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**Abstract**

Due to the heterogeneity of potential tinnitus causes, a biomarker is needed that would relate to a 'final common pathway' for tinnitus, irrespective of specific contributory mechanisms. This biomarker should differentiate between tinnitus and other comorbid conditions. Previous research suggested that Intensity Mismatch Asymmetry may be such a biomarker. The hypothesis was that downward deviant stimuli sounded more similar in intensity to the default prediction of tinnitus intensity, so people with tinnitus would show a reduced response to this change, while the upward deviant was further from the default prediction, thus making the MMN response larger. The present project aimed to systematically explore design features that may affect responses to intensity deviants in people with tinnitus, hyperacusis, and healthy controls. The main factors explored included: having only one or a combination of the aforementioned conditions, attentional direction, and presence of close or widely spread frequencies. Several conclusions were reached. Hyperacusis, tinnitus with hyperacusis, and tinnitus without hyperacusis, potentially all have different underlying mechanisms. Generally, presence of hyperacusis enhances MMN responses to upward intensity deviants and disrupts adaptation mechanisms. Tinnitus without hyperacusis showed stronger MMN amplitude in response to downward deviants at tinnitus-like frequency. Tinnitus generally has normal adaptation processes, however, interference between close frequencies limits this adaptation/habituation. A similar pattern was elicited in controls when two nearby frequencies were involved in a roving paradigm experiment, pointing towards a specific interference effect, and possibly reflecting contrast gain theory mechanisms.

The effectiveness of a novel covariance-cancelling sound therapy was tested as a potential treatment for participants with tinnitus. The aim of these sounds was to reduce synchronous activity between neurons that are responsible for frequencies around the tinnitus frequency of an individual. These sounds were successful in significantly reducing the perceived loudness of tinnitus after 6 weeks of daily listening.

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**Glossary of Abbreviations & Units:**

A_Start/End – Start/End of Listening Period with the Active Sound Stimulus

A1 - Primary Auditory Cortex

ABR – Auditory Brainstem Response

ADT – Auditory Discrimination Training

ANOVA – Analysis of Variance

ASSR – Auditory Steady-State Response

BOLD – Blood-Oxygen-Level-Dependent Signal, Detected in fMRI

C – Control Participant

CN – Cochlear Nucleus

CR – Coordinated Reset

dB - Decibel

dB HL – Hearing Level

dB SPL – Sound Pressure Level

DCN – Dorsal Cochlear Nucleus

DD – Downward Deviant

EEG – Electroencephalogram

EOG – Electrooculography

ERP – Event-Related Potential

f/MRI – Functional/ Magnetic Resonance Imaging

FCz – Fronto-Central Midline Channel of EEG

GABW – Gamma-Aminobutyric Acid

H.I. – High Intensity

HNMT – Heidelberg Neuromusic Therapy

HQ – Hyperacusis Questionnaire

Hz - Hertz

IC – Inferior Colliculus

IHS – Inventory of Hyperacusis Symptoms

IMA – Intensity Mismatch Asymmetry

kHz - Kilohertz

L.I. – Low Intensity

MEG - Magnoencephalogram

MGB – Medial Geniculate Body

MMN – Mismatch Negativity

ms – Milliseconds

mV – Microvolts

N - Number of Participants

NAc – Nucleus Accumbens

NMDA – N-Methyl-D-Aspartate

NRS – Numerical Rating Score

NTT – Neuronomics Tinnitus Therapy

PTA – Pure Tone Audiometry

RCT – Randomised Controlled Trial

RI – Residual Inhibition

ROC – Receiver-Operator Curve

RP – Repetition Positivity

RS – Repetition Suppression

S_Start/End – Start/End of Listening Period with the Sham Sound Stimulus

SD – Standard Deviation

SE – Standard Error

SR – Stochastic Resonance

T+H- - Participant with Tinnitus but without Hyperacusis (Tinnitus Only)

T+H+ - Participant with Tinnitus and with Hyperacusis

TFI – Tinnitus Functional Index

T-H+ - Participant without Tinnitus but with Hyperacusis (Hyperacusis Only)

THI – Tinnitus Handicap Inventory

TMNMT – Tailor-Made Notched Music Training

TRN –Thalamic Reticular Nucleus

UD – Upward Deviant

ULL – Uncomfortable Loudness Level

VAS – Visual Analogue Scale

vmPFC – Ventromedial Prefrontal Cortex

VNS – Vagal Nerve Stimulation

WP – Washout Period

# Chapter 1. Introduction

## 1.1 What is tinnitus?

Tinnitus is a persistent sound heard by an individual without an environmental source, which may appear as pure tone, ringing, hissing, whistling, static, or cicada-like sounds [1]. There are two major types of tinnitus: objective and subjective. Objective tinnitus is rare and appears due to a range of identifiable underlying physical conditions of the ear or nearby structures. Some examples are audible blood flow abnormalities, e.g. idiopathic intracranial hypertension, and non-vascular conditions such as middle ear myoclonus [2, 3]. It can be heard by physicians through audiological tests including tympanometry or otoacoustic emissions [4]. Subjective tinnitus, on the other hand, is a common condition where the phantom sound does not occur directly due to an underlying physical source [5]. Due to the heterogeneity of potential causes of subjective tinnitus, classification of this condition has been difficult [6]. Some tinnitus dimensions that can be classified include laterality (bilateral/unilateral), tonal vs noise type of sound, with vs without hyperacusis, somatic, intermittent vs permanent, acute vs chronic.

Tinnitus can be non-bothersome, but an estimated 20% of people with tinnitus seek treatment [1]. This can be due to the sound of tinnitus itself, or related symptoms such as depression and sleep disturbances [7, 8]. This thesis focuses on the study and treatment of subjective tinnitus.

The world tinnitus prevalence is 10-15%, with increasing numbers of the population experiencing it as they age [9, 10]. Hearing loss has been established as the largest risk factor of tinnitus [11, 12]. Hyperacusis (disorder in which normal environmental sounds are experienced as uncomfortably loud) also often co-occurs with tinnitus [13]. Some minor factors related to tinnitus include ear infections, noise exposure, ototoxicity, head/ear surgeries, diabetes, history of cardiovascular disease, heavy drinking, arthritis, and non-steroidal anti-inflammatory drugs (though this association may be present due to inflammation itself) [7, 12].

## 1.2 The auditory system

The presence of tinnitus stems at least in part from functional changes to different levels of the auditory pathway [6, 14]. Therefore, before looking into mechanisms of tinnitus, it may be helpful to understand the basic structure of the auditory pathway itself.

