 12.0 kHz to equal that of their tinnitus. The tinnitus frequency spectrum (likeness rating) was then taken for the same pure tones at the determined loudness level, followed by a brief test for residual inhibition. Control subjects completed the same intake procedure as tinnitus subjects except for procedures pertaining to tinnitus.

Four groups of subjects were studied: controls tested at 500 Hz (Cont500 Hz), tinnitus subjects at 500 Hz (Tinn500 Hz), control subjects at 5 kHz (Cont5 kHz), and tinnitus subjects at 5 kHz (Tinn5 kHz). The tinnitus and control groups were matched for age within the two stimulus frequencies and as much as possible between the two frequencies. The number, age, and gender of subjects in each group and the sound levels experienced by the subjects are given in Table 1 where properties of tinnitus are also reported for the two tinnitus groups. Figure 1 shows audiometric thresholds for each group and, for tinnitus subjects only, the tinnitus spectrum and loudness matches for sound frequencies between 500 Hz and 12 kHz. Approximately one week lapsed between the intake session and the experimental session described next.

2.2. Stimuli. The stimuli were 500 Hz and 5 kHz pure tones AM by a 40.96 Hz sinusoid (called 40 Hz, 100% modulation depth following the modulation wave). Tone duration was 975.56 ms, such that each stimulus contained 40 AM pulses. Stimuli were generated by a digital signal processor (Tucker-Davis RP.2) and presented binaurally through ear inserts (Etymotic ER2). Sound levels were determined by a loudness

Table 1: Participant demographics.

	Tin500 Hz group	Cont500 Hz group	Tinn5 kHz group	Cont5 kHz group
Characteristics of participants				
Number (male)	17 (10)	16 (5)	11 (7)	11 (8)
Age in years, mean (SE)	62.0 (3.31)	62.0 (2.29)	48.6 (4.75)	53.9 (5.86)
Age range in years	22–77	42–74	22–68	22–76
Audiometric Data				
Mean (SE) threshold @ 500 Hz (dB HL)	10.6 (3.49)	11.9 (2.90)	8.0 (2.56)	10.5 (2.88)
Mean (SE) threshold @ 1 kHz (dB HL)	18.4 (3.76)	13.1 (3.25)	7.9 (2.70)	10.7 (3.67)
Mean (SE) threshold @ 2 kHz (dB HL)	24.5 (4.24)	12.5 (3.97)	9.8 (3.65)	13.6 (3.61)
Mean (SE) threshold @ 5 kHz (dB HL)	43.8 (5.04)	33.3 (5.19)	28.5 (6.24)	25.7 (7.01)
Sound levels				
Standard for matching	1 kHz pure tone 65 dB SL	1 kHz pure tone 65 dB SL	2 kHz 40 Hz AM tone at 65 dB SPL	2 kHz 40 Hz AM tone at 65 dB SPL
Mean (SE) stimulus intensity (dB SPL)	81.2 (1.68)	78.7 (2.90)	60.0 (2.05)	58.5 (2.01)
Stimulus intensity range (dB SPL)	69–93	47–93	50–74	40–66
Tinnitus characteristics				
Mean (SE) duration in years	12.5 (2.68)		11.7 (3.03)	
Mean (SE) loudness rating Borg CR100 scale	44.8 (5.62)		57.1 (6.21)	
Mean (SE) loudness match (1 kHz tone, dB SPL)	36.7 (9.05)		53.9 (6.32)	
THQ Mean Total Score (SE)	32.5 (5.64)		48.9 (6.66)	
Tinnitus bandwidth (number of participants)				
Tonal	12		6	
Ringing	2		2	
Hissing	3		3	
Tinnitus ear				
Bilateral	15		11	
Left	1		0	
Right	1		0	

matching paradigm in which subjects in the 500 Hz groups matched the loudness of the stimulus to a reference pure tone of 1 kHz presented at 65 dB SL and subjects in the 5 kHz groups to a reference tone of 2 kHz presented at 65 dB SPL. These matching procedures aligned the groups with those of earlier research [2, 6] and equated subjective stimulus loudness between the tinnitus and control groups at each probe frequency. However, it was inevitable that probe intensity measured in SPL would vary between the 500 Hz and 5 kHz groups as a consequence of threshold shifts at 5 kHz and hyperacusis in the tinnitus groups. Possible effects of probe intensity were evaluated by regressing effects of attention expressed in each brain response on probe intensity in SPL, which was known for each subject.

2.3. Auditory Task. The auditory task is described in Figure 2. Subjects sat in a sound-attenuated (ambient noise level 16 dBA SPL) and electrically shielded booth, comfortably in a chair distanced 1.4 m from a computer monitor. There were two types of stimuli: standard stimuli and stimuli containing a target. The two stimuli were identical except that target stimuli contained a single 40 Hz pulse of variable increased amplitude (target) that occurred randomly at 415 ms, 610 ms, or 805 ms after stimulus onset. Approximately 2/3rd of the stimuli contained a target; however, because approximately 1/3rd of the targets were expected to be below or close to the threshold of detection, target stimuli likely were heard on about 50% of trials. Trials of both types (standard and target) unfolded in either active blocks or passive blocks with each block containing 54 stimuli and lasting roughly 2.5 minutes. On active blocks, the word "Listen" appeared in a text box on the computer screen, instructing participants to attend to the trial for a target event. After stimulus completion text on the screen prompted, "Did you hear a target?" As per instructions on the screen, participants pressed the left mouse button "yes" if they had detected a target and a right mouse button "no" if they had not. Correct responses (hits and correct rejections) generated a green text box for 400 ms providing appropriate feedback. Incorrect responses (misses and false alarms) produced a red text box for the same duration. An intertrial interval (ITI) varying between 1400 and 1600 ms commenced with each behavioral response, giving a variable interval of about 1900 ms including the feedback cue and depending on behavioral response latency. During passive blocks, the text "Stop responding and ignore

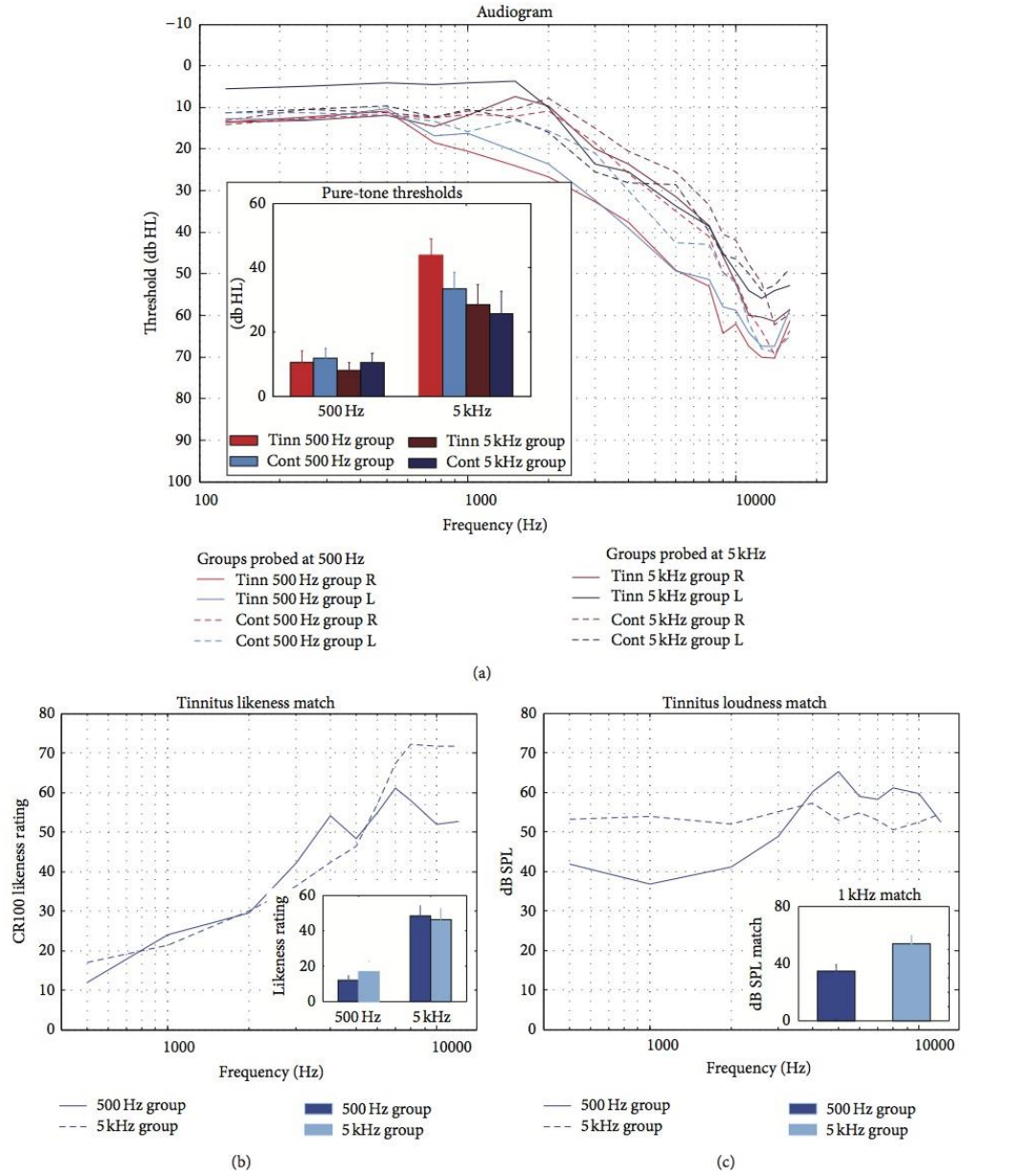

FIGURE 1: Audiogram, tinnitus spectrum, and tinnitus loudness matches. (a) Pure-tone audiograms (pulsed-tone method) from 0.125 to 16 kHz showing each ear and group separately. Comparisons of thresholds averaged across ears at 500 Hz and 5 kHz are shown in the inset bar graph (5 kHz interpolated between 4 kHz and 6 kHz) separately for groups probed with 500 Hz and 5 kHz sounds. (b) Tinnitus likeness ratings from 0.5 to 12 kHz for both tinnitus groups and an inset bar graph comparing 500 Hz ratings to 5 kHz ratings in each group. 500 Hz ratings are below the tinnitus spectrum which commences above a likeness rating of 40 (a sound beginning to resemble tinnitus; Roberts et al. 2008). (c) Tinnitus loudness matches from 0.5 to 12 kHz for both tinnitus groups. Inset bar graphs compare loudness matches at a common 1 kHz frequency.

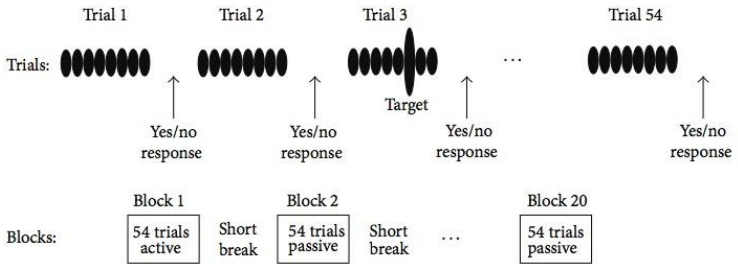

FIGURE 2: Auditory task. Upper panel: three standard 40 Hz AM stimuli and one target stimulus containing a single amplitude-enhanced AM pulse (target) are illustrated by cartoons containing 8 AM pulses (40 pulses were delivered on each trial). Approximately 2/3rd of the stimuli contained a target of variable enhanced amplitude such that not all targets were detectable. Lower panel: on active blocks participants identified whether a target was present or not; on passive blocks participants ignored the sounds and waited for the next active block. Blocks contained 54 trials and alternated between active and passive blocks for a total of 20 blocks per session.

stimulus" appeared continuously on the computer screen, indicating that participants should ignore the sounds and wait until the next active block. The ITI was randomly varied between 1600 and 1900 ms (stimulus offset to onset) for passive blocks, to be comparable to active blocks. Each session began with an active block and alternated with passive blocks for a total of 20 blocks (10 active and 10 passive) with 54 trials in each (Figure 2).

It should be noted that active trials on this task not only required attention to the stimuli but also involved other cognitive functions such as processing of target events, behavioral response selection, and perhaps also anticipation of correctness feedback. Short latency responses such as the ASSR and N1 are likely to be dominated by attention since this process was necessarily deployed commencing at trial onset with other functions following after target detection. Consistent with this expectation, Gander et al. [7] found that attention modulated ASSR amplitude in a dual auditory-visual task when all other task requirements (processing of feedback events, response selection, and correctness feedback) were held constant. We refer herein to the active/passive manipulation as one affecting attention but acknowledge that long-latency brain responses in particular may reflect overlapping cognitive functions.

Immediately prior to the session, each subject completed a staircase procedure in order to determine a set of target amplitudes suitable for the detection task. 80 stimuli were presented each containing a target, commencing with a 200% amplitude increase known to be detectable by inexperienced subjects. Target amplitude decreased after each "yes" response and increased after each "no"; target amplitude at the end of 80 trials was taken as the amplitude corresponding to the subject's threshold of detection (TH). A set of six target stimuli was then generated for each subject consisting of TH, TH ± 5%, TH ± 10%, and TH + 20% for use on the detection task. TH varied between subjects and averaged 47% over all subjects.

2.4. Electrophysiological Recording. The EEG was recorded from a 128-channel Biosemi ActiveTwo amplifier (Cortech Solutions, Wilmington, NC) and sampled at 2048 Hz. Before recording, the electrode array positions were digitized for each participant (Polhemus Fastrak). EEG data were stored as continuous data files referenced to the vertex electrode.

2.5. Signal Processing. Eyeblink and other movement artifacts were removed from raw continuous data by the spatial filtering option of BESA (version 5.1.8; MEGIS Software GmbH, Grafelfing, Germany). Responses were epoched around 100 ms pre- and poststimulus baselines.

40 Hz Steady-State Response. EEG responses for ~85% of trials (rejecting trials with artifacts exceeding 100 μV between 30 and 50 Hz) were used for analysis of the ASSR. Data were converted to the average reference and filtered 40 to 42 Hz (zero phase). For each of the 128 channels, data between 244 and 952 ms poststimulus were collapsed to a two-AM cycle average waveform for each subject (see Figure 3). Because the ASSR is reflected in most electrodes, ASSR amplitude was calculated as the total field power (TFP) determined by Fourier transform summed over 128 electrodes, following Gander et al. [20] and Roberts et al. [6].