The human auditory system is able to perceive sound frequencies between 20 Hz and 20 kHz. 'Low' frequencies are those below 2 kHz, 'high' frequencies are 2-8 kHz and 'extended high' frequencies are 8-20 kHz [5]. The organ of Corti within the cochlea in the inner ear converts sound vibrations into impulses that can be transmitted up to the auditory cortex via the ascending auditory pathway [15]. It is located in the scala media, which is filled with endolymph liquid. This organ contains basilar and tectorial membranes, which are connected by hair cells. The basilar membrane stretches along the cochlea and is tonotopically organised with specific locations responsible for responding to each frequency [16]. High-frequency sounds produce movements at the base of the basilar membrane, whereas low frequency sound regions are at its apex. This frequency organisation (tonotopy) is maintained by the inner hair cells (the main innervating cells of the auditory nerve) and throughout the auditory pathway [17]. The outer hair cells are responsible for sharp tuning of the frequencies and help to amplify low-level sound by increasing basilar membrane movements [16]. When the outer hair cells are damaged, low frequency signals cannot be amplified, whereas inner hair cell damage disrupts tonotopic mapping [18]. Hair cells do not regenerate in mammals [15]. The auditory nerve fibres connect to the base of hair cells through the spiral ganglion cells [19]. The spiral ganglion is made up of bipolar cells, which extend to make up the auditory nerve on one side and connect to the hair cells on the other [20]. There are two types of spiral ganglion cells. The more common type is Type 1 cells, which are large and myelinated. Each Type 1 dendrite innervates one inner hair cell, but every inner hair cell can have multiple afferent dendrites connected to it [21]. This allows each inner hair cell to be well represented by the fibres carrying information to the brainstem. Type 2 cells are unmyelinated and small, innervating multiple outer hair cells [22]. Each type 2 dendrite has a single contact from an afferent dendrite. Both synapse types are excitatory, using glutamate to transmit information [21]. Ninety-five percent of afferent auditory fibres leave from inner hair cells.

The auditory nerve travelling from the cochlea terminates in the cochlear nuclei (CN) of medulla oblongata in the brainstem [20]. Different auditory nerve fibres have varying levels of sensitivity to sound. There are three main groups of auditory nerve fibres: low, medium and high spontaneous firing fibres. The low spontaneous firing group is less likely to become saturated with impulses from the hair cells, which plays a role in their ability to detect change in high level sounds, for example, during ongoing background noise [23, 24]. The low spontaneous firing group is also the most vulnerable both to noise exposure and ageing [24].

The dorsal CN (DCN) sends fibres to the contralateral inferior colliculus (IC). Fibres from the ventral CN (VCN) innervate the ipsilateral lateral lemniscal nuclei and both sides of the

superior olivary complex [25]. The olivary complex has ascending and descending branches. The descending fibre tract is the olivocochlear bundle, which acts as a feedback loop that relays information back to Type 1 spiral ganglion cells [21]. The synapses between the olivocochlear bundle and the ganglion cells have receptors for neurotransmitters and neuromodulators. This may act as a signal that modifies the sound analysis carried out in the cochlea, decreasing basilar movements and responses of hair cells to the sound signals, and allowing saturated fibres to once again have the ability to signal changes in the incoming auditory information at higher levels [18]. The ascending branches of the olivary complex innervate the lateral lemniscus. Most of the fibres of the lemniscus end in IC, though some travel directly to the medial geniculate body (MGB) in the thalamic relay system. The fibres that reach IC then join the other fibres in MGB. From MGB, fibres travel towards the gyri of Heschl of the primary auditory cortex (A1).

MGB receives input from the auditory cortex, thalamic reticular nucleus (TRN), amygdala and striatum, thus integrating limbic structures in the processing of auditory information [25]. Basolateral amygdala also receives input from MGB and A1 and sends signals to the hippocampus [26]. Hippocampus also receives input from A1, which has connections to the prefrontal cortex.

### 1.3 Tinnitus subtypes

No agreement on a main tinnitus-causing mechanism has been reached, so the nature of tinnitus subtypes remains a topic of debate [14, 27]. One possible classification scheme involves 3 tinnitus subtypes in which tinnitus is initiated in the periphery of the auditory system but is maintained in a number of ways [1, 28]. This, however, is not the only way to classify tinnitus.

The first subtype is peripheral tinnitus. Here, increased spontaneous activity, before or in the cochlear nerve, is propagated up through the auditory pathway. Tinnitus is perceived if top-down modulation cannot suppress the increased activity levels [28]. Peripheral tinnitus may be initiated by abnormal endocochlear potentials, N-methyl-D-aspartate (NMDA) receptor activation due to ototoxic drugs, or increased glutamate release and depolarisation of auditory nerve fibres due to acoustic trauma [28].

Central tinnitus encompasses two subtypes; 1) peripheral-dependent (reliant on reduced cochlear input) and 2) peripheral-independent (self-sustaining and independent of reduced cochlear input) [29]. Instances of these are thought to be much more common than peripheral

tinnitus [28]. Central tinnitus is initiated by decreased cochlear activity through deafferentation of auditory nerve fibres. The classical explanation is that peripheral damage induces tinnitus through homeostatic plasticity processes, acting to preserve mean firing rates in the face of diminished input, which in turn elicit an increase in spontaneous firing, neural synchrony, or tonotopic map reorganisation. Other possible tinnitus-causing factors are changes in inhibitory processes in thalamic nuclei, or facilitation of non-auditory input into the auditory pathway [6, 28].

### *1.3.1 Neural gain*

Homeostasis is the maintenance of a dynamic system with a preferred range of states that allows it to function [30]. Coordination between excitatory and inhibitory firing allows gain to adjust by altering intrinsic excitability, receptor expression, neurotransmitter release volume or probability.

Effects of gain changes can be assessed in spontaneous and evoked activity. Individual neuron activity can be studied through firing rates or their synchrony with other nearby neurons. On a larger scale, firing patterns can be studied either invasively through local field potentials in animals, or with magneto-/electroencephalography (M/EEG) in humans. The caveat with M/EEG is that it encompasses both firing rates and neural synchrony. Indirectly, functional imaging techniques such as fMRI can study blood oxygenation changes as a proxy for large-scale neuronal activity.

While neural gain has been implicated in tinnitus, it is also an important factor in related conditions such as hearing loss and hyperacusis [31]. I discuss this in more detail in the "Hyperacusis" part of this chapter.

## 1.4 Problems with studying tinnitus

While the presence of tinnitus appears to stem from functional changes to the auditory pathway, it is not clear which mechanisms are required for tinnitus development, or instead are correlates of closely related conditions such as hearing loss and hyperacusis. Even if these changes do relate specifically to tinnitus, it then remains to be seen whether they are causes or consequences of tinnitus. Hearing loss and hyperacusis are associated with large-scale changes to brain function in most of the processes that have been attributed to tinnitus. Often these factors are not subject to stringent enough controls in tinnitus research to know which condition relates to any changes seen.

### *1.4.1 Hearing loss*

Hearing loss induces changes in tonotopic mapping, spontaneous firing rate and neural synchrony in the auditory pathway [32]. When presented with tones of different frequencies, the cortical BOLD response to an 8 kHz tone was significantly larger in people with high-frequency sensorineural hearing loss than controls [33]. However, this change was somewhat reduced in the presence of tinnitus alongside the hearing loss. Further, while the highest amplitude changes occurred at 8 kHz, tinnitus pitch was not related to the increased responsiveness, indicating that the changes were not specific to the tinnitus frequency. Significant functional reorganisation of A1 occurred in participants with hearing loss and no tinnitus compared to controls, but the co-presence of tinnitus seemed to be associated with reduced reorganisation [33].

#### *1.4.1.1 Overt Hearing Loss*

Sensorineural hearing loss stems from damage in the cochlea, whereas conductive hearing loss happens due to damage to the outer ear [34, 35]. Overt hearing loss results in reduced ability to hear quiet sounds or follow speech. High frequency sensorineural hearing loss is a particularly important tinnitus risk factor as the location of high frequency hair cells on the cochlea makes them more likely to be damaged [34, 36]. Eighty-eight percent of 85 consecutive tinnitus patients who visited a particular otolaryngology clinic and had undergone extended high-frequency audiometry testing had no hearing loss in low frequencies (<2 kHz), but did have high or extended high frequency hearing loss (2-16 kHz) [5]. Only 10/85 participants showed no hearing impairment at any of the frequencies tested. However, the group of patients with normal hearing was significantly younger than other groups. Alternatively, studies may have used insufficiently detailed hearing tests [37]. For example, when testing tinnitus patients with normal audiograms, 49% showed hearing impairment at a specific frequency but not at frequencies at +/- ⅓ octave different from the impaired tone. Most of these “hearing notches” were not in the octave frequencies. However, while some of the patients with a “hearing notch” in this precision-pure tone audiometry showed potential impairments in outer hair cell function, others did not, meaning that mechanisms other than peripheral damage could be related to tinnitus. Otherwise, these participants may have cochlear damage that does not impair overt hearing ability measured by pure tone audiometry thresholds.

#### *1.4.1.2 Hidden hearing loss*

Noise overexposure can lead to short-term hearing loss due to oversaturation of hair cells [24]. However, some progressive impairments in auditory nerve fibre function remain after audiometric thresholds return to normal. For example, young adults with normal audiometric thresholds for frequencies above 8 kHz but who were considered high risk for ear damage, needed significantly more hair cell activity to create action potentials sufficient to relay information to the brainstem despite normal hearing levels, possibly due to cochlear neuron degeneration [24]. Fibres with low spontaneous activity and high thresholds seem particularly vulnerable to excitotoxicity, but such damage may go unnoticed as these fibres are responsible for differentiation of sounds in loud environments [38]. While hair cell function returns to normal soon after exposure, some of the synapses remain dysfunctional, as has been shown through auditory brainstem response (ABR) studies [19]. ABR is an evoked potential that consists of a number of waves. The earliest waves represent activity generated by the auditory nerves, whereas wave V is generated by synapses between lateral lemniscal tracts and IC [39]. ABR can therefore be used to study evoked activity in different parts of the auditory pathway, both in animals and humans. Hidden hearing loss mechanisms may explain tinnitus presence in seemingly normally hearing humans, the outcome to the auditory pathway function is similar to that in overt hearing loss because both types are a form of reduced sensory input that might predispose to tinnitus. Importantly, a recent review suggested that implementing extended high frequency audiometry testing may be clinically significant as participants with tinnitus are more likely to have elevated thresholds in these frequencies compared to controls [40]. Additionally, this testing may help with early detection of susceptibility to tinnitus and early prevention programmes.

### *1.4.2 Hyperacusis*

Hyperacusis causes normal environmental sounds to be experienced as uncomfortably loud [9]. Hyperacusis is likely to occur with hearing loss and in the ageing population, though it also occurs in people with normal audiograms [26, 41]. Some studies have suggested that the presence of hyperacusis in tinnitus is related to worse reactions to tinnitus (e.g. shown by higher Tinnitus Handicap Inventory scores) [42]. As with tinnitus, increased central gain has been proposed as a mechanism for hyperacusis, but possibly through different mechanisms [43].

One of the theories is based around the loudness growth function, in which intensity is the input vs perceived loudness is the output (Figure 1.1) [43]. These models include central gain,

internal central noise and central variance playing specific roles in tinnitus and hyperacusis [31, 43]. Loudness growth models argue that a compression mechanism occurs in the ear and an expansion mechanism in the brain, which allows the transformation from intensity to perceived loudness. In normal hearing, there is a linear relationship between the base hearing threshold and a set uncomfortable loudness level (ULL). In tinnitus, there is additive noise (can occur at multiple different levels) which possibly compensates for overt hearing loss and raises the base threshold of intensity. This may create the phantom noise (but does not alter the slope of loudness by changing ULLs). This central noise may be a part of homeostasis maintenance within the brain, in part through a bottom-up process of adaptive stochastic resonance (SR). SR theory states that addition of uncorrelated neural noise allows a particular narrow range of signals that are weak, for example, due to impaired cochlea neurons, to increase via SR and reach a threshold in the nonlinear auditory system [44]. If the linear gain shifts with the new base intensity but keeps its linearity, there will be changes the ULLs at the same time along with the additive noise, and then both auditory conditions can be generated. When no tinnitus is present, and base hearing threshold (intensity) is unchanged (though there is a possibility of hidden hearing loss), multiplicative non-linear gain is necessary to alter ULLs which would then cause hyperacusis. Overall, this model argues that tinnitus is related specifically to the increased additive noise level whereas hyperacusis is related to altered gain (either linear or non-linear). There is also a more recent proposal of central variance that affects both central noise and gain [31]. Central variance is a process by which the brain alters amounts of compensation and neural synchrony that usually occur in response to hearing loss. Increased central variance reduces the amount of possible central accommodation, especially during the additive noise process in tinnitus but also in hyperacusis. The total variance of the central activity increases nonlinearly by the square of central gain, but proportionally with the increase of central noise. Neither of the resulting variance amounts are optimal for restoring normal base loudness levels or especially the normal loudness growth.

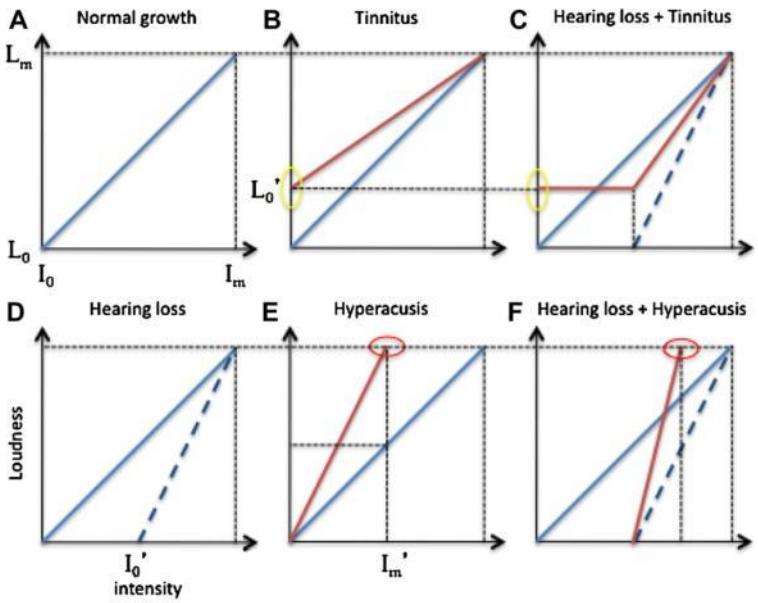


***Figure 1.1 (Found in Zeng, 2013 [43]). Loudness growth function.*** *Dashed line represented hearing loss, with Io showing the minimum threshold of intensity (x-axis) and Lo representing the minimum loudness that can be heard (y-axis). The red lines represent either tinnitus or hyperacusis effects on loudness growth function. Im shows the maximal intensity that is acceptable and Lm represents the maximal acceptable loudness. Any values with ' at the end represent altered values due to an auditory condition stated in the title of each graph.*

In a study comparing tinnitus and hyperacusis, increased responses in IC and MGB were more likely to be associated with hyperacusis than tinnitus [45]. On the other hand, increase in auditory cortical responses was related to both tinnitus and hyperacusis at 70 dB (decibel). Tinnitus dependence of A1 activation became even more prominent at 50 dB and was not related to hyperacusis. These findings complicate interpretation of much of the tinnitus literature, as both MGB and IC have been heavily implicated in tinnitus research that did not control for hyperacusis. As one of a great many examples, enhanced sound-evoked activations in right CN and left MGB of tinnitus patients were found in a study that controlled for hearing loss, but not hyperacusis [46]. Due to findings implicating IC and MGB in hyperacusis and not tinnitus [45], it is possible to argue that findings in the latter study did not really reflect tinnitus, or only partially reflected tinnitus-related subcortical changes [46].