Transient Responses. EEG responses for ~80% of trials (rejecting trials with artifacts exceeding 150 μV between 1 and 20 Hz) were used for analysis of transient responses. Epoched data were averaged and interpolated to the 81-channel "reference free" average reference montage of BESA using each participant's digitized electrode array positions, which reduced individual differences in electrode cap placement between subjects. Data were then filtered from 0.2 to 20 Hz (zero phase). The latencies of P1 (from time window 30–85 ms), N1 (85–140 ms), P2 (140–230 ms), and N2 (250–350 ms) transient responses were identified from electrode Fz where the responses typically reached their amplitude maximum [7]. TFP for each response was calculated as the sum of each channel's squared voltage at the peak latency of electrode Fz (Figure 3). The auditory sustained response (SR) was calculated as the TFP over the time interval 400–900 ms (Figure 3). Two subjects (both in the 500 Hz tinnitus

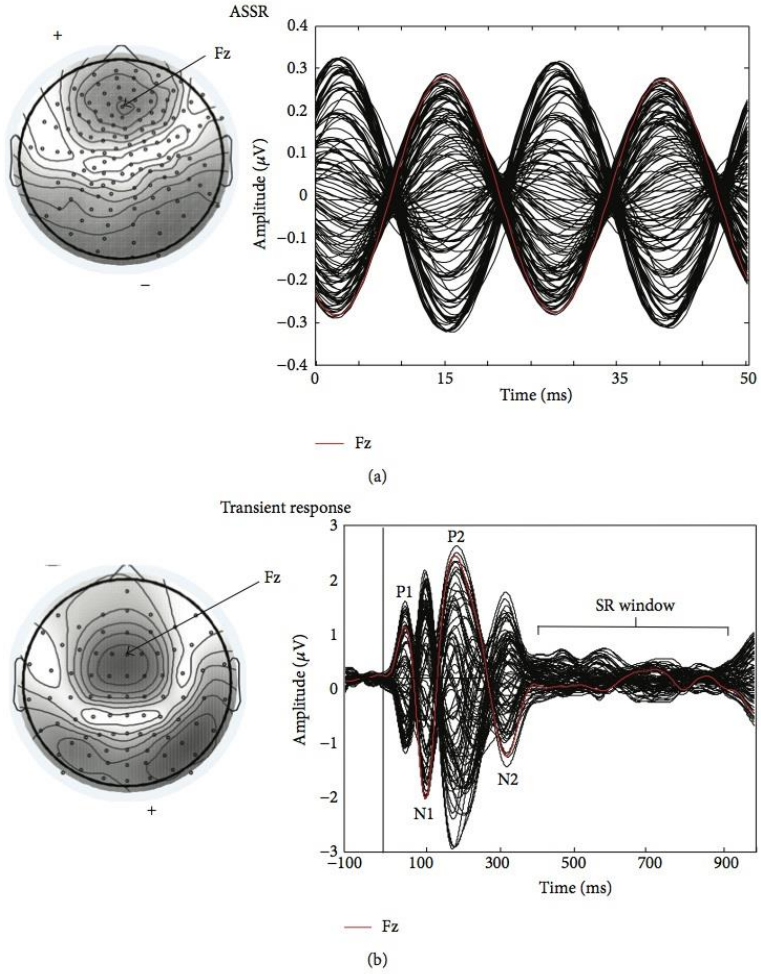

FIGURE 3: Representative topographies and time domain waveforms for the 40 Hz auditory steady-state response (ASSR) and N1 response derived from the grand average of active trials from control subjects probed with a 500 Hz stimulus. (a) shows the ASSR during the interval 244–952 ms poststimulus, collapsed down to two 40 Hz AM cycles. An alternating dipolar waveform is observed (one for each AM cycle). ASSR amplitude was calculated as the total field power of all electrodes in the two-cycle AM waveform. (b) A dipolar N1 is seen peaking at 100 ms poststimulus. N1 amplitude was calculated as total field power at the peak of the dipolar waveform. The transient responses P1, P2, and N2 and the time range for the auditory sustained response (SR) are also labeled in the waveform. For the purpose of visualization, the trace in the right panel is high pass filtered at 2 Hz to distinguish N2 from the SR which is attenuated as shown here. In each panel the Fz electrode is shown in red.

group) were omitted from the analysis of the SR because of the electrode drift exceeding −50 μV past 400 ms.

2.6. Statistical Evaluation. Repeated measures ANOVAs were performed using the General Linear Model of Statistica (version 6.0). Least significant difference (LSD) tests were used to describe significant main effects and interactions. Group comparisons not addressed by ANOVA were evaluated by t-tests. Significance level was set at $\alpha = 0.05$. Further details regarding statistical approach are reported where appropriate in Section 3.

3. Results

3.1. Behavioral Responses. Performance on the behavioral task is presented in Figure 4. The probability of a hit (P(H)) exceeded the probability of a false alarm (P(FA)) for all subjects with no differences between the tinnitus and control

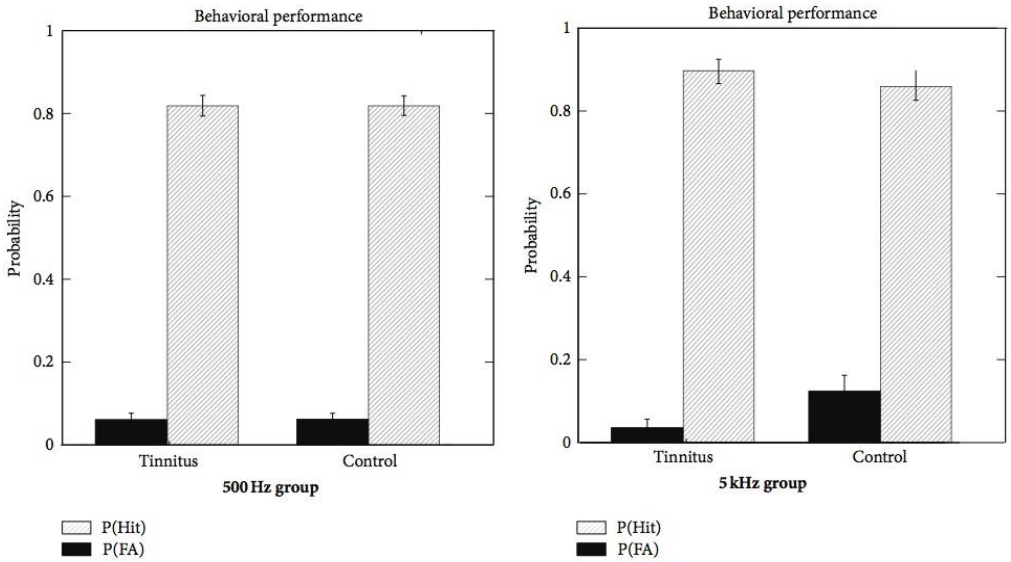

FIGURE 4: Performance on the behavioral task for both tinnitus and control groups probed at 500 and 5 kHz. The probability of a hit (P(Hit)) is averaged across the six target amplitude enhancements. The probability of a false alarm (P(FA)) was determined from trials with no target.

groups or the two carrier frequencies on this measure. P(H) averaged 0.85 overall indicating that for most subjects at least one of the six target stimuli was not detectable.

3.2. Electrophysiological Responses

3.2.1. Effects of Carrier Frequency and Group on Passive Blocks. In the first analysis, ANOVAs including the variables group (tinnitus/control) and frequency (500 Hz and 5 kHz) were applied to passive blocks for each brain response to identify effects of these variables on brain activity in the absence of attended performance. ANOVA returned main effects for carrier frequency (500 Hz versus 5 kHz groups) on these blocks for the ASSR ($F(1, 51) = 10.38$, $P = 0.002$), P1 ($F(1, 51) = 11.87$, $P = 0.001$), N1 ($F(1, 51) = 10.17$, $P = 0.002$), P2 ($F(1, 51) = 12.93$, $P = 0.001$), and the SR ($F(1, 49) = 5.31$, $P = 0.025$), with similar results for N2 ($F(1.51) = 2.40$, $P = 0.127$). For each response TFP was larger in the 500 Hz groups than in the 5 kHz groups in accordance with the known dependence of the amplitude of the ASSR and transient responses on carrier frequency [21]. No main effects involving group reached significance for any response on passive blocks, although P2 tended to be larger in control subjects than in the tinnitus groups ($P = 0.078$) on these blocks. Interactions between carrier frequency and group did not reach significance for any response on passive blocks.

3.2.2. Effects of Attention (Active versus Passive Blocks). Effects of attention were evaluated first by comparing response TFP on active blocks where attention to the probe stimuli was required with that on passive blocks where subjects were instructed to ignore the stimuli and rest. No main effects or interactions involving active/passive were found for P1 and P2 responses, and these responses are not discussed further. However, the main effects of attention were found for the ASSR ($F(1, 51) = 10.38$, $P = 0.002$), N1 ($F(1, 51) = 7.51$, $P = 0.008$), N2 ($F(1, 51) = 29.12$, $P < 0.001$), and the SR ($F(1, 49) = 28.71$, $P < 0.001$).

Effects of attention on these responses were examined in more detail, as follows. For each subject and response, the effect of attention was calculated (1) as the difference in TFP between active and passive blocks (passive subtracted from active) and by (2) representing the attention effect as TFP on active trials divided by TFP on passive trials (this ratio minus 1, to represent no effect of attention as zero). Distributions of these measures ($n = 55$ subjects) were then examined for kurtosis, which can be pronounced for the ASSR where large but repeatable individual differences are known to occur (test-retest reliability $r > 0.90$, [2]), likely reflecting summation of ASSR fields across two tonotopic maps sharing a common low frequency border in Heschl's gyrus. For ASSR amplitude kurtosis was lower for the ratio measure (2.94) than for the difference measure (19.4), whereas the reverse was true for N1 (5.95/2.37), N2 (13.4/10.37), and the SR (9.50/2.64). Thus for the additional analyses reported below, effects of attention were analyzed as the ratio measure for the ASSR and as the difference in TFP between active and passive blocks for N1, N2, and the SR. Effects were evaluated statistically by t-tests and by ANOVA applied to

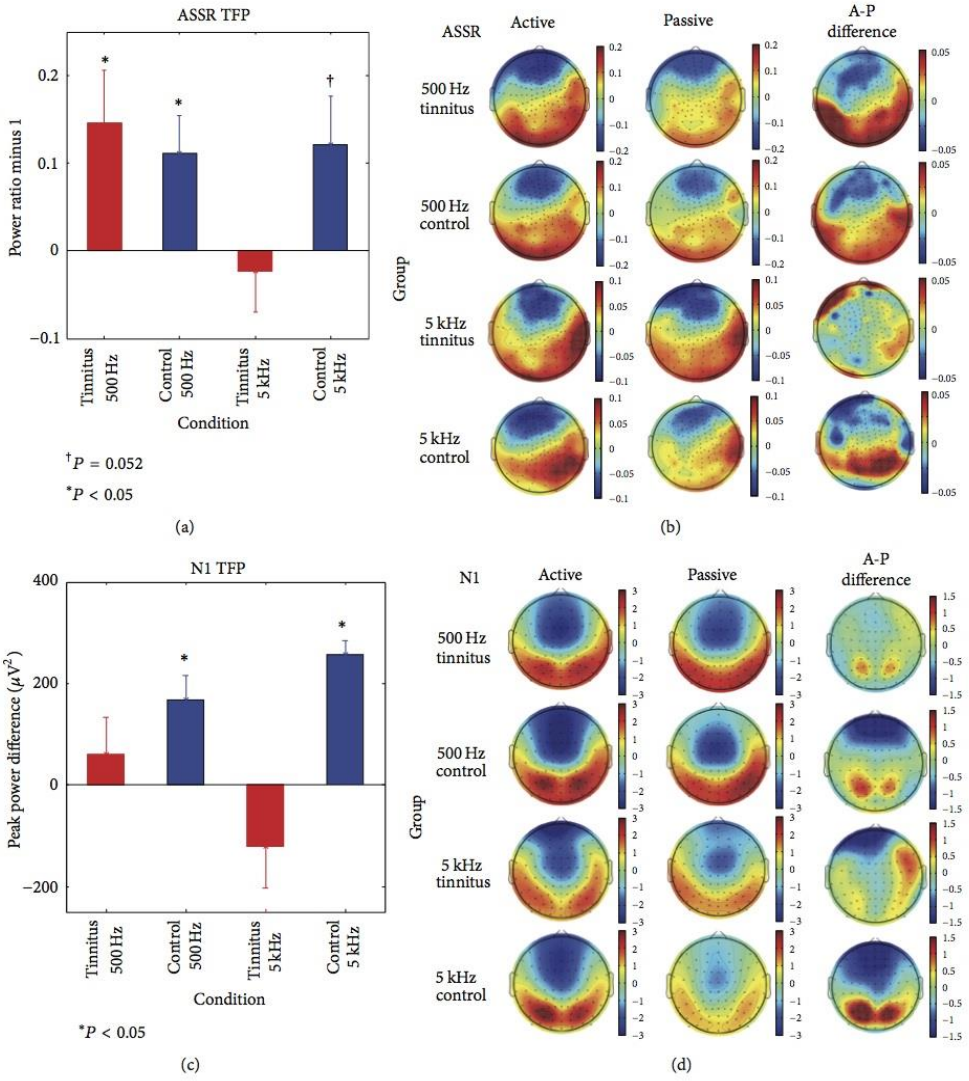

FIGURE 5: ASSR and N1 attention effects. (a) Effect of attention on ASSR TFP in each group (A/P TFP-1). (b) Voltage map of the ASSR taken at the time point of maximum total field power on active and passive blocks and the voltage difference map (active-passive blocks). (c) Effect of attention on N1 TFP in each group (active-passive blocks). (d) Active, passive, and difference voltage maps for N1 at the peak latency of electrode Fz. The error bars in (a) and (c) are one standard error ($^*P < 0.05$; $^\dagger P = 0.052$).

these measures. In addition, the topography of TFP on active and passive blocks and the difference in TFP (active-minus passive) are shown for all responses.

ASSR. Effects of attention on the ASSR are shown in each group as TFP ratios in Figure 5(a) and as voltage difference maps in Figure 5(b). TFP ratios increased on active compared to passive blocks in the Cont500 Hz group ($t(15) = 2.53$, $P = 0.023$), Cont5 kHz group ($t(10) = 2.199$, $P = 0.052$), and in the Tinn500 Hz group ($t(16) = 2.42$, $P = 0.028$), but the TFP ratio did not increase on active blocks in the Tinn5k group ($t(10) = -0.49$, $P = 0.628$). This pattern can also be seen in the voltage difference maps presented for the four groups in Figure 5(b) (right column) where the voltage difference was minimal in the Tinn5 kHz condition. When the four groups were collapsed into one, the TFP ratio

differed significantly from zero ($t(54) = 3.54$, $P = 0.001$) confirming the sensitivity of ASSR amplitude to attention. An ANOVA applied subsequently to TFP ratios with group and frequency as between-subjects variables found no significant effects, although the interaction of group and frequency approached significance ($F(1, 51) = 2.69$, $P = 0.106$) reflecting the pattern seen in Figure 5(a). LSD tests within this interaction found the 5 kHz and 500 Hz tinnitus groups to be different from one another ($P = 0.032$) whereas contrasts of the Cont500 Hz group and the Cont5khz group to the Tinn5 kHz group reached $P = 0.09$ in each case. Effects of attention on ASSR amplitude were unrelated to ASSR amplitude on passive blocks when correlations were calculated between the two responses for the total sample ($r = 0.09$, $P > 0.53$) or for the tinnitus and control groups separately collapsing over probe frequency ($rs = 0.24$ and 0.09, resp., $Ps \geq 0.21$).

N1. Effects of attention on the N1 are shown for each group in Figure 5(c) (TFP difference between active and passive blocks) and in Figure 5(d) (voltage difference maps, right column). TFP increased on active compared to passive blocks in Cont500 Hz group ($t(15) = 3.35$, $P = 0.043$) and in the Cont5 kHz group ($t(10) = 9.48$, $P < 0.001$), but this difference did not reach significance in tinnitus groups probed at either frequency ($Ps > 0.17$) notwithstanding a weak posterior modulation which can be seen in the voltage difference map for the Tinn500 Hz group. ANOVA applied to the difference in TFP between active and passive blocks returned the main effects of group ($F(1, 51) = 13.37$, $P = 0.001$) and a significant interaction between group and frequency ($F(1, 51) = 4.12$, $P = 0.048$). LSD tests within the interaction found that the N1 TFP difference was larger in the Cont5 kHz group than in either tinnitus condition ($P < 0.04$ or better) and also larger in the Cont500 Hz control group than in the Tinn5 kHz group ($P < 0.004$). Correlations between N1 TFP on passive blocks and the effect of attention on N1 TFP did not reach significance when the four groups were collapsed into a single sample ($r = -0.23$, $P = 0.09$) or when correlations were calculated for the tinnitus and control subjects separately collapsing over probe frequency ($rs = -0.26$ and -0.13, resp., $Ps \geq 0.19$).

N2. Effects of attention on N2 are shown for each group in Figure 6(a) (TFP difference measure) and as voltage difference maps in Figure 6(b) (right column). TFP increased on active compared to passive blocks in Cont500 Hz ($t(15) = 4.42$, $P < 0.001$), Cont5 kHz ($t(10) = 4.47$, $P = 0.001$), and Tinn500 Hz ($t(16) = 2.21$, $P = 0.042$) groups, while the difference in Tinn5 kHz approached significance ($t(10) = 1.94$, $P = 0.081$). Comparison of the groups by ANOVA found no significant main effects or interactions involving group or frequency, although the TFP difference between active and passive blocks tended to be larger in the control groups than in the tinnitus groups at both probe frequencies (main effect of group $P = 0.105$). The voltage maps of Figure 6(b) show further that N2 reached its maximum negativity at central electrodes, as did the TFP difference between active and passive blocks. This contrasts with the ASSR and N1 where amplitude maxima were focused frontocentrally on active trials (see Figures 5(b) and 5(d), resp.), particularly for the ASSR whose sources are localized tonotopically in the region of Heschl's gyrus.

Sustained Response. SR TFP increased on active compared to passive trials in all groups (Figure 6(c)). The results for each group were Tinn500 Hz ($t(14) = 2.27$, $P = 0.039$), Cont500 Hz ($t(15) = 2.78$, $P = 0.0139$), Tinn5 kHz ($t(10) = 3.07$, $P = 0.012$), and Cont5 kHz ($t(10) = 5.46$, $P < 0.001$). While active-passive differences in SR TFP tended to be larger in the control groups than in tinnitus, SR TFP differences for each group subjected to ANOVA revealed no main effects or interactions of group or frequency. On active blocks the SR showed a predominant negativity at central electrodes (Figure 6(d)) where the effect of attention was also predominantly expressed.

3.3. Demographics. The mean age of the subjects, their hearing thresholds at four sound frequencies, the intensity of the probe stimuli they received, and, where applicable, properties of their tinnitus are summarized for each group in Table 1. Correlations between several of these variables and (1) ASSR and N1 responses measured on passive blocks in the absence of attended performance and (2) effects of attention on ASSR and N1 TFP are reported in Table 2.

3.3.1. Age. Subjects in the 500 Hz groups of Table 1 were on average 60.0 years old and those in the 5 kHz groups were 51.3 years old, a difference that was significant ($F(1, 51) = 8.33$, $P = 0.005$). However, age range was similar among the four groups, and the tinnitus and control groups within each frequency were matched with no significant differences found in age between them. Age did not correlate significantly with ASSR and N1 responses measured on passive blocks or with effects of attention expressed in these responses when the tinnitus and control groups were collapsed at each frequency (Table 2).

3.3.2. Hearing Thresholds. The audiograms for each group and ear measured to 16 kHz are reported in Figure 1(a). All groups exhibited thresholds exceeding 25 dB HL above 3 kHz while for the Tinn500 Hz group this criterion was met at 2 kHz. Threshold shifts were similar in both ears, with the only difference being thresholds about 7 dB greater in the right ear than in the left ear in the Tinn5 kHz group at the audiometric frequencies of 500 Hz and 1 kHz. To compare audiometric thresholds across all groups, 5 kHz thresholds were interpolated from 4 and 6 kHz thresholds, collapsed over left and right ears, and submitted to repeated-measures ANOVA with 500 Hz thresholds (see inset, Figure 1(a)). ANOVA returned the main effect of audiometric threshold frequency confirming higher thresholds at 5 kHz than 500 Hz in each subject group ($F(1, 51) = 66.23$, $P < 0.001$). The main effect of group (tinnitus versus control) on 500 Hz and 5 kHz audiometric thresholds was not significant. Audiometric thresholds at 500 Hz and 5 kHz did not correlate with ASSR or N1 amplitude measured on passive blocks or with effects

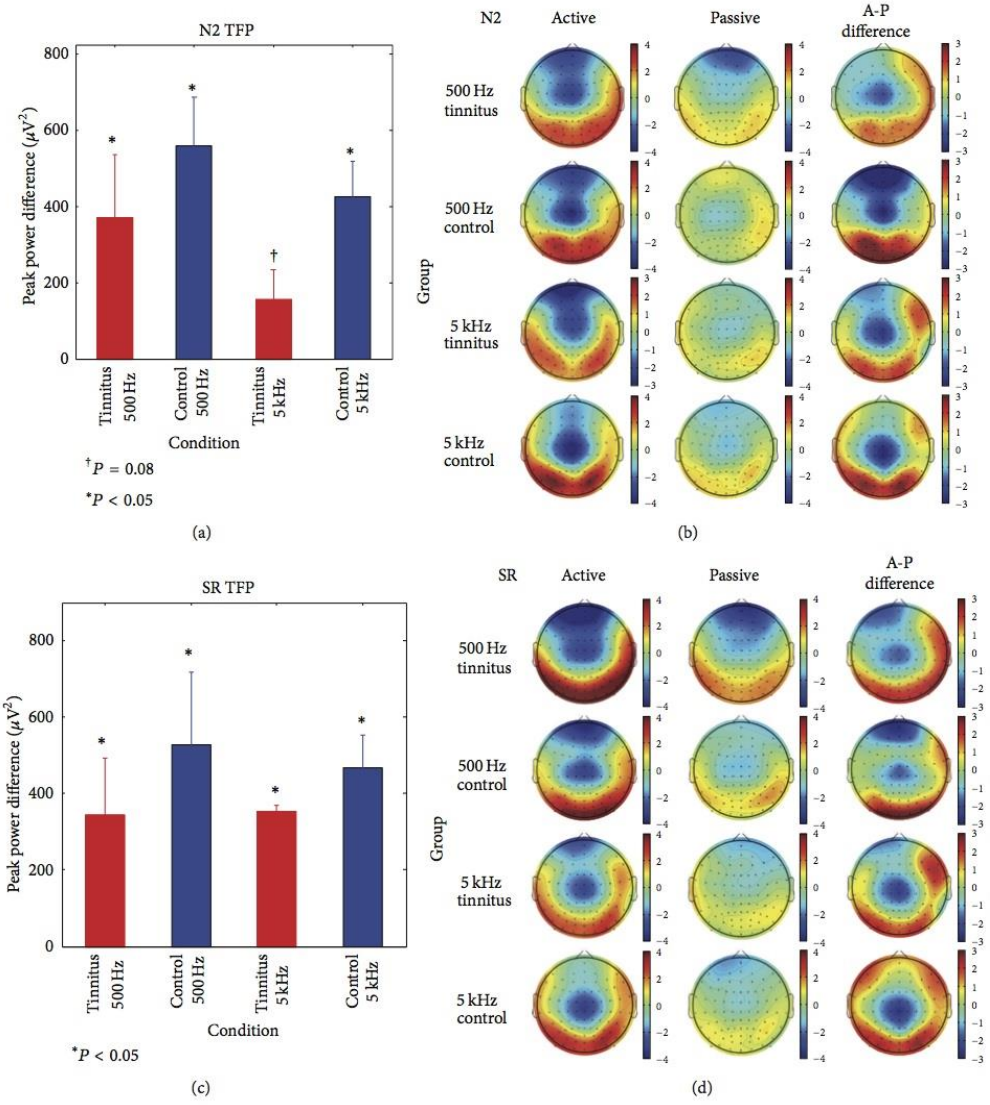

FIGURE 6: N2 and auditory SR scalp topography and attention effects. (a) Effect of attention on N2 TFP in each subject group (active-passive blocks). (b) Active, passive, and difference voltage maps for N2 at the peak latency of electrode Fz. (c) Effect of attention on SR TFP in each subject group (active-passive blocks). (d) Active, passive, and difference voltage maps for SR averaged from 400 to 900 ms. The error bars in (a) and (c) are one standard error ($^{*}P < 0.05$; $^{\dagger}P = 0.08$).

of attention in these responses when the tinnitus and control groups were collapsed at each frequency (Table 2).

3.3.3. Probe Intensity. Probe intensity ranged from 47 to 93 dB SPL (M = 79.9) in the 500 Hz probe groups and from 40 to 74 dB SPL (M = 59.3) in the 5 kHz groups. Differences in probe SPL between the tinnitus and control groups tested at each carrier frequency averaged 2.5 dB or less (Ps > 0.51), indicating that sound level matching between the groups was achieved within the 500 Hz and 5 kHz conditions. However, probe intensity collapsed over the tinnitus and control groups differed between the 500 Hz (80.0 dB SPL) and 5 kHz (59.2 dB SPL) conditions ($F(1, 51) = 73.05$, $P < 0.001$). This difference was a function of several factors including a 15.7 dB HL threshold shift at 1 kHz in the 500 Hz groups (who

Table 2: Relationship of ASSR and N1 responses on passive blocks and ASSR and N1 attention effects to subject and tinnitus variables. The table entries are product-moment correlations reported for the 500 Hz and 5 kHz conditions separately.

	Subject variables*				Tinnitus variables			
	Age	500 Hz threshold†	5 kHz threshold†	Probe SPL	Loudness match (1 kHz)	Borg CR100	THQ	Years with tinnitus
				500 Hz condition				
ASSR TFP passive	0.16	0.30	0.06	0.55‡	0.44	−0.03	0.44	−0.24
N1 TFP passive	0.10	0.24	−0.14	0.07	−0.25	−0.52‡	0.04	0.14
ASSR TFP ratio	−0.30	0.27	0.12	0.32	0.14	0.14	0.14	0.13
N1 TFP diff.	−0.28	0.06	0.05	0.02	−0.08	−0.26	0.10	0.13
				5 kHz condition				
ASSR TFP passive	0.25	0.11	−0.19	−0.01	0.24	0.19	−0.40	0.05
N1 TFP passive	0.18	0.32	0.14	−0.04	0.52	0.19	−0.62‡	0.57
ASSR TFP ratio	−0.13	0.02	−0.20	−0.04	0.43	0.23	−0.41	0.27
N1 TFP diff.	−0.07	−0.10	−0.18	−0.06	−0.46	−0.11	0.46	−0.43

*Tinnitus and control subjects combined.
†Left and right ears combined.
‡$P < 0.05$.

would have experienced their 500 Hz probes at about 65 dB SL when matching to a 1 kHz 65 dB SL standard), a tendency for subjects to find 5 kHz 40 Hz AM sounds perceptually more salient than 500 Hz 40 Hz AM sounds, the presence of threshold shifts at 5 kHz in groups tested at this frequency, and some degree of unreported hyperacusis for a 5 kHz sound in the 5 kHz groups (which would have reduced probe SPL when matching a 65 dB SPL 2 kHz standard).

To assess whether probe intensity affected the brain responses, probe SPL was correlated with ASSR and N1 amplitude on passive blocks in the absence of attended performance and with effects of attention observed for these two responses. A correlation between probe level and ASSR amplitude was found on passive blocks in the 500 Hz group ($r(31) = 0.55$, $P = 0.001$; Table 2), indicating that louder 500 Hz probe stimuli evoked large ASSR responses in this group on passive trials. Probe intensity did not correlate significantly with ASSR responses evoked by 5 kHz probes or with N1 evoked by probes of either frequency on passive blocks. We also correlated probe intensity with effects of attention on ASSR and N1 amplitude collapsing the tinnitus and control groups within the 500 Hz and 5 kHz conditions. There was a weak tendency for stronger probe stimuli to be associated with larger effects of attention on ASSR amplitude in the 500 Hz groups ($r = 0.32$, $P < 0.07$), but no correlations between probe intensity and ASSR and N1 attention effects reached significance in the 500 Hz and 5 kHz conditions (see Table 2).

3.3.4. Tinnitus Characteristics. The tinnitus likeness matches obtained in the Tinn500 Hz and Tinn5 kHz groups are shown in Figure 1(b) where a likeness rating of 40 indicates a sound that is beginning to resemble tinnitus [12]. In each group the likeness matches given for 500 Hz sounds were well below the tinnitus spectrum and those for 5 kHz sounds well within it (effect of sound frequency $F(1, 26) = 58.74$, $P < 0.001$) with no difference observed between the likeness matches of the groups at either frequency. Tinnitus loudness was assessed by a Borg CR100 scale (range zero to 100) and by loudness matches obtained using a 1 kHz tone (after Roberts et al. 2008) and tinnitus handicap by the THQ (total score range zero to 100). Loudness matches given by Tinn5 kHz group were higher at 1 kHz (mean = 53.9 dB SPL, see Table 1) than those of Tinn500 Hz group (M = 36.7 dB SPL, $t(26) = 2.61$, $P = 0.014$), although when all matching frequencies were considered the groups did not differ from one another ($F(1, 26) = 1.13$, $P > 0.71$, Figure 1(c)). Loudness ratings on the BorgCR100 scale were nonsignificantly higher in the Tinn5 kHz group ($P = 0.16$) while THQ scores were significantly worse in this group compared to the Tinn500 Hz group ($t(26) = 2.14$, $P = 0.042$). To assess whether these results suggesting a stronger tinnitus in the Tinn5 kHz group may have influenced the attention effects, pairwise correlations were calculated between tinnitus loudness matches at 1 kHz, BorgCR100 ratings, and the THQ, on one hand, and ASSR and N1 attention effects, on the other hand. The resulting correlations were directionally inconsistent and did not reach significance either in the Tinn500HZ and Tinn5 kHz groups considered separately (see Table 2) or when the two groups were combined into one sample. When passive trials only were considered, N1 TFP correlated negatively with the BorgCR100 loudness in the Tinn500 Hz group and with the THQ score in the Tinn5 kHz group reflecting lower TFP for a more disturbing tinnitus (Table 2). When the tinnitus groups were collapsed together, correlations involving tinnitus loudness measures and brain responses on passive trials were near zero and not significant. The duration of tinnitus was similar in the Tinn500 Hz and Tinn5 kHz groups (M = 12.5 and 11.7 years, resp., Table 1) and did not correlate significantly with the two brain responses in either group (Table 2) or when the two groups were combined.

4. Discussion

We previously reported that the amplitude of the ASSR (localizing to cortical sources in A1) and the N1 transient

response (localizing to cortical sources in A2) was not modulated by top-down attention in tinnitus sufferers when the probe frequency was 5 kHz, a frequency known to be in the region in which tinnitus sufferers experience their tinnitus [6]. Conversely, age and hearing-threshold matched controls successfully modulated the amplitude of both responses [6] in accordance with prior evidence showing the responses to be sensitive to attention in normal hearing subjects [7, 8, 20]. It was suggested that tinnitus-related neural activity in central auditory pathways may have prevented modulation of the two responses by attention in the tinnitus sufferers. In the current experiment we tested this possibility by determining whether attention modulates these brain responses normally when evoked by a 500 Hz sound in tinnitus sufferers, which is a sound well below the TFR where tinnitus-related neural activity is believed to occur. The procedure used to assess modulation by attention was the same for the two groups, and the 500 Hz and 5 kHz datasets were combined into a single analysis which also included the long-latency auditory evoked potentials N2 and SR. We found that top-down attention modulated ASSR amplitude normally in tinnitus and control subjects probed with 500 Hz sounds and for control subjects probed with a 5 kHz sound, but not for tinnitus subjects probed with a 5 kHz sound. N1 amplitude was modulated by attention for control groups tested at each probe frequency, but modulation of N1 amplitude by attention failed for tinnitus groups tested at both frequencies. The amplitude of N2 and SR responses was modulated by attention in all groups. We discuss how attention may work in tinnitus sufferers compared to normal hearing individuals and consider how differences between these groups may be expressed in ASSR and N1 amplitude in the absence of attended performance.

4.1. Auditory Attention in Normal Hearing and in Tinnitus. Several lines of evidence have suggested that mechanisms that support auditory attention are persistently aroused in tinnitus [2]. One approach has been to compare the performance of subjects with chronic tinnitus with that of control subjects matched for age and verbal intelligence on cognitive tasks that require divided attention and access to memory. The rationale has been that obligatory attention to the tinnitus percept may deplete the cognitive resources needed to perform such tasks. Following this approach it has been shown that, while subjects with tinnitus perform as well as controls on tasks such as simple word naming, they do not perform as well on more complex tasks requiring retention of words in working memory over a series of sentences [22] or on Stroop tasks that divide attention between word naming and color naming [23]. The performance deficits observed in the tinnitus groups in these studies remained intact when measures of anxiety, depression, and hearing level were regressed out by covariate analyses. A more direct approach was followed by Cuny et al. [24]. In an initial demonstration based on research by Schröger [25], Cuny et al. presented normal hearing subjects with S1 stimuli in one ear that were to be ignored while they categorized S2 stimuli presented to the other (attended) ear. Performance on the S2 task was disrupted by infrequent deviant S1 stimuli, which appeared to draw attention away from the S2 task presented to the other ear. Cuny et al. subsequently found that when this task was presented to subjects with unilateral tinnitus, the interfering effect of deviant S1 stimuli was diminished when the S2 task was presented to the tinnitus ear compared to the reverse arrangement. It was suggested that persistent top-down auditory attention was directed to the tinnitus ear, such that deviant S1 stimuli presented to the nontinnitus ear could not draw attention away from it [24]. These results are in agreement with functional imaging studies of tinnitus [26, 27] which have reported increased activity in A1 and in auditory association areas that are modulated by attention when normal hearing subjects perform auditory detection tasks [2].