Perhaps importantly, mild hyperacusis was measured in [45]. They noted that it was difficult to find participants with tinnitus and normal sound level tolerance, which may be a problem for hyperacusis questionnaires as most participants in their study did not self-recognise as having abnormal sound level tolerance. Authors suggested that different activation in A1 may

be due to enhanced auditory selective attention processes, or over-attention, which in turn allows tinnitus to be heard.

### *1.4.3 Animal studies*

Tinnitus research has been heavily guided by animal auditory research, but there is no standardised animal model, due to limited correlation between different measures, such as the acoustic startle reflex and conditioned behaviour [6]. A reliable diagnostic test for tinnitus in animals, however, is needed to allow tinnitus research in animals and humans to be better corroborated by findings in the two fields.

Researchers use noise trauma, ototoxic drugs such as salicylate, or conditioning to induce tinnitus in animals [1, 6]. Salicylate is an anti-inflammatory drug that, when delivered in high doses, induces temporary hearing loss, tinnitus and hyperacusis-like behaviours by inhibiting movement of the outer hair cells [41]. Salicylate also acts on spontaneous firing within the central auditory system. Upon inducing potential tinnitus in animals, behavioural tests are used to assess its presence. Animals may be trained to stop/display a specific behaviour, e.g. drinking, while background noise is present. Alternatively, behavioural tests may involve a startle reflex if a sound is presented to the animals [47]. The acoustic startle reflex is a brainstem response to a sudden loud noise. This response can be inhibited if there is a silent gap in a sound played before the startling stimulus; this is called prepulse inhibition [48]. Animals with tinnitus were argued to be less able to detect the silent gap. It is not clear whether the responses are due to tinnitus, or other factors such as hyperacusis, changes in the baseline startle response or altered temporal processing [13]. Further, human gap detection studies showed limited differences in gap detection thresholds between humans with and without tinnitus, independent of frequency of the stimuli [49].

Four main issues with animal research into tinnitus have been identified [1]:

1) Ototoxic drugs may cause damage beyond the targeted areas. As exposure to concentrated ototoxic medications in humans is rare, the damage done to the animal may not be similar to processes in the human brain [6]. Additionally, the tinnitus induced in animals is acute, whereas much of human tinnitus research is done on chronic tinnitus.
2) While noise trauma is a more accurate model of human tinnitus, results using this model have been more variable. Furthermore, noise exposure in animals is not representative of human tinnitus as it tends to be traumatic and acute, whereas noise damage in humans progresses over the lifespan [6]. Additionally, noise trauma can induce

hearing loss, which may then alter brain networks, rather than tinnitus itself being the cause of observed brain changes [50].

3) The use of anaesthesia, which alters neuronal spontaneous activity in the central auditory networks of both animals and humans [51, 52].

4) The inability to characterise perceptual and psychological attributes of tinnitus in animals. While there are behavioural indications of tinnitus presence, researchers cannot be certain that the behaviour is due to tinnitus. These difficulties undermine translation of findings into human research.

Animal studies tend to focus on small numbers of neurons, whereas large neuronal populations are usually studied in humans [6]. So, while animal studies are useful in understanding tinnitus at a fine-grained level, it may be difficult to relate them to the systems-level changes seen in humans, e.g. in EEG recordings.

Overall, animal tinnitus models show inconsistent results; results from different models do not always correlate with each other, or with human research. Animal models are further complicated by the difficulty of distinguishing effects of tinnitus from related disorders, e.g. hyperacusis, or effects of stress from noise trauma [26].

## 1.5 Tinnitus mechanisms

### *1.5.1 Spontaneous firing rates and neural synchrony*

Both spontaneous firing activity and neural synchrony have been implicated in tinnitus. An increased spontaneous firing rate allows for more neural synchrony, but the two can be differentiated, especially in thalamocortical rhythms [6].

### *1.5.2 Peripheral tinnitus*

Peripheral tinnitus stems from increased spontaneous firing rates in the inner ear. A translabyrinthine section of the cochlear nerve improved tinnitus in some tinnitus sufferers (though tinnitus was the same or worse in other tinnitus patients from the same sample groups) [53, 54]. House & Brackman (1981) suggested that this procedure was more successful in tinnitus caused by a vascular lesion or a tumour, but less successful in other subjective tinnitus [48]. Other treatments included furosemide, a loop diuretic which influences the inner ear by reducing the endocochlear potential but does not alter central auditory activity. The endocochlear potential is related to cochlear spontaneous activity and is therefore important in tinnitus perception. Oral furosemide suppressed tinnitus in 85/180

participants [55]. Patients who benefited from furosemide had vestibular schwannomas: tumours made of Schwann cells in the auditory canal of the inner ear. Notably, patients were selected on the basis of reacting positively to an injection of furosemide prior to the oral drug testing, so the data may have been biased towards a more positive outcome of the treatment.

### *1.5.3 Central tinnitus*

Central tinnitus stems from altered activity within the central auditory system. DCN, IC and MGB have often been implicated in maintaining the neural activity responsible for tinnitus perception.

DCN has been called the site of tinnitus induction, where hyperactivity is induced by reduced input from cochlea [6, 56]. DCN hyperactivity may be caused by decreased levels of glycine and gamma-aminobutyric acid (GABA), as well as an increase in glutamate transporters [57]. The main DCN output neurons are fusiform cells. Immediate tinnitus-related spontaneous firing rate increases have been noted in fusiform cells within exposed frequency regions, despite limited response threshold changes, thus suggesting that the change in firing rate was not hearing loss dependent [58]. Both increased synchronous activity and burst firing were found in fusiform cells in tonotopic regions related to tinnitus [56]. Additionally, bilateral DCN ablation prior to noise exposure in rats prevented initiation of tinnitus [59]. As DCN is earlier in the auditory pathway, its activity may also affect activity in IC and MGB [56]. However, when the animal already displayed tinnitus-related behaviours, DCN lesions did not reduce tinnitus [60]. As such, DCN hyperactivity may be important in acute tinnitus but structures higher up in the auditory system become more important in established tinnitus. Notably, DCN lesions in [60] were not complete, so it is possible that some signals allowed further transmission of tinnitus [57].

Increased IC spontaneous firing and neural synchrony have been seen in animals with tinnitus, but this initially may be dependent on the increased input from CN [1, 61]. Further, studies of IC after noise exposure often did not use behavioural testing for tinnitus, or used varying methods of tinnitus induction, which may explain some of the varied levels of IC spontaneous firing changes found [57]. Studies have also shown an increase in spontaneous firing in MGB caused by excitatory IC inputs, which drive tinnitus-related change in auditory cortex activity [62]. MGB is involved in gating of sensory information as it receives input from a variety of structures. Increased spontaneous activity and burst firing were found in MGB of rats with tinnitus compared to unexposed rats [63].