The presence of tinnitus did not impair behavioral performance during auditory discrimination under the conditions of our test, likely because there was no competing task requirement and most of the targets presented on the discrimination task were easy to detect. However, while ASSR and N1 responses known to be attention sensitive were modulated normally by attention in our control groups, modulation of these responses by attention was modified in tinnitus subjects. The pattern of impairment we observed could reflect differences in the functional organization of A1 and A2 and aberrant neural activity occurring in these regions in tinnitus sufferers. Unlike ASSR sources in A1 that show a frequency (tonotopic) organization in the region of Heschl's gyrus, N1 sources localize to lateral aspects of the superior temporal gyrus [18], are weakly or not tonotopic [28], and appear to reflect contributions arising from several cortical areas that comprise A2. A2 regions exhibit a heterogeneous cytoarchitectonic structure [29, 30] in which layer II/III pyramidal neurons receive inputs from diverse regions of the brain and in turn form intrinsic contacts that are more distal than in A1 where links are made in more localized modules [31]. Frequency representations which are prominent in A1 are virtually absent in A2, which appears to be specialized for processing of multidimensional auditory objects and for conveying perceptual information to higher cortical structures [30, 32, 33]. Hence it is possible that neural changes related to tinnitus (such as reduced intracortical inhibition [34], increased spontaneous activity [34, 35], and increased synchronous firing [34]) occurring in tonotopic regions of A1 may have diffusely activated A2, impairing modulation of N1 responses at both probe frequencies in tinnitus subjects. However, because A1 regions coding 500 Hz sounds are below the frequency region of A1 where tinnitus-related activity is presumed to occur, attentional modulation of the ASSR was expressed normally when tinnitus subjects were probed with this sound frequency. This interpretation is consistent with evidence from animal [1, 36] and human [37] studies which suggests that aberrant neural activity occurring in frequency regions of A1 affected by hearing impairment contributes to tinnitus percepts. It can also be aligned with previous results [38] showing that the mismatch negativity (a brain response initiated in A1 by bottom-up auditory attention, [39]) was increased in individuals with tinnitus when evoked by unexpected frequency deviants adjacent to

the audiometric edge but not one octave below it. Overall it appears that persistent tinnitus-related activity occurring in the frequency region of A1 affected by hearing loss may impair modulation of the ASSR by top-down attention in this frequency region in tinnitus, but bottom-up disparities may still evoke larger responses near the lesion edge where cortical reorganization may be present [36].

Notwithstanding prior evidence for persistent auditory attention in tinnitus [24], this interpretation suggests that mechanisms of top-down auditory attention functioned normally in tinnitus sufferers under the conditions of our test, but their expression was modified by the presence of tinnitus-related neural activity occurring in central auditory pathways. Other findings of the study can be aligned with this interpretation. Subjects in the Tinn5 kHz and Cont5 kHz groups received an additional six sessions of training on the auditory detection task in the earlier study of Roberts et al. [6]. ASSR amplitude increased over training sessions in the tinnitus subjects but not in their matched controls [6] nor in previous studies using subjects with normal hearing [11, 20], possibly reflecting reduced lateral inhibition in the tinnitus subjects [1]. As training progressed, ASSR amplitude began to modulate on active blocks compared to the passive baseline in tinnitus subjects revealing an effect of attention on this response, although this modulation subsequently declined and was weak compared to that seen in controls (N1 did not modulate with attention during any session of training in the tinnitus subjects). New analyses reported in the present paper have gone further to show that the long-latency responses N2 and SR (which reach their negative maxima at central electrodes) were modulated between active and passive trials in our tinnitus groups as well as by control subjects. It is possible that these responses reflect communication between auditory regions and global networks in frontoparietal cortex that are involved in memory processing and response preparation [40]; moreover, the performance deficits cited above in tinnitus [22, 23] may derive in part from competition for resources in these pathways. In this respect we note that, while N2 and SR responses were modulated by attention in our tinnitus subjects, there was a tendency toward stronger effects in the control groups at both probe frequencies.

4.2. Group Differences in the Absence of Attention. Neuromodulatory systems in the basal forebrain and midbrain tegmentum are widely believed to be activated by tasks requiring attention and serve to make neurons more sensitive to their afferent input [2]. On this basis, evidence for persistent auditory attention in tinnitus could be expected to modulate the amplitude of brain responses evoked by auditory stimuli under passive conditions where tinnitus sufferers would experience tinnitus but control subjects would not. In a previous study using 40 Hz AM stimuli similar to those used here but different groups of subjects [2], we found that ASSR amplitude was larger in a tinnitus group than in controls when the carrier frequency of the probe was 500 Hz ($P = 0.004$), but this difference was reversed in groups for whom the carrier frequency was 5 kHz ($P = 0.045$). Reduced ASSR amplitude at 5 kHz was attributed to tinnitus-related synchronous activity occurring in the TFR of the tinnitus subjects (a busy line effect). Additionally, N1 amplitude was larger in the tinnitus groups compared to controls at both probe frequencies ($P = 0.023$). These results were obtained during a continuous 20-minute baseline condition in which individuals in the tinnitus groups would have heard their tinnitus. To compare these findings with the current dataset, we performed paired t-tests contrasting the tinnitus and control groups on passive blocks for the ASSR measured as TFP and N1 amplitude measured at electrode Fz (as in the previous work). ASSR TFP tended to be smaller in tinnitus than control subjects at 5 kHz ($P = 0.26$) and N1 larger ($P = 0.18$) at this frequency in qualitative agreement with previous results, but no group differences in ASSR or N1 amplitude reached significance in the present dataset. Overall, current evidence suggests that ASSR amplitude is larger in tinnitus subjects than in controls, at least for sounds below the TFR [2, 37]. Results regarding N1 are less consistent [2] and may reflect differences among studies with regard to the conditions of testing, stimulus procedure, and other variables that have yet to be identified.

4.3. Limitations and Future Directions. Within each probe frequency, our tinnitus and control groups were well matched for tinnitus characteristics, age, hearing status, and stimulus levels. Group differences in the effects of attention on brain responses at each probe frequency could not be attributed to these variables which did not differ between tinnitus and control subjects. However, while our 5 kHz and 500 Hz groups were well matched for hearing function, age range, and years of tinnitus, subjects in the 500 Hz groups tended on average to be 10 years older and their THQ scores lower than subjects in the 5 kHz groups. The intensity of the probe stimuli also differed between the 500 Hz and 5 kz conditions, in part because of the presence of threshold shifts at 5 kHz in the Tinn5 kHz and Cont5 kHz groups. To assess whether differences in these variables may have influenced our results, we correlated each variable with the effects of attention on ASSR and N1 responses at each probe frequency, collapsing tinnitus and control subjects within each frequency to increase the likelihood of uncovering alternative explanations for the findings. None of the variables correlated significantly with the effects of attention on ASSR and N1 responses, at either probe frequency. Within the limits of this analysis we conclude that differences between tinnitus and control groups in the effect of attention on ASSR and N1 amplitude reflected the presence of tinnitus in the tinnitus subjects and not the other attributes or the conditions of testing. Although interactions among different stimuli could be a limiting factor, looking forward it could be informative to modify our stimulus procedure to allow examining effects of tinnitus on attention-sensitive responses when both probe frequencies are tested within the same subjects.

A further possible limitation to consider is the extent to which a given brain response reflects the operation of an attention mechanism rather than brain processes concerned with other cognitive or behavioral functions. Active trials in our procedure required not only the deployment of attention

but also the processing of target events using memory, the preparation of behavioral responses depending on target occurrence or nonoccurrence, and likely the anticipation of correctness feedback depending on outcome. As we have noted, auditory attention is known to increase ASSR amplitude when these additional factors are held constant [7], confirming the sensitivity of this response specifically to attention. Although the transient N1 response is widely believed to be sensitive to attention, as far as we are aware similar detailed analyses precluding contributions from other task features are surprisingly lacking this response. In the absence of such studies it is reasonable to assume that brain responses with short latencies are likely to reflect attention, assuming that on any attention task this process is deployed at trial onset.

Many individuals with tinnitus also experience some degree of hyperacusis expressed either by verbal reports of sensitivity to environmental sounds [41] or by loudness growth functions that are steeper than those observed in individuals with similar audiometric profiles [42]. Because we did not have a basis in the present study to distinguish between these two conditions, failure of attentional modulation could relate in principle either to the presence of tinnitus or hyperacusis or to both. It is not easy to disentangle these correlated factors in tinnitus research. However, the current findings are not easily explained in terms of altered perceptual responses to the probe stimuli in the tinnitus groups. ASSR and N1 responses might have been expected to reflect such differences under passive conditions, but the differences we observed between tinnitus and control groups were small and did not reach significance. Our practice of requiring subjects to adjust probe sound intensity to comfortable-level standard sounds presented in the frequency range of normal hearing may have attenuated effects attributable to hyperacusis in our tinnitus samples. It is also relevant that effects of attention on ASSR and N1 responses did not correlate with physical sound intensity within tinnitus and control subjects tested at 500 Hz or 5 kHz. Had perceptual responses to the probe stimuli affected attentional modulations, such correlations might have been expected but did not occur.

5. Conclusion

Previous studies have provided behavioral evidence of impaired performance on tasks involving control of attention in individuals with tinnitus compared to individuals without tinnitus. Our study extended the analysis to compare, between age and hearing-level matched tinnitus and control groups, the effect of attention on brain responses known to be sensitive to attention in normal hearing subjects. We focused in particular on the 40 Hz ASSR which localizes to sources in tonotopically organized primary auditory cortex (A1) and the N1 transient response which localizes to sources in nontonotopic secondary auditory cortex (A2). We found that, unlike in controls where all responses were modulated by attention, the presence of tinnitus impaired attentional modulation of the ASSR evoked by a 5 kHz but not a 500 Hz sound and the N1 evoked at both sound frequencies. We suggest that impairments of auditory attention are expressed preferentially in the 5 kHz region of tonotopically organized A1 where tinnitus-related neural activity is typically expected to occur and more diffusely in nontonotopic A2 where neuron response properties are more broadly tuned for spectrotemporal and multisensory integration.

Abbreviations

A1: Primary auditory cortex
A2: Secondary (nonprimary) auditory cortex
AM: Amplitude modulated
ANOVA: Analysis of variance
ASSR: Auditory steady-state response
DCN: Dorsal cochlear nucleus
EEG: Electroencephalogram
ITI: Intertrial interval
SPL: Sound pressure level
SL: Sensation level
SR: Auditory sustained response
TH: Threshold
THQ: Tinnitus Handicap Questionnaire
TFP: Total field power
TFR: Tinnitus frequency region.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Chapter 4: Top-down attention modifies a spectral estimate of phase shifts during resets of the auditory steady state response.

Paul, B. T., Bruce, I. C., Roberts, L. E. (Submitted). Top-down attention modifies a spectral estimate of phase shifts during resets of the auditory steady state response.

4.1 Preface

Findings in Chapter 3 suggested that top-down attention operates normally for tinnitus sufferers, but tinnitus-related activity in the TFR may have prevented the expression of attention in A1 and broadly in A2. The wider implication of this interpretation is that A1 neurons in the TFR are perhaps resistant to their inputs because they are constantly engaged in the synchronous hyperactivity that is associated with the presence of tinnitus. For this claim to be supported and the role of attention to be clarified within the deafferentation model, a direct test of the insensitivity of neurons in the TFR of A1 to input—such as from the bottom-up—needs to be conducted.

As discussed in Section 1.5.3, one procedure that would permit a view into how A1 neurons respond to bottom-up input is by evoking the ASSR reset response, which occurs when the ASSR is interrupted by a silent gap or noise burst and recovers over a 200 ms interval (Ross et al., 2005; Ross & Pantev, 2004). This response likely represents how cortical neural networks rebuild the representation of the AM envelope after it has been disrupted (Ross et al., 2012). One quality of the ASSR reset phenomenon that is not known is whether the

response dynamics are sensitive to top-down attention, which might be observable when comparing conditions of active and passive listening. It is important to understand this effect in control subjects before bringing it into use with tinnitus subjects, so that hypotheses can be formed regarding how tinnitus-related changes in A1 might affect normal modulation of the reset response. For that reason, subjects in Chapter 4 do not have tinnitus.

Accordingly, the experiment in Chapter 4 tested the hypothesis that ASSR resets evoked by silent gaps are sensitive to top-down attention. As will be seen, one challenge we faced was that noise in the electrophysiological measurement did not permit a valid analysis of phase for individual subjects, which is an important dynamic in the reset phenomenon. However we dealt with that issue by calculating changes in spectral centroid, a novel frequency spectrum analysis of EEG determining the frequency where most power is concentrated in the ASSR signal, in order to infer phase shifts. The centroid effects we observed were consistent with expected changes in phase found previously for ASSR resets (Ross et al., 2012).

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4.2 Abstract

We tested the hypothesis that top-down auditory attention modulates rapid interruptions (resets) of the 41-Hz auditory steady state response (ASSR), an evoked potential localizing to tonotopic primary auditory cortex (A1). While recording the electroencephalogram (EEG), 15 subjects heard 41 Hz amplitude modulated (AM) tone bursts containing brief silent gaps of short (~12 ms) or long (61 ms) duration that were known to evoke ASSR resets. In one condition participants indicated whether the sound had contained one or two long gaps (attention condition), while in another condition subjects did not respond and ignored sounds (passive condition). Each subject completed the procedure twice, once for a 400 Hz and once for a 5 kHz carrier frequency. To compare attended and passive conditions, we measured ASSR power and the spectral centroid, a novel frequency spectrum analysis of the steady-state EEG that reflects the frequency where most power is concentrated in the signal. The centroid analysis is used here to infer changes in ASSR phase. Before gap interruptions, ASSR power for both carrier frequencies was larger and centroids were closer to the 41 Hz AM rate during attended compared to passive conditions, consistent with known attention effects on the stable ASSR. After the gap, the centroid decreased from baseline toward a lower frequency (analogous to a phase lengthening) under active conditions, while centroid shifts under passive conditions trended upward but were less appreciable. ASSR power did not differ between attended and passive conditions during the reset period. These results suggest that attention is expressed on the phase but not amplitude of the ASSR reset response.

4.3 Introduction

In complex environments, listeners require auditory attention to track acoustic signals that may contain information about desired behavioral goals. One brain response known to be sensitive to attention is the 40-Hz auditory steady state response (ASSR), a scalp-recorded oscillatory potential that reaches its maximum amplitude when a carrier frequency is amplitude modulated (AM) at rates near 40 Hz. The cortical sources of ASSRs evoked by different carrier frequencies exhibit a coarse low-frequency-anterolateral to high-frequency-posteriomedial spatial organization in the region of A1when measured intracortically (Bidet-Caulet et al. 2007; Godey et al. 2001) or determined by electromagnetic source imaging (Gander et al., 2010a, Pantev et al., 1996; Wienbruch et al., 2006). This organization may reflect the summation of evoked fields across two tonotopic maps that have been found to share a low frequency border situated anterolaterally in the region of Heschl's gyrus (Kaas & Hackett, 2000; Langers et al., 2007).

Studies have shown that the amplitude of the ASSR increases when subjects are required to attend to targets in the AM signal in an active condition, compared to conditions in which subjects ignore the targets and do not respond (Gander et al., 2010b; Paul et al., 2014; Ross et al., 2004). An interpretation of these findings suggests that top-down attention enhances neural responsivity in the auditory cortex, such that a larger population of neurons tuned to the carrier frequency of the AM sound responded on active blocks. The mechanism of

attention-related effects is not presently known, but could reflect activation of neuromodulatory systems (such as cholinergic projections from the basal forebrain) that are known to increase the sensitivity of auditory neurons to their afferent input under conditions of attention (Sarter et al., 2005, 2009; Zhang et al., 2016).

The ASSR can be briefly disrupted by inserting a gap in the AM stimulus, creating a response known as the ASSR reset. Immediately following a gap, the ASSR rapidly uncouples its phase from the AM rhythm and loses amplitude. After the gap ends and the tone resumes, the ASSR synchronizes to the reinstated AM rhythm and recovers its phase and amplitude over ~200 ms (Makeig & Galambos, 1989; Ross & Pantev, 2004). ASSR reset responses are believed to reflect how auditory cortical networks rebuild sensory representations after a change occurs within the stimulus (Ross et al., 2012). By studying the phase and amplitude recovery dynamics of the ASSR within the 200 ms integration time window, studies have inferred limits of temporal integration (Ross & Pantev, 2004), mechanisms of sound localization (Ross, 2008), and effects of noise on detecting stimulus change (Ross et al., 2012; Ross & Fujioka, 2016) in A1. Despite these findings, the effects of top-down attention on the ASSR reset response have not been directly investigated.

In the present study we examined how the amplitude and phase of the ASSR are modified by top-down attention, and how these attributes of the responses are affected by gaps of unpredictable duration inserted into the ASSR

stimulus. In one condition we required subjects to actively listen and respond to gaps in AM stimuli and in another condition had subjects passively listen and not respond. ASSR resets evoked in two carrier frequencies (400 Hz and 5 kHz) were tested in separate conditions to determine if attention-related effects were frequency specific. We found that before gap-evoked resets when the ASSR was stable, ASSR power at both frequencies was larger under the active listening condition, consistent with known effects of top-down attention on ASSR amplitude. However after gap-evoked resets when ASSRs were recovering, attention deployed under the conditions of our test modified a spectral measure of phase dynamics that was not explained by changes in power, and power during the reset interval was not different between attended and passive listening conditions. Results are discussed in terms of known task-related changes observed in A1 and attention-related neuromodulatory systems that assist with stimulus representations in auditory cortex.

4.4 Methods

4.4.1 Participants

Fifteen participants (3 male, 1 left handed) aged between 18–30 years (M = 20.21, SD = 3.04) from the McMaster University undergraduate psychology subject pool enrolled in the study. We obtained audiometric thresholds in all participants up to 8 kHz using the pulsed-tone method on a GSI 61 audiometer (Telephonics TD-39 296 D200 supra-aural headphones). Of the 15 subjects, 11 had thresholds $\leq$ 25 dB HL to 8 kHz. Of the four remaining subjects, two had

thresholds of 40 dB HL at 6 and 8 kHz, one had a threshold of 30 dB HL at 3 kHz, and a final subject had a 30 dB HL threshold at 500 to 750 Hz. At all other frequencies tested in these individuals, thresholds were < 25 dB HL. Because we used a procedure that equated stimulus loudness across probe frequencies and individuals (see below) we did not exclude these four subjects from the total sample. No subjects reported a history of head trauma or hearing problems nor reported the use of medication during the time of the study. All procedures complied with the Declaration of Helsinki and were approved by the Research Ethics board at McMaster University. Subjects provided informed consent and received course credit for participation.

4.4.2 Stimuli and Materials

During all procedures participants sat in a chair distanced 1.4m from a computer monitor in a sound attenuated (ambient noise level 16 dBA SPL) and electrically-shielded booth. Etymotic Research ER-2 transducers were inserted in each ear. Sounds were generated by a Tucker-Davis RP2.1 digital signal real-time processor.

All stimuli were 40.96 Hz (herein referred to as 41 Hz) AM tone bursts (100% AM depth) lasting 2329.25 ms in duration, with carrier frequencies of either 400 Hz or 5 kHz (Figure 4.1a). During the course of the experiment participants received stimulation to the left ear only, since the ASSR is evoked in the hemisphere contralateral to the stimulated ear and has been shown to be larger in the right hemisphere (Ross et al., 2005b). Before the experiment began each

participant matched the loudness of the 400 Hz and 5 kHz probes to a 1 kHz pure tone presented at 65 dB SL. This procedure was designed to equate the perceived loudness of each stimulus to a common reference frequency (1 kHz) where hearing thresholds were ≤15 dB HL for all subjects. Mean stimulus sound pressure levels (SPLs) established by this procedure were 74.2 dB SPL (SD = 9.91) for the 400 Hz carrier and 74.1 dB SPL (SD = 8.33) for the 5 kHz carrier.

Each tone burst contained three silent gaps known to evoke ASSR resets (Ross and Pantev, 2004). Gap durations were either 12.2 ms (short) or 61 ms (long), whose onset and offset occurred at the zero crossings of the amplitude modulation cycle such that the AM rate following each gap was shifted by 180 degrees in phase from the pre-gap rate. We chose these gap lengths because durations that are integer ratios of the AM period (e.g., 24.4 or 48.8 ms) may produce weaker ASSR resets, since their onset and offset occur at the stimulation rate (Ross and Pantev, 2004). It should be noted that these gap lengths are above the normal threshold for gap detection in the ASSR which is typically around three ms (Ross & Pantev, 2004). For the purposes of our classification task described below, we chose gap durations higher than the threshold so that each gap instance had a high likelihood of being perceptually detected. Gaps commenced every 550 or 575 ms (randomly chosen) following either the probe onset or the preceding gap. Intervals greater than 500 ms permit an adequate amount of time for the ASSR to re-establish after desynchronization (Ross & Pantev, 2004). Tone bursts contained either one long gap with two short gaps, or

two long gaps with one short gap. The type of gap (long or short) and location of gap within the tone burst were unpredictable and randomized for each tone burst presentation.

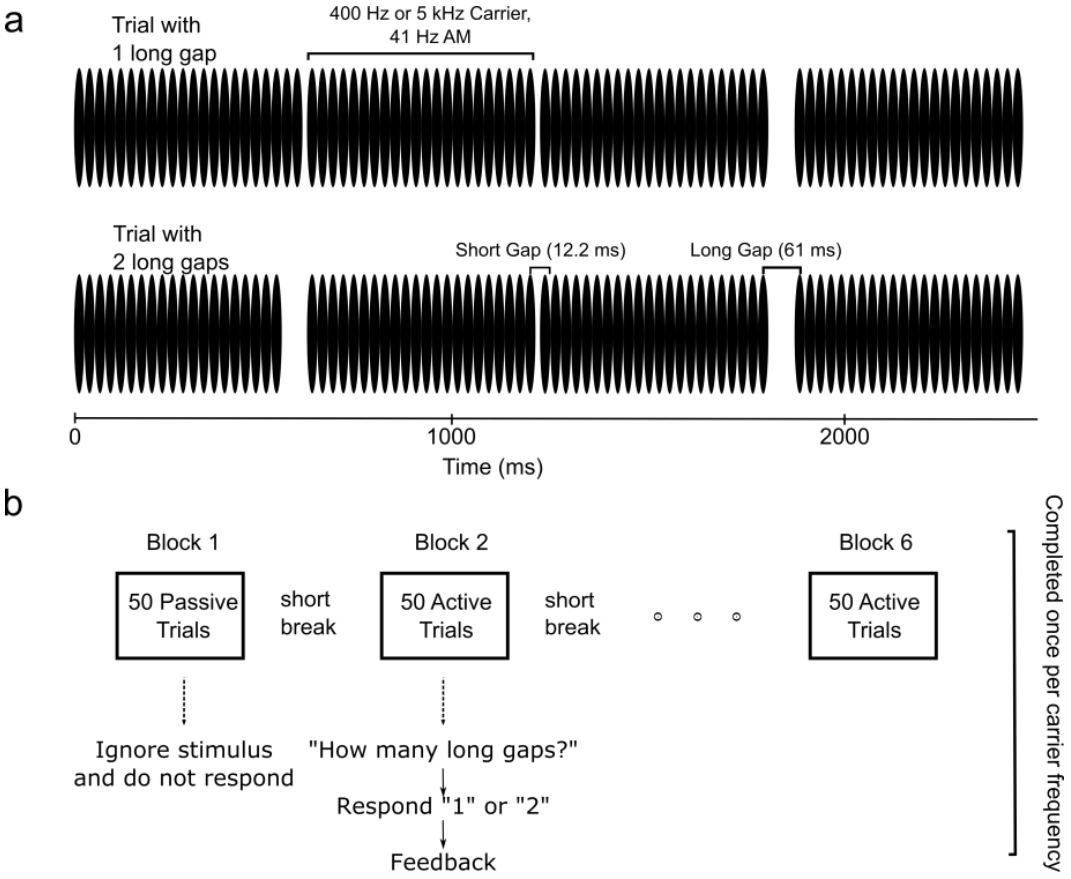

Figure 4.1. Stimulus and task design. a) A pure tone of either 400 Hz or 5 kHz carrier frequency was AM by 41 Hz, lasting nearly 2400 ms in duration and presented to the left ear only. After loudness matching procedures, stimuli were near 74 dB SPL. Three gaps were randomly interspersed in the tone with at least 500 ms between onset and offset of the probe. Gaps were either short or long, and trials always contained either one or two longer gaps. b) Subjects completed blocks of 50 trials in which they were to passively listen to the sounds, or respond to the number of long gaps present in the signal. A short break unfolded between each block. For each carrier frequency, there were 6 trial blocks that alternated in active and passive conditions.

4.4.3 Procedure

Trials were grouped into active and passive blocks consisting of 50 trials each (Figure 4.1b). During active blocks subjects performed a classification task in which they listened to stimuli and indicated after trial offset whether they heard one or two long gaps in the stimulus. For each active trial, the text "Listen" appeared on the computer screen before and during tone burst presentation. After sound offset participants were prompted by text on the computer screen to classify each stimulus by clicking the left computer mouse button if they heard one long gap or the right mouse button if they heard two long gaps. If participants responded correctly, a green box highlighting the word "Hit" appeared on the screen; if participants responded incorrectly, a red box with the word "Miss" appeared. A variable interval between 1400 and 1600 ms followed the feedback cue. The inter-trial interval (stimulus offset to onset) varied between 1900 and 2100 ms depending on the subject's reaction time. During passive trial blocks, the text "Ignore stimulus and stop responding" appeared on the screen throughout the duration of the block. The experimenter instructed each subject to keep their eyes open at this time but not respond to the stimuli. The passive inter-trial interval varied randomly between 1900-2100 ms, comparable to active blocks. For each carrier frequency, blocks alternated between active and passive trials until a total of six blocks had been presented (Gander et al., 2010b; Paul et al., 2014). The first carrier frequency for each session was chosen at random.

Before the trial blocks commenced, the experimenter described the stimulus and task to the participants. Samples of the stimuli with one or two long

gaps coupled to a visual depiction (similar to Figure 4.1a) iterated repeatedly. Subjects could listen to each type as many times needed to understand the task. After the experimenter received verbal confirmation that subjects understood the task, the main experiment began.

4.4.4 Electrophysiological Recording and Spatial Filtering

The EEG was sampled by a 128-channel Biosemi ActiveTwo amplifier (Cortech Solutions, Wilmington, NC) at 2048 Hz and was referenced online to the vertex electrode. Before recording, electrode positions were digitized for each participant (Polhemus Fastrak). In BESA (version 6.0; MEGIS Software GmbH, Grafelfing, Germany) short and long gaps for active and passive trials were epoched from −200 ms before and 300 ms after gap onset. Trials with ocular, muscle or other artifacts in the 25–57 Hz band exceeding a threshold of ±75 μV were verified by visual inspection and were removed. On average ~80% of trials were retained. Long and short gap trials for each subject were averaged separately for active and passive conditions for both carrier frequencies and attention conditions.

We used inverse modeling in BESA to determine dipole source locations for the ASSR. We computed sources in both hemispheres but for the analysis only considered sources localizing to the right hemisphere, as ASSR generators are strongest contralateral to the ear of stimulation (Ross et al., 2005b). Source models were computed individually for each subject using the 41 Hz ASSR from the passive condition during the 200 ms pre-gap ASSR baseline, with the goal of estimating the cortical source during the most stable period of stimulation and

without interference from attention-related activity that may add variance to the source model. Pre-gap periods before all gap types were collapsed into one average separately for each person and carrier frequency, and then bandpass filtered between 39 and 43 Hz. Dipole source locations were identified in a two-step procedure. In step one, two principal components were calculated and added to the solution. The first component accounted for most field variance (>80% on average) and likely represented ASSR sources while the second component (< 20%) represented primarily noise and non-ASSR variance. In step two, we used the second principal component (non-ASSR component) to spatially filter noise while a dipole model was fit to the remaining field variance.

The coordinates of the dipole model were computed first by seeding two unconstrained regional sources (each consisting of three orthogonal dipoles) in left and right auditory cortex, and their 3D locations were determined by inverse modeling. The sources were then reoriented so that one dipole accounted for the maximum field variance (this vector typically oriented toward channel FCz). Residual variance of all model fits averaged over all subjects amounted to 12.8% (SD = 11.3%) for the 400 Hz solutions and 14.7% (SD = 15.6%) for the 5 kHz solutions. The dipole in the right hemisphere localized in the region of Heschl's gyrus for each subject and carrier frequency. The grand averaged source waveforms and their 3D locations are presented in Figure 4.2. The coordinates and orientations for each individual's right hemisphere source were used as a linear spatial filter through which unfiltered averages for both active and passive

stimulus epochs were passed. Thus, our analysis focused on spatially-filtered potentials and not dipole moments.

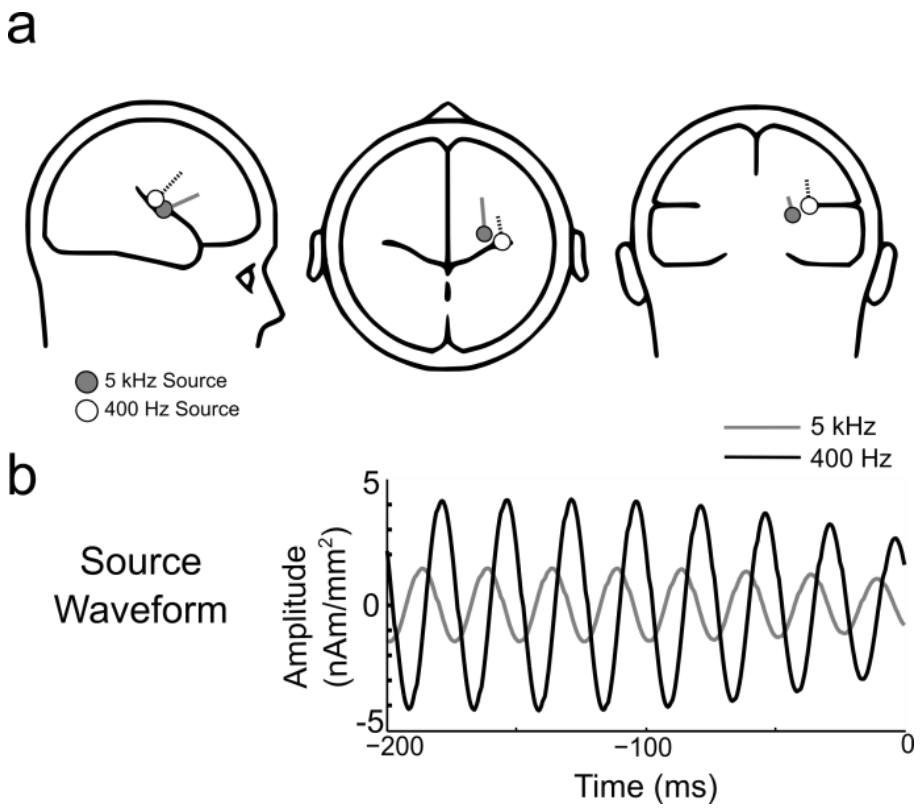

Figure 4.2. Source locations and waveforms a) Source locations for the 5 kHz and 400 Hz probe tones computed from the grand average waveform of the 200 ms pre-gap interval for the passive condition only in BESA. The 5 kHz source seated more medially than the 400 Hz source, consistent with previous reports (Brugge et al., 2009; Pantev et al., 1996; Wienbruch et al., 2006). b) An example source waveform of the -200 ms pre-gap interval, showing a larger dipole moment for the 400 Hz probe than at 5 kHz, also consistent with known effects of carrier frequency on the ASSR (Roberts et al., 2015; Paul et al., 2014).

4.4.5 Signal Processing

All signal processing was performed in MATLAB (The Mathworks, Inc., Natick, MA) using custom code and functions from the Digital Signal Processing toolbox. As discussed, gaps embedded in the AM tone are expected to evoke ASSR resets

characterized by an immediate drop in power and phase shift away from the 41 Hz modulation frequency. Once the tone resumes, the ASSR rebuilds over a 200 ms interval in which the ASSR phase realigns and amplitude recovers to its pre-gap value (Ross & Pantev, 2004). In past studies (Ross & Pantev, 2004; Ross et al., 2012) the reset interval was analyzed by computing the Hilbert transform over the signal to obtain the instantaneous amplitude and phase. In initial analyses of the present ASSR data, noise in the signal precluded a valid analysis of the instantaneous phase on averaged waveforms of individual subjects. It is noteworthy that in all previous studies ASSR resets were measured by the magnetoencephalogram (MEG), which has a greater signal-to noise ratio than EEG.

In order to estimate phase shifts of ASSRs on individual averages, we instead examined changes in the spectral frequency of the ASSR that occurred before and after gaps. The power spectrum of the ASSR is expected to peak at 41 Hz where phase locking to the 41 Hz stimulus is maximal. Disruptions of the ASSR are known to produce a temporary phase shift in the steady-state neural signal (Ross & Pantev, 2004; Ross, 2008; Ross et al., 2012), which would shift power in the spectrum to higher or lower frequencies during the reset period. This follows the principle that the instantaneous frequency is the temporal derivative of the instantaneous phase (Picinbono, 1997). As an example, Figure 4.3a shows the power spectrum of the ASSR for each individual (thin lines) and the grand averaged ASSR (thick line) during a 200 ms epoch pre- and post-gap, for the 5

kHz probe when subjects were attending to stimuli. The power spectrum expectedly peaked at 41 Hz for most subjects during the pre-gap baseline (left panel) while the AM tone was present. During the post-gap interval following the gap (right panel), power expectedly decreased, and the spectrum shifted away from the 41 Hz peak. However it is clear during the post-gap interval that for each individual there are multiple peaks in the spectrum, making it difficult to determine which peak represents the shift away from 41 Hz pre-gap interval. Thus, phase shifts cannot be easily determined by measuring changes in the peak of the power spectrum in the ASSR reset phenomenon.