Animal models of noise trauma show that spontaneous firing rate increase appears in A1 at the later stages of potential tinnitus development [47]. Conversely, neural synchrony is enhanced in areas related to the frequencies above the tone of the trauma straight after the noise exposure. However, despite some studies linking neural synchrony to tinnitus, few studies sufficiently control for hearing loss to make any definitive conclusions [30].

#### *1.5.3.1 Thalamocortical dysrhythmia*

A popular theory states that conditions such as tinnitus, depression and Parkinson's disease may share increased theta-frequency synchrony that modifies brain organisation [64]. The coupling between theta (4-7 Hz) and gamma (35+ Hz) activity in the auditory thalamus and auditory cortex contralateral to the tinnitus ear was argued to underlie tinnitus [27]. This is because the theta burst firing allows access to thalamocortical focal information about a sound, and the sound is brought to consciousness through coupling with gamma activity [65]. This theory suggests that in limited deafferentation (<20 dB), the cortex is responsible for the tinnitus whereas in more severe hearing loss, parahippocampal memory may be responsible for retrieval of missing information. However, some studies related to thalamocortical dysrhythmia did not control well for hearing loss, as well as other potential correlates such as attention [66]. A tinnitus retraining therapy study showed that reduced gamma activity was correlated with reduced tinnitus awareness and reaction, rather than the loudness or presence of tinnitus itself [66]. Additionally, a study showed that gamma oscillations can show either positive or negative associations with perceived tinnitus loudness under different circumstances [67].

## 1.6 Evoked neural synchrony

### *1.6.1 Auditory steady state response (ASSR)*

ASSR is an evoked auditory potential which acts as a marker of the strength of auditory responses at the level of A1 input and initial processing. It is induced by repeated, amplitude modulated at 40 Hz, stimuli [1]. ASSR amplitudes rise with intensity of the stimulus but decrease as stimulus frequency increases. Larger ASSR amplitudes were seen in response to sounds below 2 kHz in people with tinnitus and hearing loss compared to controls [68]. In a different study, ASSRs in the tinnitus sample were larger than in controls when presented with 500 Hz sounds, but the opposite was seen for 5 kHz [61]. The inconsistency may have occurred because participants with tinnitus tested in [68] had sensorineural hearing loss, which may affect ASSRs differently to tinnitus. ASSR responses to 5 kHz, but not 500 Hz,

sounds were also affected by residual inhibition, which occurs when a sound similar to tinnitus frequency (5kHz in this study) temporarily suppresses tinnitus [69]. Interestingly, the tinnitus groups had increased ASSR amplitude at the tinnitus frequency. In controls, masking increased ASSR responses to 500 Hz but had no effect on responses to 5 kHz. As such, authors argued that residual inhibition modulates aberrant neural activity at tinnitus frequencies.

Successful residual inhibition was related to larger ASSR increases across frequencies compared to poor residual inhibition in participants with tinnitus, a finding that was not seen in controls [69]. A pilot study found that higher amount of stress caused by tinnitus, as assessed by THI (tinnitus handicap inventory), was associated with lower ASSR amplitudes in response to 4 kHz stimuli [70]. So, residual inhibition potentially eased the stress caused by tinnitus and the alteration in ASSR amplitudes was due to the mental state of tinnitus sufferers rather than the percept itself. However, as there were only three participants in each group, more research is needed to understand these findings.

### *1.6.2 Auditory brainstem response*

ABR represents synchronous neural activation in response to a stimulus [71]. Elevated wave V:I amplitude represents increase of central gain [30]. A computational model based on simulated and human data linked ABR responses to tinnitus perception [11]. One study showed a significant reduction in ABR wave I in the tinnitus group, but no differences in wave V between tinnitus and control groups. Researchers argued that between the auditory nerve and IC, excitatory gain was increased, and inhibitory gain was reduced in order to enhance spontaneous activity to bring activity back to the expected levels by increasing their own responsiveness. However, as a side effect of the hyperactivity in non-damaged neurons, a percept of tinnitus was generated [24]. All participants in this study had normal audiograms, arguably confirming that the high-threshold, low spontaneous activity auditory nerve fibres were damaged. However, only women were studied, thus excluding half the population. Furthermore, findings have not always been replicated in numerous similar studies that have followed, and the majority did not rule out hyperacusis as a potential factor in the findings [72] [13, 73]. Interestingly, another study found that there was a significant difference in latencies of waves III and V between participants with single feature versus multiple feature tinnitus [74]. Contrary to the previous research, a study with rat and human data showed that tinnitus was related to a failure to increase neural gain sufficiently to compensate for reduced signal to noise ratio of the input [13]. Authors suggested that reduced neural gain may lead to

lack of contextualisation of the auditory information by attentional and stress-regulating networks. Despite the disparity, longer latency and reduced amplitude of wave I in tinnitus with normal hearing has been a consistent finding in past research, though this may indicate presence of hidden hearing loss in tinnitus participants [71]. Human studies described above are cross-sectional. On the other hand, a longitudinal study with a large cohort indicated that, once confounding factors such age sex, age, hearing and hyperacusis are controlled for, only increased wave V latency in the left ear is associated with the presence of constant tinnitus, whereas wave I results were very affected by transducer systems [75]. The increase in wave V latency was very small, and may have occurred due to some residual differences in hearing loss not fully corrected for by the analysis.

In mice, noise trauma could predict (accelerated) loss of spiral ganglion cells in the future [76]. As animal studies are carried out on a microscopic level cochlear damage may not yet be noticeable in younger human groups but may have had the tinnitus-inducing effect from the temporary peripheral damage and potential ongoing progression of the impairments.

## 1.7 Neurochemistry

Balance between excitatory and inhibitory neurotransmission is implicated in tinnitus [56]. Some of the main neurotransmitters involved in tinnitus are glutamate and GABA.

### *1.7.1 Glutamate*

NMDA receptors are glutamate receptors that have been implicated in higher cochlear spontaneous firing rates, especially in animal studies using the salicylate model of tinnitus. A biochemical model of peripheral tinnitus has been proposed, in which glutamate is released either spontaneously or in response to a stimulus and binds to NMDA receptors [77]. Opioid peptides are released during physical or emotional stress from efferent terminals of the olivary complex and potentiate the excitatory abilities of glutamate at NMDA receptors. This excitation may then travel up through the auditory pathway, affecting central firing rates as well. This enhances neural responses to stimuli or increases spontaneous firing rates. Redistribution of NMDA receptors in fusiform cells in MGB has also been implicated in reduced inhibition of their spontaneous firing [56].

### *1.7.2 GABA*

Two theories of GABA-related abnormalities have been associated with tinnitus. The more widely accepted is the gain control theory, in which tinnitus is associated with reduced

thalamic inhibition [62]. Reduced inhibition enhances thalamocortical synchrony, potentially altering the functionality of TRN gating. Decreased GABA and glutamic acid concentrations were found in MGB in rats with tinnitus [59]. As extrasynaptic GABA receptors in the sensory thalamus contribute to the tonic inhibitory postsynaptic activity in the thalamic neurons, the reduced concentrations of GABA in MGB may allow tinnitus signals to travel up the auditory system [62, 65]. However, enhanced tonic inhibition caused by an increased concentration of extrasynaptic GABA receptors in MGB was found 2 months post-noise exposure, leading researchers to argue that increased tonic inhibition leads to abnormal burst firing rates as shown in the thalamocortical dysrhythmia theory [78].