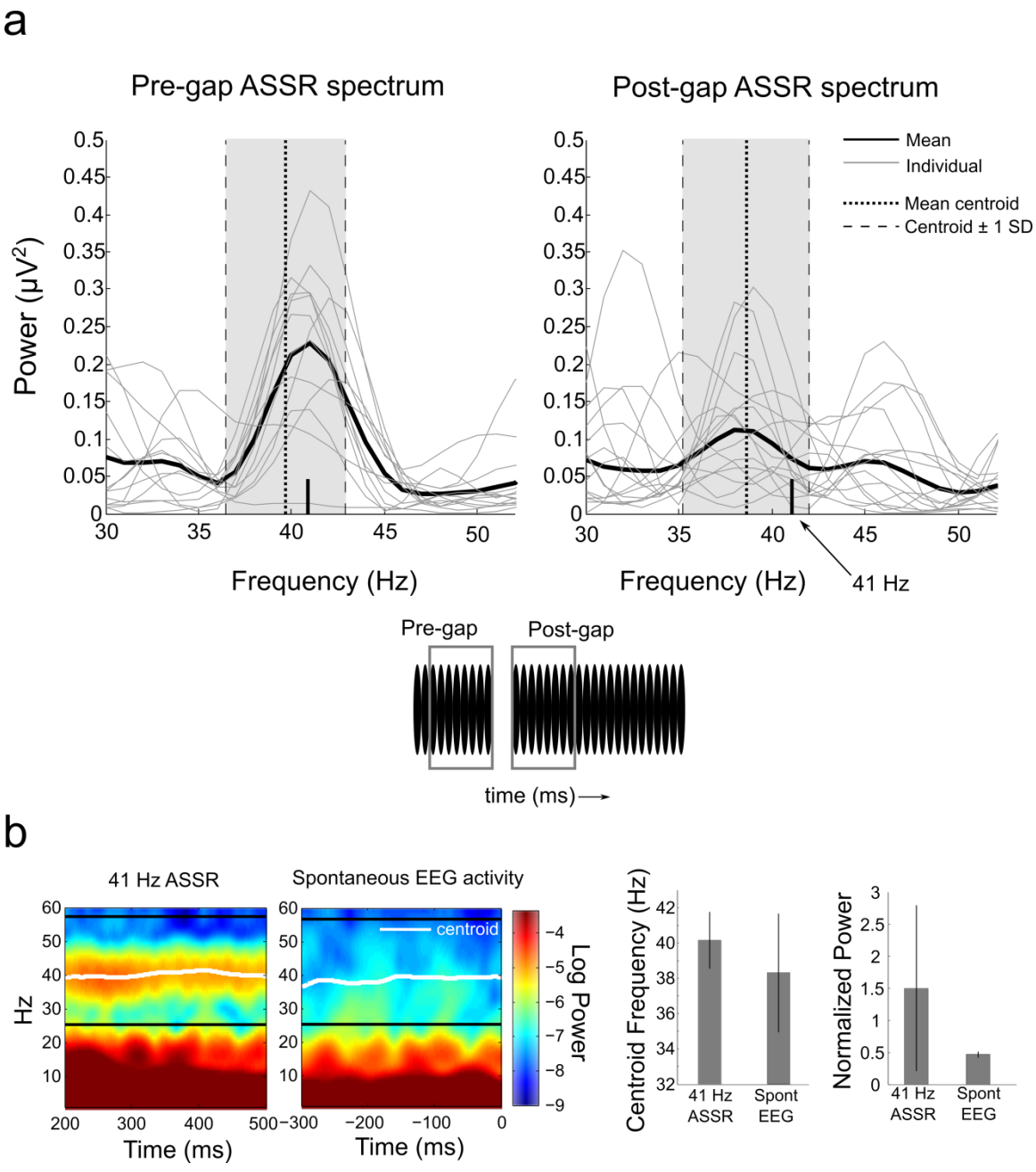

Figure 4.3. Comparison of spectral power and centroid measured for the ASSR. a) Power spectra of the ASSR during the 200 ms pre-gap period (stable ASSR) and the 200 ms post-gap period (ASSR reset) for the active condition during the 5 kHz probe tone. The thick black line represents the grand average mean, with

individual subjects depicted as gray traces. The centroid frequency for each period is shown as a vertical dotted line, with 1 standard deviation bounded by vertical dashed lines and shaded in between. b) Grand-average time frequency representations of the ASSR spectrum and spontaneous (resting-state) EEG at and before the 400 Hz, 41 Hz AM tone in the passive condition for all subjects are shown in the left two panels. Black lines represent the bandwidth of the filter over which the centroid and wideband power were calculated. The white line depicts the centroid trace. Bar graphs in the right two panels depict the mean centroid and normalized power values for the ASSR and spontaneous EEG spectrum. Error bars are 1 SD.

A solution to this problem is to measure the *spectral centroid*, the mean frequency weighted by the power at each frequency, which reflects the frequency around which most power of the signal spectrum is distributed. To our knowledge the centroid has not been previously used to measure ASSRs, and only one other study used the centroid to characterize resting-state EEG (Ali et al., 2014). An example of the ASSR centroid is overlaid on the time-frequency spectrum in Figure 4.3b, leftmost pane, which is the grand average power spectral density (PSD) spectrum of the 400 Hz ASSR for all subjects in the passive listening condition, between 200-500 ms after the onset of the probe. As can be seen power is concentrated at and around 41 Hz (the stimulation rate), while ongoing sustained activity is limited to the frequency band below 25 Hz. For this reason the centroid is calculated across the 25 to 57 Hz band (black lines on spectra in Figure 4.3b), the widest symmetrical bandwidth around 41 Hz that excludes contribution from sub-25 Hz sustained activity. The resulting centroid, shown as a white trace overlaid on the power spectrum, localizes close to 41 Hz, but noticeably the centroid sits lower than the peak frequency. This minor low-

frequency bias results from the 1/*f* power spectrum of the EEG known to be present in the absence of stimulation (Ward, 2002). To demonstrate, the centroid calculated in the 25–57 Hz band of the grand-average PSD plot of the EEG during the 300 ms interval before stimulus onset (when no AM tone was present; herein referred to as the spontaneous EEG) for all subjects sits around 38-Hz (3 Hz below the filter center) because of the 1/f influence (Figure 4.3b, rightmost spectrogram). Comparisons of the mean and standard deviation of the centroid and power for the ASSR and spontaneous EEG spectra are shown in the rightmost bar graphs, where it can be seen that the centroid is closer and less variable around the stimulation rate when the ASSR is present, but the centroid for the spontaneous EEG is lower and more variable (Figure 4.3b, left bar graph). Power is expectedly larger and more variable in the presence of 41 Hz stimulation, compared to the spontaneous EEG which has less power and variance (Figure 4.3b, right bar graph).

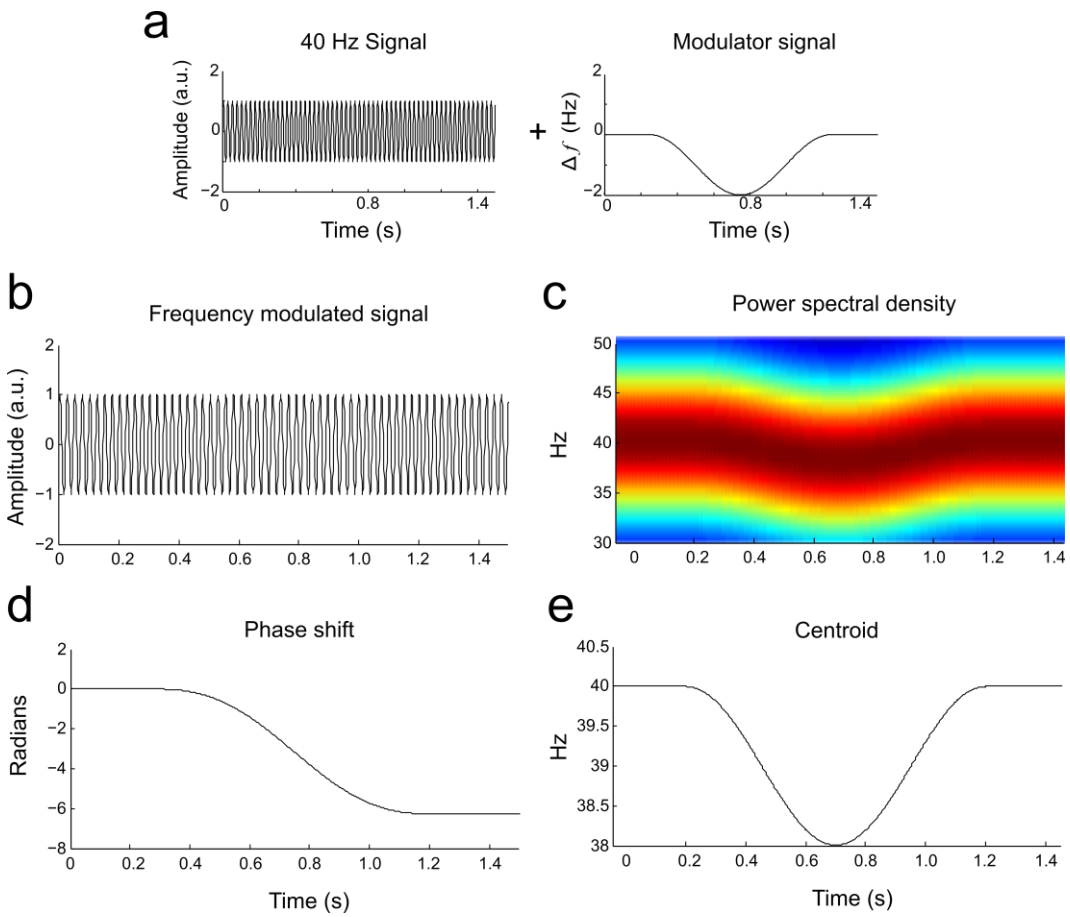

Figure 4.4. Relationship of a phase shift to the spectral centroid. a) The frequency of a 40 Hz sinusoid (left) is modulated by a signal (right) such that a transient 2 Hz downward frequency shift is produced. b) The frequency modulated signal resulting from (a). c) The power spectral density (PSD) of the frequency modulated signal as a function of time (color corresponds to power of arbitrary units). A downward frequency shift in the signal spectrum can be observed. d) The instantaneous phase of the Hilbert transform was taken over the 40 Hz signal in (a) and the frequency modulated signal in (b), and the phase angles as a function of time were subtracted and plotted. By the end of the frequency modulation, the phase of the frequency modulated signal is delayed by 6 radians away from the original 40 Hz frequency. e) The centroid frequency, computed as average frequency weighted by power of the PSD, shows a transient 2 Hz downward shift. Thus, the temporary drop in the centroid corresponds to the phase shift in d).

These principles may be used to infer phase shifts of the ASSR after gap perturbation. A demonstration describing how the centroid relates to phase shifts

within a simulated signal is provided in Figure 4.4. A 40 Hz signal shown in Figure 4.4a is frequency modulated by a Hamming window such that a transient 2 Hz downward frequency shift is produced (the composite of these signals is presented in Figure 4.4b), which may represent a likely scenario involving the reset of a 40 Hz ASSR. It is clear in the PSD plot in Figure 4.4c that a transient 2 Hz shift of the signal is present. In past reports (e.g., Ross et al., 2012) shifts in phase of the ASSR signal after a gap-evoked reset were evaluated by computing the instantaneous phase of the Hilbert transform of both an unmodulated 40 Hz reference signal and the resetting ASSR signal. Subtracting the unwrapped instantaneous phase angles of both signals reveals a phase shift of the ASSR reset response away from the 40 Hz signal, here shown in Figure 4.4d as a phase delay of 6 radians by the end of the frequency modulation. The spectral centroid calculated over the power spectral density in 4.4c depicts the transient 2 Hz downward frequency shift of the signal as a consequence of the frequency modulation. Thus, the phase delay corresponds to a transient downward shift in the spectral centroid.

Returning to the ASSR reset example in Figure 4.3, prior to the gap (Figure 4.3a, left panel) the centroid calculated over the range 25–57 Hz (dotted line, Figure 4.3a) was situated at ~40 Hz, close to 41 Hz where power peaked for most subjects. During regeneration of the ASSR following a gap, spectral power distributed to other frequencies thus pulling the centroid downward (Figure 4.3a right panel) to ~38 Hz, notwithstanding that 41 Hz was the AM rate of the

stimulus. A transient downward shift can be thought of as a phase lengthening of the ASSR during the recovery period. Because the centroid is somewhat influenced by background EEG and noise in the signal, it is important to concurrently measure overall power between 25–57 Hz to determine if changes in the centroid reflect a) a shift in the frequency content of the ASSR without a substantial change in its power or b) an increase or decrease in the power of the ASSR relative to the background EEG. These comparisons are provided in the analysis below.

For all conditions the centroid was measured as a time series (such as in Figure 4.3b, left panels) derived from a PSD analysis. After filtering the signal from 25–57 Hz, the PSD was estimated for all signals using a short-time Fourier transform (STFT) with a 250-sample (5 ASSR pulses) Tukey window function. This window function applies a cosine ramp to the first and last 35% of samples of each window to reduce spectral leakage, but for the middle 30% the function is flat so that temporal resolution is preserved. Each window of the STFT overlapped by 249 samples. Through the Goertzel algorithm (Goertzel, 1958) we defined frequency bins in 1 Hz steps from 1 to 80 Hz. We calculated the spectral centroid *C* in each window as

$$C = \frac{\sum_{f=1}^{N}(f * P_f)}{\sum_{f=1}^{N} P_f},$$

where *P* is the PSD value for every *f* 1 Hz frequency bin, and *N* is the number of frequency bins per window. The measures compared between attended and passive blocks were the centroid, power values averaged across the 25–57 Hz

band, and also power averaged across 40–42 Hz (to afford comparison of our results with those previous studies of the effect of attention on ASSR power).

4.5 Results

4.5.1 Behavioral results

All subjects performed the task above chance, averaging 74.6% percent correct (pc) across all trials (1 within-subjects standard error (SE) = 5.75). Task performance for the 400 Hz carrier (mean pc = 77.5%, SE = 3.74) was somewhat better than for the 5 kHz carrier (mean pc = 71.6%, SE = 4.36), a difference that while small was statistically significant ($t(14) = 2.58$, $p = 0.021$, two-tailed test).

4.5.2 Electrophysiological results

Inspection of the grand average time domain EEG waveforms seen in the top row of Figure 4.5a shows that the ASSR at 5 kHz briefly reset following both short and long gaps. The waveforms in the second row show spectral power averaged across the 40–42 Hz narrow band (this frequency range used by Gander et al. 2010b to reveal an effect of attention on ASSR amplitude). Power in this band decreased shortly after gap onset but recovered over a ~200 ms period (thick bracket) to the pre-gap value. Narrowband power did not appear to differ between active and passive conditions after gaps but there was a tendency for it to be larger during active conditions in the pre-gap baseline. In the third row of Figure 4.5a the filter has been widened to 25–57 Hz to explore power changes over the frequency range in which the centroid was calculated. It can be seen that compared to the pre-gap baseline power 25–57 Hz tended to decrease after gaps

but did not appear to differ between the attention conditions. The fourth row of Figure 4.5a shows the spectral centroid across the 25–57 Hz band, and describes how the spectral distribution changed. Before gaps, the centroid localized to a higher frequency during the active compared to the passive condition. During active but not passive trials the centroid decreased by ~1.5 Hz after gaps. In contrast, under the passive condition the centroid was lower than on active trials during the pre-gap baseline, and tended to shift up in frequency after gaps.

ASSR resets during the 400 Hz probe followed a similar pattern to results at 5 kHz. The top row of Figure 4.5b shows a clear reset and recovery of the ASSR time domain waveform after short and long gaps. 40–42 Hz power traces in the second row show a drop in power after each gap, with a tendency toward larger pre-gap power during the active condition compared to the passive condition on long gap trials. Narrowband power in the post-gap interval did not appear to differ between attention conditions. Power in the wider 25–57 Hz band in the third row of Figure 4.5b was only weakly modulated by the gaps and did not appear to differ between the attention conditions. In the last row of Figure 4.5b, centroids during the active condition were close to the 41 Hz stimulus rate during the pre-gap interval, and like centroids of the 5 kHz probe, decreased from their baseline after each gap. Centroids under the passive condition sat lower than the AM rate particularly on long gap trials and did not appear to change from their dominant pre-gap baseline at either gap interval.