### 1.8 Noise cancelling mechanisms

As not everyone with cochlear damage experiences tinnitus, and some people with tinnitus have clinically normal hearing, non-auditory regions may be involved. Underactive gain control via the TRN may allow the tinnitus signal to become consciously perceived [79]. In this model, the subcallosal area (including nucleus accumbens (NAc) and ventromedial prefrontal cortex (vmPFC)) is involved in cancellation of the tinnitus percept signal at the thalamic level by linking limbic systems with thalamocortical perceptual systems through gain control. NAc and its ability to modulate limbic systems through serotonergic neurons aid in evaluation of emotional significance and relevance of stimuli, thus potentially being able to filter out irrelevant signals such as tinnitus [6]. NAc and vmPFC are interconnected and have been related to inhibitory control. VmPFC exerts modulatory influence on NAc and the auditory system, which is reduced if vmPFC becomes dysfunctional [80]. Subcallosal serotonergic neurons innervate TRN, telling it when to inhibit thalamic relay neurons. TRN controls the firing modes of relay neurons of MGB, which transmit signal from IC to the auditory cortex. Furthermore, TRN can act on very specific modalities and topographical areas, thus allowing it to accurately control gain for only specific sound frequencies. So, if these brain regions are well functioning, subcallosal regions can use TRN to reduce the gain in the tinnitus-related frequencies. Limbic region failure after cochlear damage, however, allows tinnitus to be perceived.

P50 is an evoked potential that indicates responses to redundant stimuli. Noise cancellation has been studied through a p50 paired stimulus paradigm, where the first response indicates initial responsiveness, and the ratio between the first and second tones indicates sensory gating ability. Notably, in a study on participants with tinnitus and normal hearing, p50 component recordings suggested that reduced inhibition of sensory gating compared to

controls was related to tinnitus severity [81]. P50 responses in mild tinnitus were comparable to controls. Therefore, it is possible that underactive gating may be related to more severe tinnitus, rather than tinnitus in general. A different interpretation of the results has been suggested, where participants with tinnitus have persistently active gating even after the first stimulus, unlike controls who dynamically activate gating after the first stimulus, unlike controls who dynamically activate gating after the first stimulus [30].

### 1.9 Predictive coding

Predictive coding may unite the different mechanisms that potentially lead to subjective tinnitus, or at least help explain why some but not other individuals develop tinnitus under seemingly equivalent predisposing conditions such as hearing loss [82]. Predictive coding explains perception as a hierarchy of inferences about environmental states. From the lower levels, the auditory system receives sensory input. Higher level predictions about the sensory input are based on prior beliefs and expectations. The bottom-up sensory information ascends through the auditory pathway via excitatory neurons, and top-down predictions are relayed down by inhibitory neurons [83]. At each level, the expected state and the incoming sensory information are compared by error units (Figure 1.2), with discrepancies between the two generating a prediction error signal. Prediction error is precision-weighted; precision reflects how confident the system is in the prior belief. Precision is the postsynaptic gain that results from the interaction between ascending and descending neurons. Postsynaptic gain is modulated by involved networks, such as attention, memory or emotion [30, 79, 84]. The more synchronous the signalling of other networks is, the higher the effect of precision is as such activity would be more likely to reach a threshold of a prediction error signal. If the expected and the incoming sensory information are not fully matched, a prediction error is generated. The error signal allows the higher levels to adjust their predictions, forming a new (posterior) expectation that is better matched to the sensory information. This updating of generative models based on comparing their predictions to incoming sensory signals *is* perception. This way, the next comparison has more precision, through the newly formed expectation that is better matched to the sensory information. There has been much empirical support for predictive coding. Just one example of the plethora of possible examples was a study on single neurons in an auditory oddball paradigm showed a hierarchical organisation of prediction error signals that ascend up the auditory pathway [85]. Increase in prediction error values occurred as the signal travelled from IC to MGB and to the auditory cortex. These prediction errors were responsible for generating large-scale responses to auditory stimuli.

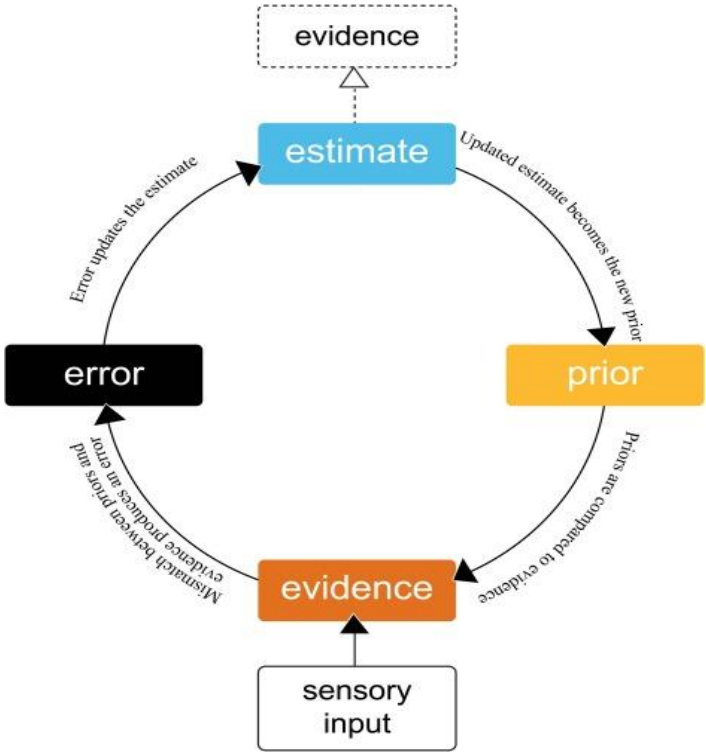


***Figure 1.2 (Found in Hullfish et al, 2019 [82]).*** *Sensory input is presented as evidence to be compared to the prior belief. If there is a mismatch between the two signals, a prediction error is created, which updates the estimate information. Estimate information is relayed to the next level in the hierarchy as the evidence, where the same process will occur. The new estimate at the current level also becomes the new prior for the next time such sensory input is presented at this level.*

The Sensory Precision Integrative Model of Tinnitus is based on predictive coding [83]. Spontaneous activity is always present in the auditory system; however, its signal tends to be incoherent and weaker than most externally-generated sensory input [86]. According to this model, “tinnitus precursors” are prediction errors generated by spontaneous activity in the auditory pathway. As the tinnitus precursors are not correlated with any sensory signals from the external environment, and have no intrinsic behavioural relevance, they have low precision. As such, when compared against top-down sensory predictions, the tinnitus precursor is usually inhibited, i.e. explained away as noise. However, a tinnitus precursor may become too intense or be given too much precision, leading to a false rejection of the default null hypothesis of silence. A number of reasons for increased intensity of a tinnitus precursor could be accounted for by this model, for example increased firing rates and central gain due to cochlear damage, or reduced TRN inhibition due to affective and gating influences.

Precision-weighted prediction errors are reflected by gamma oscillations [65]. As such, there is an inverse relationship between gamma amplitude and tinnitus precursor loudness.