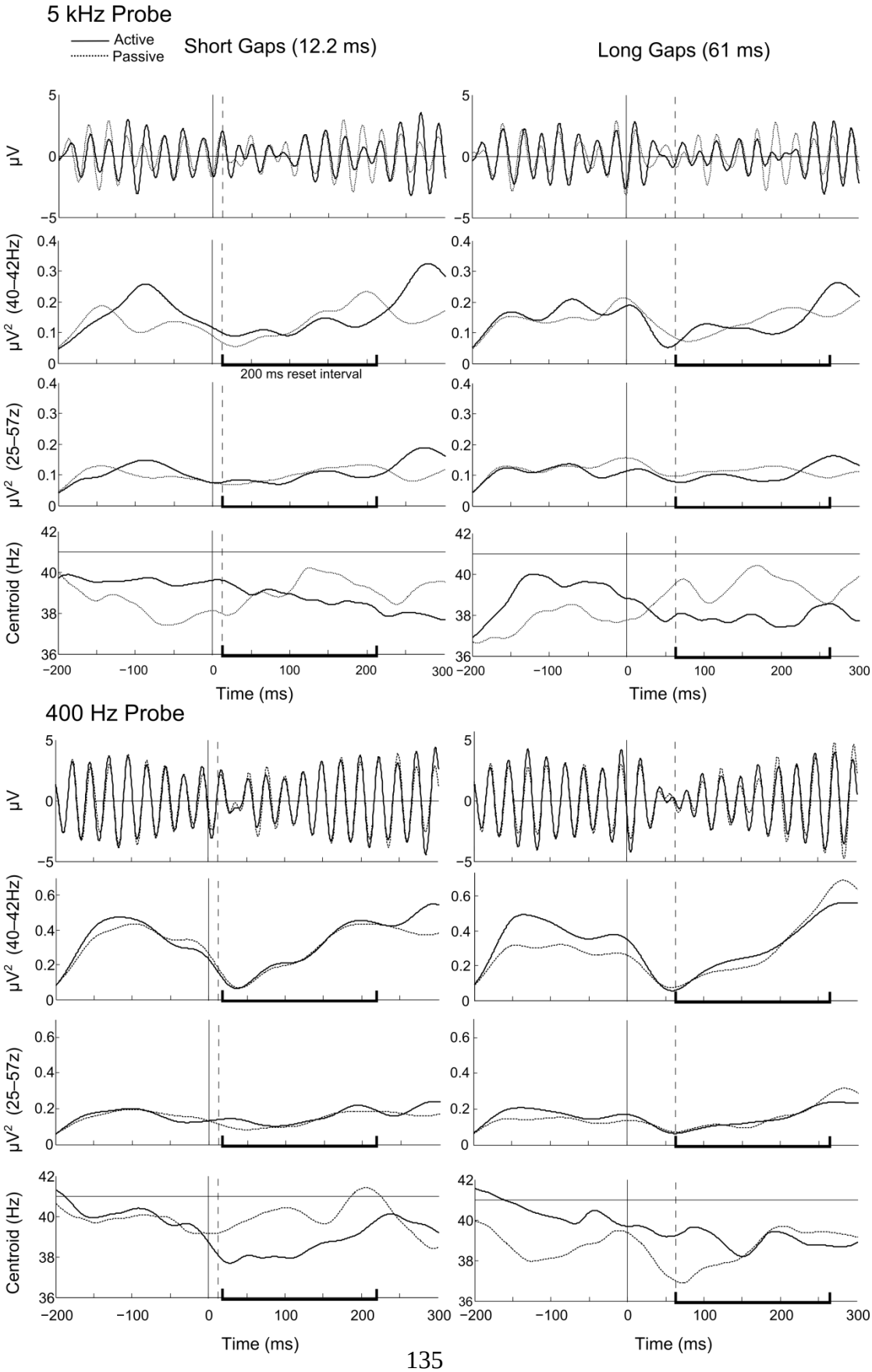
5 kHz Probe
Active
Passive
Short Gaps (12.2 ms)
Long Gaps (61 ms)
μV
μV² (40–42Hz)
200 ms reset interval
μV² (25–57z)
Centroid (Hz)
Time (ms)
400 Hz Probe
μV
μV² (40–42Hz)
μV² (25–57z)
Centroid (Hz)
Time (ms)

Figure 4.5. Time series of ASSR resets for the 5 kHz and 400 Hz probe around short gaps (left column) and long gaps (right column). The top row under each probe frequency shows the 25–57 Hz bandpass filtered time domain waveforms of the ASSR, the second row the narrowband 40–42 Hz power, the third row the 25–57 Hz band power, and the bottom row the spectral centroid over time for the signal. The thick vertical black line at the 0 ms mark on each time series indicates the onset of the silent gap. The thin vertical line after indicates gap offset and probe onset. Intervals outlined by thick brackets correspond to the 200 ms ASSR reset period that was compared to the 200 ms pre-stimulus period.

To statistically evaluate the results during the pre-gap period when the ASSR was stable, we averaged values across the −200 to 0 ms interval separately for narrowband power, wideband power, and the spectral centroid. For each of these signal measurements, a 2x2x2 repeated measures analysis of variance (ANOVA) was performed comparing conditions of attention (active vs. passive), gap type (long gap vs. short gap), and carrier frequency (400 Hz vs 5 kHz stimulus). No main effects or interactions with gap type or were found in these analyses, so a new 2x2 ANOVA model was run only examining effects of attention and carrier frequency. In this model, a main effect of attention was found ($F(1,14) = 6.12$, $p = 0.027$) showing that active attending produced significantly higher 40–42 Hz power during the pre-gap interval compared to passive listening. This result is portrayed in the left panel of Figure 4.6a, where it can be seen that power was larger under active listening for both carrier frequencies. In contrast, power averaged over 25–57 Hz did not significantly differ between the attention conditions or show interactions (all ps > 0.27, results not shown). However, centroids were higher in active compared to passive conditions during the pre-gap baseline (main effect of attention, $F(1,14) = 9.01$, $p = 0.009$). This result is shown

in the right panel of Figure 4.6a, again with similar findings for the two carrier frequencies.

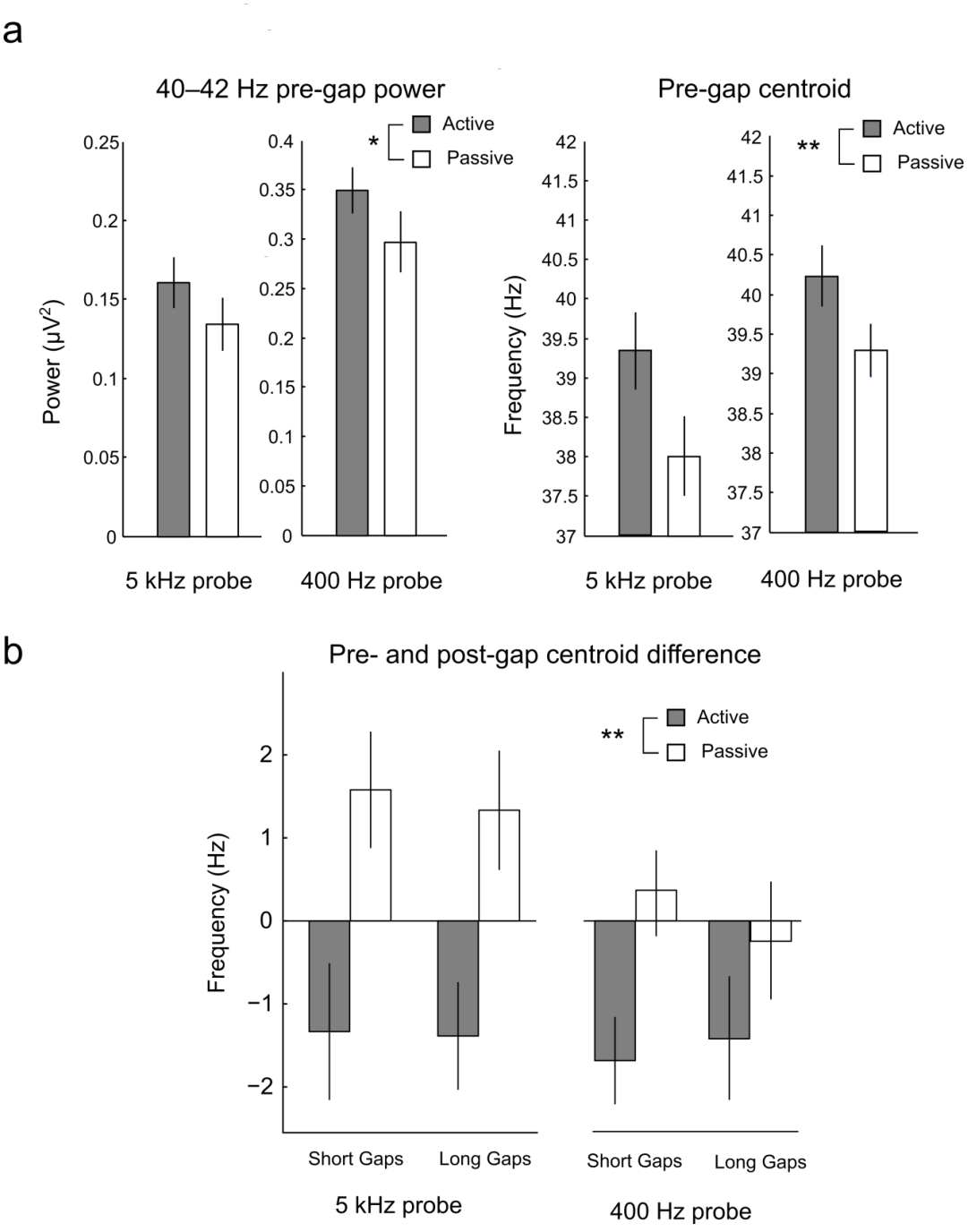

*Figure 4.6. Bar graphs depicting pre-stimulus and pre- and post-gap differences of power spectral centroids for the 5 kHz and 400 Hz carrier. Error bars are 1 within-subjects standard error. * P < 0.05; ** P < 0.01.*

In a second ANOVA model again using a 2x2x2 design comparing conditions described above, we examined changes in the spectral centroid before and after each gap type. For this purpose we subtracted the pre-gap baseline from the averaged values across the 200 ms recovery period of the ASSR reset (post-gap interval). The active condition (see Figure 4.6b) produced a downward shift in centroid frequency in response to the gap compared to the passive condition, which tended to produce an upward frequency shift instead (main effect of attention, $F(1,14) = 9.64$, $p = 0.008$). Least Significant Difference tests revealed that the downward shift of the centroid under active conditions was significant ($p = 0.02$) while the upward shift under the passive condition while visually prominent at 5 kHz did not reach significance ($p = 0.18$). Although the increase at 400 Hz was smaller, there were no significant main effects or interactions with carrier frequency. No main effects or interactions with gap type were found. There were no significant effects or interactions found for wide or narrowband power for the pre−post gap difference.

4.6 Discussion

4.6.1 Summary

The goal of our study was to determine the effect of top-down attention on the dynamics of the gap-perturbed 41 Hz ASSR reset response, revealing how

attention might facilitate the temporal processing of rapid stimulus changes in auditory cortex. ASSRs were evoked by either a 5 kHz or 400 Hz tone that was amplitude modulated by 41 Hz. We measured separately the ASSR power and spectral centroid (a measure indicating the frequency where power is concentrated in the signal spectrum which could be used to infer changes of phase) after a gap disrupted the ASSR to determine how these properties changed when subjects attended to gaps (active listening) versus when they were not (passive listening). During the reset period, the centroid shifted toward lower frequencies in the attended condition and in contrast tended to shift upwards or remain unchanged in the passive condition. This pattern did not differ significantly between stimuli of high and low carrier frequency that were tested within subjects in separate conditions. Narrowband (40–42 Hz) and wideband (25–57 Hz) power did not differ between active and passive conditions during the reset, indicating that centroid shifts downward toward a lower frequency under active attending were likely a result of a phase lengthening of the ASSR as the ASSR recovered. These results imply that attention is expressed on the phase but not power of the ASSR recovery interval. Subjects' behavioral performance was slightly but significantly better for the 400 Hz versus 5 kHz tone, suggesting frequency-specific differences in gap perception in an AM tone.

Replicating previous findings, we found that 40–42 Hz narrowband ASSR power in the pre-gap interval when the ASSR was stable was larger in the active compared to passive listening condition (Gander et al., 2010b; Paul et al., 2014).

A new finding was that within the pre-gap period the centroid during the active condition was significantly closer to 41 Hz than during the passive condition. Overall power in the 25–57 Hz band (the band over which the centroid was calculated) during this time was not significantly different between active and passive conditions, suggesting that pre-gap centroid effects could either be due to stronger phase locking or more power concentrated at 41 Hz stimulus rate at times when subjects were actively listening.

4.6.2 The effect of attention on the ASSR recovery after gap desynchronization

Although attention did not affect power of the ASSR recovery interval after the reset, the decrease and 200 ms recovery of ASSR narrowband power during this interval is consistent with the time course of ASSR resets previously reported (Makeig & Galambos, 1989; Rohrbaugh et al., 1989, 1990a,b; Ross & Pantev, 2004; Ross, et al., 2004; Ross et al., 2012). Similar to amplitude, phase changes evoked by gaps reach a maximum deflection 100 ms after the perturbation onset which realign within 200 ms after the reinstatement of the AM stimulus (Ross & Fujioka, 2016; Ross et al., 2012; Ross & Pantev, 2004), consistent with spectral centroid effects observed in the current results. Ross and Fujioka (2016) and Ross et al. (2012) were the only studies to require subjects to actively listen and respond to gap stimuli that evoked ASSR resets. Both studies used a design in which the ASSR was evoked by stimulating one ear with a 40 Hz AM tone while in the other ear either multi-talker noise or silence was presented. In the presence of contralateral noise, phase shifts were less pronounced and the amplitude

recovered within 50 ms, far shorter than the 200 ms recovery interval found without contralateral noise. Although task attention was held constant across these conditions, the pronounced phase shift in quiet may in part be attributed to attentional facilitation at times when gap stimuli were not centrally masked.

To our knowledge only Rohrbaugh et al. (1989) have compared ASSR resets between active and passive listening conditions. In the active condition of that study, the click-evoked 40 Hz ASSR was reset by the presence of standard and oddball pure tone stimuli of different frequencies that the participants had to detect and report, while in the latter condition there were no oddball stimuli and participants passively listened to ASSR disruptions by only a standard stimulus. Oddball stimuli in the active condition were associated with longer ASSR latency shifts (lengthening of ASSR phase) compared to standard stimuli in the passive condition, and ASSR amplitude did not differ between conditions. However it was not possible to dissociate if latency shifts resulted from active listening itself or the novelty of the oddball. The present study however did not use an oddball paradigm and stimuli were identical between active and passive conditions, but the results of both studies align, suggesting that attention is expressed on the phase and not amplitude of the ASSR reset.

At present it is not clear how to interpret the role spectral shifts play in stimulus processing for the two attention conditions. One possibility is that the shifts could reflect the different cognitive operations that were required of the subjects during active and passive blocks. Alternatively, an interaction of top-

down (active) and bottom-up (involuntary) attention could be suggested. On active blocks it appears that gaps uncoupled neural networks in A1 coding the AM envelope leading to a significant phase lengthening over the 200 ms recovery interval. The shift could reflect a pronounced disinhibition of neural networks by a failure of predictive coding (Winkler et al., 2009; Arnal & Giraud, 2012), which may serve to increase the capacity of neural networks to represent unstable auditory scenes. Subsequently the ASSR redeveloped under active attention, shifting the focus of spectral power back to the AM frequency as seen in the pre-gap interval (Figure 4.6a). On passive blocks where active attention was not deployed, the amount and concentration of pre-gap power was less centered on the AM rate which may also be a consequence of poorer phase locking. Under this condition, bottom-up attention triggered by the gaps may have shifted the phase to align more closely with the AM rate for a brief period of time, although this trend did not reach significance. The trend was more visually pronounced for the 5 kHz carrier, possibly because (as indicated by their behavioral performance) the task was somewhat more difficult for the subjects for this carrier frequency, suggesting a greater engagement of attention mechanisms.