Once the tinnitus precursor is perceived, eventually, the repeating rejection of silence may create a new default prediction of tinnitus. One potential explanation for this is that the tinnitus precursor gains enough precision to be perceived (or enough for the consequent tinnitus prior to be perceived). In that view, silence is simply the absence of any auditory percepts. Alternatively, the more precision is given to the tinnitus precursor, then less weight is assigned to bottom-up influences that attempt to relay the silence in the environment.

Learning, such as associative plasticity (e.g. via connections with parahippocampal cortex) at higher levels, may allow the perception of tinnitus to continue even after the factors that increased the intensity, and/or precision, of the precursor are removed, and are argued to be particularly important in cases of severe or profound hearing impairment [65]. Notably, computationally the model requires the tinnitus-inducing factors to only be temporarily present while learning takes place. This therefore would account for people with tinnitus who do not have a history of lasting cochlear damage, or reorganised tonotopic maps [73, 86]. Although these changes in the tinnitus precursor (which may manifest as altered spontaneous neural activity in the forms of firing rates, neural synchrony, large-scale oscillations, and metabolic or blood flow changes), may be temporary, and therefore may not be detectable with conventional neuroimaging methods, the skewed default predictions may be detectable as altered prediction error responses to specific auditory stimuli around the tinnitus frequency. One such commonly used measure of prediction violation is mismatch negativity (MMN), which is widely used in neuroscience research, and is detectable with EEG.

### *1.9.1 Mismatch Negativity*

MMN is an evoked potential that indicates sensory change detection based on recent sensory context, irrespective of whether attention is aimed away or towards the stimulus [87-89]. It is most often elicited using auditory stimuli. It is represented in EEG data as a negative waveform component, which for auditory responses is located over the vertex. MMN is usually studied using oddball paradigms. In the oddball paradigms, a number of standard stimuli are repeated, with an unexpected deviant stimulus embedded in between repetitions. It is calculated by subtracting the responses to standard auditory events from the response to a deviant auditory event, around 150-250 ms after the onset of the deviant [87, 90]. However, small perceptual changes, as well as different types of deviants (frequency, intensity, duration, location and silent gap), can create delayed and prolonged MMN curves, so the time frames in

which MMN is studied may differ depending on the paradigm [91]. Echoic memory may underlie MMN, as it allows comparison between the incoming information and recent events and appears as a result of changes in extrinsic and intrinsic connectivity in bilateral primary auditory cortices, superior temporal gyri and the right inferior frontal gyrus [92].

Predictive coding encompasses two theories of MMN [87]. The first is the Model Adjustment Hypothesis. MMN is commonly considered to be a neural correlate of error detection as it indicates a break of regularity of the stimuli (so the two signals are mismatched). In this hypothesis, the fronto-temporal network allows top-down modulation (predictions) to interact with sensory inputs and create a prediction error that results in an MMN response. Second is the Adaptation Hypothesis, in which the neurons of the auditory cortex adapt and attenuate N1 responses during repetitions of the standard stimuli, thus appearing as a separate response. However, MMN appears even in the absence of an N1 response and has a different scalp distribution to N1 [87]. According to predictive coding, the prediction error from the model adjustment hypothesis is created by synaptic plasticity changes in the adaptation model.

Auditory event-related potentials, including MMN, may differ in chronic tinnitus participants from normal hearing, age and gender matched controls (with similar hearing threshold on a group level, but not individually matched) across all deviant types [91]. However, differences in peak MMN response latencies were not consistent across deviant types. For example, in the study cited above where participants used frequencies around 1 kHz, MMN amplitudes were found to be smaller for frequency, duration and silent gap deviants in tinnitus participants compared to controls. Authors suggested that the differences between groups revealed abnormal central auditory processing and impaired pre-attentive sensory memory. These results have been supported by studies with similar MMN paradigms, which elaborated that people with bothersome tinnitus had smaller MMN amplitudes than either non-tinnitus controls or people with non-bothersome tinnitus [93]. However, not all studies found differences between tinnitus and non-tinnitus groups using MMN. For example, in a frequency deviant paradigm with normal hearing participants within the non-extended audiometry testing range, where the standard was 1 kHz tone and the deviant was a 1.1 kHz tone, no statistically significant differences in MMN amplitude were found [94]. This may be because the tone frequency was much lower than usual tinnitus frequencies, so the processing of this particular sound was unaffected.

## 1.10 PhD project background research

To understand the inconsistencies between previous MMN studies, and in light of the development the principled hypothesis about intensity deviants based in the Sensory Precision Integrative Model of Tinnitus, an experiment was developed to investigate a potential biomarker that could be used to bridge human and animal research. A roving paradigm was used to explore MMN in people with chronic tinnitus compared to age and hearing matched controls [95]. The roving paradigm is a type of oddball paradigm, but with two types of standard stimuli, and where deviants are defined as pseudo-random transitions between one standard type and the other (Figure 1.3). The high intensity (loud) standard was interrupted by a quieter (downward) deviant, while a low intensity (quiet) standard was interrupted by a louder (upward) deviant. The stimuli were further divided into centre frequency of tinnitus of each participant and edge frequency. The edge frequency of tinnitus was just noticeably below the tinnitus range of each participant. The two stimulus frequencies were usually less than ¼ octave apart in the original paradigm. The stimuli were 300 ms pure tones, except for randomised 150 ms duration deviants which played instead of a standard tone. Interstimulus intervals lasted 300 ms. Each experimental block was based fully on one of the two frequencies. The EEG recording session lasted about an hour overall.

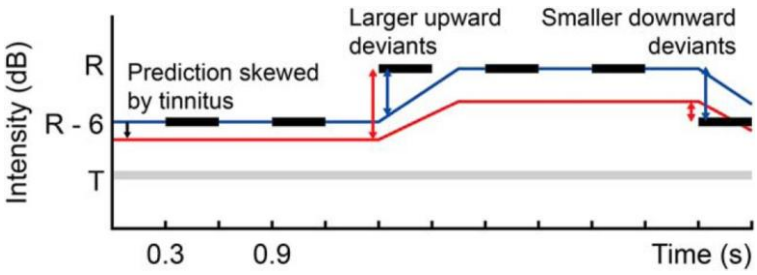


***Figure 1.3 (Found in Sedley et al, 2019 [95]). The experimental paradigm used in the original study.*** *Black bars represent the pure tone stimuli, gaps between which represent the inter-stimulus intervals. R is the intensity chosen by the participant as the loud intensity for each tone. Intensity roves between R and -6 dB (y-axis). The blue line represents the hypothesised optimal predictions for the intensity of the next stimulus based on the previous stimuli, which should be made by participants without tinnitus. However, a mixture between the tinnitus intensity (grey line) and the optimal prediction might be expected to create an intermediate prediction (red line) of a tone intensity, which is created because tinnitus intensity and stimulus intensity both exert influence on the net prediction. Due to the prediction of an intermediate intensity in participants with tinnitus, the prediction error in response to an upward deviant is larger (when stimuli become relatively further from that prediction), while the prediction error to a downward deviant is smaller (where stimuli become more like the prediction).*

Further studies were carried out using this paradigm. These were 1. Studying participants with acute tinnitus (started <4 weeks prior to testing), 2. Studying people without tinnitus, for whom the experience of tinnitus was simulated through a sound that was added in the background during the roving paradigm.