4.6.3 Neuromodulatory systems underlying attention effects in A1

The effects of attention on the ASSR in the current and previous results are consistent with animal studies in which single unit activity was recorded from A1 during attention tasks. A1 neurons in animals trained to detect amplitude modulations in a sound showed higher evoked firing rates and higher phase

locking to the AM sound compared to animals that listened passively with no reward given (Niwa et al., 2012; Dong et al., 2013). Similarly, ferrets that were rewarded for discriminating sound frequencies showed changes in the receptive fields of neurons in A1 over the course of minutes such that these neurons were more sharply tuned to target sounds and less so to non-target sounds (Fritz et al., 2003; Fritz et al., 2005; David et al., 2012). These modulations likely involve activation of neuromodulatory systems such as cholinergic projections from the basal forebrain that are known to gate neural plasticity (Ramanathan et al, 2009) and to increase the sensitivity of auditory neurons to their afferent inputs on attention tasks by targeting muscarinic and nicotinic acetylcholine receptors (Sarter et al., 2005, 2009).

Recently, Zhang et al. (2016) demonstrated that blocking muscarinic receptors of A1 neurons decreased the amplitude and phase locking of the ASSR in rat auditory cortex, while blockage of cholinergic reuptake (leaving acetylcholine to persistently activate both muscarinic and nicotinic receptors) increased ASSR phase locking amplitude. Manipulations that simultaneously block muscarinic receptors and prevent acetylcholine reuptake for nicotinic receptors interestingly enhanced only phase locking of the ASSR but not power. The results support the view that cholinergic modulation mediates attention-related effects in A1, and suggest that ASSR phase can be modulated independently of amplitude. A role for inhibitory GABAergic effects and interactions with cholinergic modulation must also be considered.

4.6.4 Conclusions

We provided evidence that active listening can modify a spectral estimate of phase shifts during the gap-evoked ASSR reset recovery interval that is independent of changes to amplitude. When the ASSR was stable before gaps, power was larger, consistent with previous results. During this time the centroid was closer to the 41 Hz AM rate which could indicate better phase-locking and/or more spectral power concentrated to the AM rate. These results may suggest that under the active listening condition, gaps more strongly desynchronized A1 neural networks coding the AM rate than when subjects were passively listening. As the ASSR regenerated, more power became focused at the frequency of the stimulus when the subject was actively attending.

4.7 Acknowledgements

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Chapter 5: Discussion

5.1 Summary and implications of findings

5.1.1 Summary

The chapters of this thesis aimed to address two issues raised under the deafferentation model of tinnitus. The first issue was that audiometric threshold shifts (indicating hearing loss) do not always accompany tinnitus, and tinnitus can arise without threshold shifts. The second issue was that although tinnitus negatively impacts the ability of individuals to attend to their environment, it is not clear if the attention system itself is affected in tinnitus or if the presence of tinnitus in the auditory system resists or detracts from the normal expression of top-down attention on auditory neurons. In brief and in order of Chapters 2, 3 and 4, the findings suggested that a) tinnitus is associated with peripheral loss of high- and low-SR fibers that may be undetected by the audiogram, b) top-down attention likely operates normally in tinnitus but its expression on auditory cortical neurons is impeded by tinnitus-related hyperactivity in the hearing loss region of auditory cortex, and c) in normal hearing individuals, top-down attention modulates phase shifts during the ASSR reset recovery interval (a bottom-up response), suggesting that this procedure can be used in tinnitus to test the sensitivity of tinnitus-affected auditory cortical neurons to bottom-up input.

5.1.2 Tinnitus and loss of peripheral synapses or fibers

The study in Chapter 2 provided the first human evidence for loss of both low- and high-SR fibers in individuals with tinnitus and normal audiograms. A

conceptual model in Figure 2.6 introduced the possibility that this pattern of peripheral fiber loss could also be present in tinnitus sufferers with high-frequency threshold shifts in the audiogram, assuming that most of the threshold shift is accounted for by outer hair cell loss and/or hair cell stereocilia damage. One notable implication of this view is that the discussion of factors behind the development of tinnitus is focused back to the auditory periphery. Many tinnitus researchers have taken the stance that the incongruence between hearing loss and tinnitus points to central factors that distinguish between tinnitus and non-tinnitus cases, and a large body of research has attempted to determine what these central factors are (e.g., Adjamian et al., 2009, Vanneste & De Ridder, 2016). Most animal research in tinnitus reported in Chapter 1 measured temporary or permanent threshold shifts in noise-exposed animals, both of which are associated with tinnitus, and concluded that central hyperactivity must distinguish tinnitus since animals in these groups were matched in the amount of noise exposure and degree of threshold shift. However counts of peripheral synapses or spiral ganglion cells are rarely conducted, meaning that in many of these studies there is the possibility that the tinnitus animals had more high- and low-SR fiber loss than non-tinnitus animals that was not expressed in thresholds.

From previous animal research and the findings in Chapter 2, it is not yet clear how high-SR fiber loss would precisely trigger the development of tinnitus. Bauer et al. (2007) described one interpretation based upon the function of high- and low-SR fiber input to the DCN. Fusiform cells (the primary input/output

cells) in the DCN receive most of their excitatory drive directly from low-SR auditory nerve fibers, whereas high-SR nerve fibers innervate small cap cells deep in DCN which provide an inhibitory drive onto fusiform cells (Liberman, 1993). Bauer et al. (2007) interestingly observed high- but not low-SR fiber loss in animals with behavioural tinnitus, so it was assumed that disinhibition of fusiform cells indirectly through high-SR fiber loss could account for tinnitus-related changes. Considering both low- and high-SR fiber loss, this view can be interpreted such that loss of direct excitatory drive and indirect inhibitory drive on fusiform cells may combine to account for the downregulation of inhibitory neurotransmission (Wang et al., 2009; Middleton et al., 2011), upregulation of somatosensory sensory inputs (Zeng et al., 2012), and changes in the timing rules of STDP (Koehler & Shore, 2013) on fusiform cells in animals with behavioural tinnitus. These changes are likely implicated in the increased SFRs, burst firing, and neural synchrony that have been together shown to correlate with tinnitus-like behaviour in animals (Wu et al., 2016). In contrast, low-SR fiber loss alone may be insufficient to trigger these changes, possibly because of the indirect inhibitory drive onto fusiform cells provided by intact high-SR fibers. The VCN also receives input from low- and high-SR fibers and is known to provide inhibitory and excitatory drive onto fusiform cells of the DCN (Oertel & Golding, 1997), meaning that a role for this region has yet to be integrated. There has been less focus on correlating tinnitus-related behaviors to VCN changes after noise-

exposure in animals to speak to further interpretations, but neurons in this region are known to increase SFRs after noise damage (Vogler et al., 2011).

Findings in Chapter 2 also speak to clinical applications. There are no current standard clinical tests that indicate loss of peripheral auditory fibers or synapses, but a protocol could be developed involving EFRs (as done in Chapter 2) complemented by or in conjunction with ABRs. For example, low-SR fibers have thresholds between 35 and 40 dB SPL (Liberman, 1978), meaning that decrements in EFRs and wave I of ABRs evoked at levels just below the low-SR fiber threshold are likely to indicate high-SR loss in individuals with normal hearing thresholds. In contrast, a 40 dB spectrum level background noise will likely saturate most high-SR fibers (Yates et al., 1989), meaning that EFRs evoked in noise by tones > 40 dB SPL can predominantly reflect the status of low-SR fibers (see stimulus design in Chapter 2). In the presence of audiometric threshold shifts, which are most likely due to the loss of cochlear amplification provided by OHCs, these stimulus levels must be adjusted by threshold values such that each fiber type is properly stimulated. For example, an individual with a 25 dB HL shift at a given frequency in the audiogram will need at least a 60 dB SPL probe (35 dB probe + 25 dB threshold shift) to test high- but not low-SR function. It is important to note that peripheral damage is unlikely to be present in every tinnitus case. The subject reported in Chapter 2 experiencing loud, intermittent tinnitus produced excellent AM sensitivity in quiet and noise, inconsistent with peripheral synaptic loss. Aside from noise exposure and aging,

tinnitus may be caused by somatosensory injuries, illness, and diseases of the auditory periphery (Langguth et al., 2013). These considerations further highlight the importance of developing more sensitive clinical tests for peripheral neuronal degeneration to determine the most likely cause of an individual's tinnitus.

5.1.3 Tinnitus and attention

As stated, the tinnitus research field has placed a considerable focus on both auditory and non-auditory central factors behind the generation of tinnitus because hearing loss does not reliably accompany tinnitus. One debate revolves around the causal relationship between tinnitus-related neural activity in auditory areas and broader brain networks involved in consciousness, arousal, and attention that must interact to bring tinnitus into perception (De Ridder et al., 2013). The model and supporting evidence presented by Roberts et al. (2013), for example, describes a role for auditory attention in the generation of tinnitus, suggesting that hearing damage precipitates central hyperactivity, but neural plasticity directed by persistent attention organizes hyperactive neurons into synchronous ensembles that are responsible for the tinnitus percept. One aspect of this model suggests that attention is broadly engaged across the whole of auditory cortex in tinnitus because the cortex is attempting to resolve uncertainty in the acoustic scene in a frequency nonspecific manner. This suggests the possibility of attentional disruption at frequencies outside the TFR. Inconsistent with this view, Chapter 3 suggested that top-down attention in tinnitus sufferers appears to normally modulate responses below TFR, as well as late-latency responses which

have distributed sources within and anterior to auditory cortex (Scherg et al., 1989).

Effects of tinnitus on attention may relate not to persistent arousal of top-down attention, but to the interfering effect of tinnitus-related neural activity on the expression of attentional modulation in individuals with tinnitus. The interpretation of results in Chapter 3 was that populations of neurons in the TFR of A1 are occupied with coding the tinnitus percept and are (at least initially) insensitive to modulation by active listening. This "busy-line" effect would similarly explain smaller ASSRs in the TFR under passive listening conditions alone, since phase locking of affected neurons to AM sounds is likely to be poor because of the presence of hyperactivity (Roberts et al., 2012; Roberts et al., 2015). In principle the busy-line effect would suggest that these neurons would also be insensitive to detect changes in the acoustic environment. Chapter 4 introduced groundwork for the ASSR reset paradigm that could describe how attention plays a role in rebuilding a stimulus representation in auditory cortex following a brief disruption. The results indicated that in young non-tinnitus subjects, top-down attention shifts spectral estimates of ASSR phase after gap-induced reset in a manner that implies a phase lengthening, but power is not affected. It is not immediately clear what phase shifts of the ASSR reset signify under the condition of active listening. One possibility is that the shifts represent the bottom-up disinhibition of auditory cortical networks that are initially sensitized by attention (evidenced by attentional enhancement of the stable

ASSR), briefly broadening neuronal filtering properties so that the auditory stimulus change is best represented.

The findings of Chapter 4 establish a few notable hypotheses regarding tinnitus and attention under the conditions of the ASSR reset. First, if A1 neurons are busy coding tinnitus-related activity and are less sensitive to input, ASSR resets of tinnitus subjects evoked in the absence of task attention might be expected to show weak resets (less decrement of ASSR amplitude and weak phase transitions/decreases in the centroid) for 5 kHz probes, but at 400 Hz, resets would not be different from controls. These effects would likely not be different between active and passive listening conditions if neurons in the TFR are insensitive to attentional influence. A second alternative could be that reset responses in tinnitus subjects for both active and passive listening conditions inside and outside the TFR are not different from controls, suggesting that A1 neurons in tinnitus are normally sensitive to bottom-up information. These findings would contrast with the frequency-specific effects of top-down attention in tinnitus on the stable ASSR shown in Chapter 3, implying that tinnitus-related neural activity only interferes with top-down attentional modulation of A1.

Irrespective of which of these hypotheses receives support, it could be useful to clarify the role of attention in tinnitus, relevant to clinical interventions that may reduce tinnitus-related neural activity. For example, training-related plasticity in cortex directed by tasks focusing top-down attention away from the

TFR might channel inhibition into hyperactive areas, mitigating the tinnitus percept (Engell et al., 2016).

5.2 Future directions

5.2.1 Efferent contributions to peripheral damage

A question left unresolved by the experiment in Chapter 2 was the role of efferent contributions to cochlear function in tinnitus. Knudsen et al. (2014) reported that some individuals with tinnitus and evidence of hyperacusis have increased suppression of DPOAEs under contralateral stimulation compared to controls and tinnitus subjects without evidence of hyperacusis, suggesting that MOC hyperactivity may be suppressing cochlear output. Since tests of hyperacusis or MOC function were not conducted in Chapter 2, diminished EFRs may in part result from an affected efferent system. Future studies measuring suprathreshold ability to infer peripheral loss should also include measures of the MOC system such as contralateral suppression of DPOAEs and middle ear reflexes (which enhance the impedance of the middle ear by acting on the ossicles), which are known to be affected by low-SR loss (Valero et al., 2016).

5.2.2 The role of subcortical structures in human tinnitus findings

One of the most unexplored areas in human tinnitus research concerns the role of subcortical structures in known peripheral and cortical correlates of tinnitus. There has been an intense focus on CN, IC, and MGB in animal research but few human studies are available to speak to these results (Section 1.4.6). One reason for this disparity is that there are not strong noninvasive measurement techniques

in humans that can isolate distinct subcortical functions in a frequency-specific manner. The resolution of fMRI is inadequate to detect fine changes in these regions (especially in CN where there is a wealth of tinnitus evidence in animals) and there are few electrophysiological studies focusing on lower structures of the auditory pathway (Adjamian et al., 2009) because it is difficult to separate individual subcortical sources from a composite neuroelectric potential (Shaheen et al., 2015).

These disparities can be brought to light when considering the results in Chapters 2, 3, and 4. The EFR evoked by an 85 Hz AM tone is consistent with neural generators in the auditory midbrain (IC; Herdman et al., 2002), an area known to have tinnitus-related increases in hyperactivity following noise exposure (Section 1.4.5). Discussion in Section 2.6.4 brought up the notion that degraded AM coding in this region could in part result from disinhibition, which could not be distinguished from AM deficits that resulted from peripheral synaptic loss. A way in which future studies could bypass this issue is by measuring EFRs in tinnitus sufferers evoked by higher rates (> 200 Hz AM) which better approximate contributions from the auditory nerve (Shaheen et al., 2015). However, EFRs to higher AM rates can only be obtained for higher carrier frequencies, and the magnitudes of EFRs in humans at higher AM rates may not produce favourable SNRs. Hyperactivity in the TFR of MGB and IC known in tinnitus animal models may suggest that like EFR reductions, 40 Hz ASSR reductions in the TFR may result from poor subcortical phase locking to the AM

envelope. However Boyen et al. (2014) found reduced connectivity between IC and A1 in tinnitus subjects compared to controls. Although the affected frequency region could not be determined, the results could suggest that peripheral or subcortical changes related to tinnitus may be less influential on cortical processing.

Notwithstanding the effects of tinnitus, a second consideration is that effects of top-down attention on AM processing may commence in lower auditory structures. There are known cholinergic inputs to MGB (Motts & Schofield, 2010; Hallanger et al., 1987) and IC (Motts & Schofield, 2009), which may similarly perform an attention-like modulation as observed in A1. Attentional influence may also descend from AC to IC and MGB by way of corticofugal connections (Suga & Ma, 2003). Evidence for the effect of selective attention (in these cases requiring subjects to attend to one stimulus and ignore the other) on subcortical processing in humans has been found in the IC using fMRI (Rinne et al., 2008) and in the EEG on the frequency following response (FFR; Lehmann & Schönwiesner, 2014) which is known to have subcortical sources (Bidelman, 2015). One report however did not find evidence of selective attention on the 97 or 113 Hz EFR (Varghese et al., 2015), meaning that the effect of attention on subcortical steady-state responses remains to be resolved.

5.3 Conclusions and general importance

The increasing prevalence of tinnitus (Gilles et al., 2013) and hearing loss (Shargorodsky et al., 2010) in adolescents suggests that a significant future health

challenge will be managing and treating tinnitus. These statistics are likely to increase due to poor knowledge of the long term effects of recreational and occupational noise exposure in this age cohort (Gilles et al., 2013) and a recent report finding that 42% of adolescents are engaged in dangerously high levels of everyday recreational listening (Dehnert et al., 2015). Because there is not yet an effective treatment or cure (Langguth et al., 2013), it is critical that the research community develops a better scientific understanding of factors causing and modulating tinnitus. The empirical research presented in this thesis contributes to this understanding by characterizing a pattern of peripheral damage that may be necessary for tinnitus development, and clarifying how effects of tinnitus on attention tasks may be realized.

5.4 References

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