Participants with tinnitus had larger MMN responses to upward deviants, but smaller MMN responses to downward deviants, compared to the control group (Figure 1.4), in line with the motivating hypothesis. No relationship was seen between MMN, and THI or numerical rating scale (NRS) loudness score of tinnitus loudness; this finding, termed ‘Intensity Mismatch Asymmetry’ (IMA) did not appear to be reliant on negative feelings about tinnitus or hyperacusis. The working interpretation was that downward deviant stimuli sounded more similar in intensity to the default prediction of tinnitus intensity, so people with tinnitus showed a reduced response to this change as it was a more expected sound. However, the upward deviant was further from the default prediction, thus making the MMN response larger. Area under the receiver operator characteristic (ROC) curve was 0.77 (fair diagnostic accuracy). Analysis of the group with acute tinnitus showed a main effect of upward intensity

deviants yielding larger MMN responses than downward deviants, similar to those with chronic tinnitus. No such findings were shown in simulated tinnitus, showing that it is not just any background noise that could skew brain responses to the paradigm.

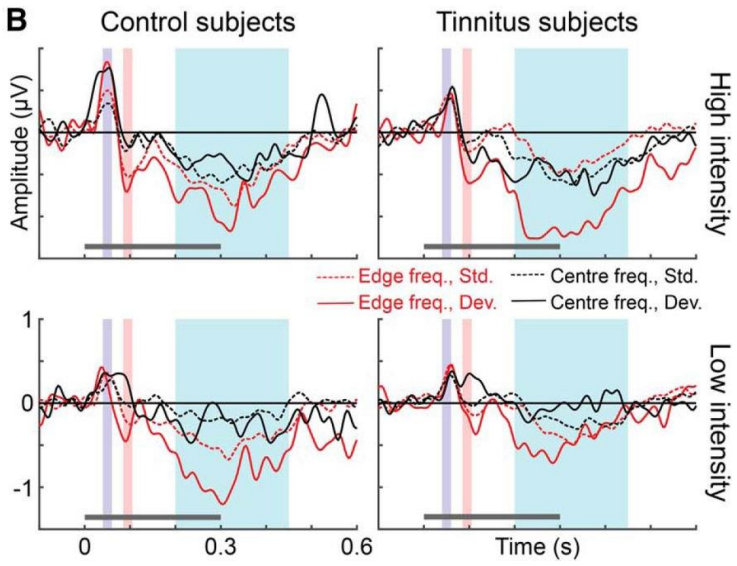


***Figure 1.4 (Found in Sedley et al, 2019 [95]). MMN response waveforms.*** *The waveforms (amplitude indicated on y-axis) from FCz electrode location are shown in the blue-coloured time-window (x-axis). Dashed lines are the responses to standard tones and solid lines are the responses to deviant tones. Red lines show responses to the centre frequency and black lines represent responses to the edge frequency. Grey horizontal bars show when the stimulus tone was played.*

### *1.10.1 PhD project aim*

The heterogeneity of potential tinnitus causes described previously may explain why finding a treatment for tinnitus has been difficult [96]. The heterogeneity of tinnitus mechanisms, and the existence of specific subtypes of tinnitus, remains controversial, and therefore it is highly possible that many potential biomarkers of tinnitus are applicable only to certain individuals. As such, there is a need for a biomarker that would indicate the presence of tinnitus across all the potential subgroups. This would need to relate to mechanisms forming part of a 'final common pathway' for tinnitus, irrespective of specific contributory mechanisms. Such a biomarker might help to better understand tinnitus mechanisms and allow treatment studies to determine the effectiveness of their treatment across tinnitus groups. A biomarker based on the Sensory Precision Integrative Model of Tinnitus in humans may contribute towards this as it encompasses the variety of causes of abnormal neural activity [83]. It may also be possible to translate this biomarker into animal models. However, prior to this, we need to understand

the mechanisms and any possible confounds of this approach. For example, understanding whether hyperacusis, attentional direction or specific paradigm contexts may affect the MMN responses to the IMA paradigm.

The primary aim of this PhD was to work towards a neurophysiological biomarker of tinnitus based on intensity mismatch asymmetry, through the replication of previous findings, and the systematic investigation of the impact of differences in subject traits and states, and of features of the paradigm design, on the results obtained.

Due to the pandemic that occurred during the earlier stages of this project, a part of the focus was diverted to an online sound therapy study for tinnitus. This remained relevant, however, as the creation of the sound modulation was also based in predictive coding as a motivating principle (though not reliant on it).

# Chapter 2. Nuances in intensity deviant asymmetric responses as a biomarker for tinnitus

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## 2.0 Introduction

Subjective tinnitus is a persistent sound heard by an individual without an environmental source, which may appear as pure tone, ringing, hissing, whistling, static, or cicada-like sounds [1]. Due to the variety of potential causes of subjective tinnitus, classification of this condition has been difficult [97]. Heterogeneity of tinnitus mechanisms, along with the existence of specific categories, or even a continuous spectrum of tinnitus, remains controversial [14, 27, 98]. It is possible that many potential biomarkers of tinnitus and treatment options are applicable only to certain individuals [99-102]. As such, there is a need for a biomarker that would indicate the presence of tinnitus across all the potential tinnitus subgroups or dimensions. This would need to relate to mechanisms forming part of a 'final common pathway' for tinnitus, irrespective of specific contributory mechanisms. Such a biomarker might help to better understand tinnitus mechanisms and allow treatment studies to determine the effectiveness of their treatment across different tinnitus groups [103]. It may also be possible to translate this biomarker into animal models, which would then allow the differentiation of hearing loss, hyperacusis and tinnitus more accurately than presently possible, and thus improve tinnitus research in animals [1].

A biomarker based on the Sensory Precision Model of Tinnitus in humans might contribute towards this goal of a single invariant biomarker, as it encompasses the variety of causes and contributors, and specifies a single mechanism through which they interact to cause tinnitus [83]. The Sensory Precision Integrative Model of Tinnitus is based on predictive coding [83, 104]. In this model, spontaneous activity is always present in the auditory system; however, its signal tends to be incoherent, and weaker than true sensory input [104]. According to this model, such a 'tinnitus precursor' generates spontaneous prediction errors. As the tinnitus precursor is not correlated to internal or external events, and are not behaviourally relevant, it has low 'precision'. As such, when compared against top-down prior predictions, or competing bottom-up inputs, the tinnitus precursor is usually explained away as noise. However, a tinnitus precursor may become sufficiently intense, or be given too much precision, leading to a false rejection of the default null hypothesis of 'silence'. Accepting the tinnitus precursor as a true 'signal' thereby reduces the prediction error it generates. Once the

tinnitus precursor is perceived, eventually, the repeated rejection of silence as the baseline perceptual state may create a new default prediction of tinnitus. Associative plasticity and other forms of learning at higher levels (e.g., parahippocampally mediated memory) might allow the perception of tinnitus to continue even after the factors that increased the intensity, and/or precision, of the precursor are removed. Although these changes in the tinnitus precursor (which may manifest as altered spontaneous neural activity in the forms of firing rates, neural synchrony, large-scale oscillations, and metabolic or blood flow changes), may be temporary, and therefore might not be detectable in the long-term with conventional neuroimaging methods, the skewed default predictions may be detectable as altered prediction error responses to specific auditory stimuli around the tinnitus frequency. One such commonly used measure of prediction violation is the mismatch negativity (MMN). MMN is an event-related potential that indicates auditory change detection based on recent auditory context, irrespective of whether attention is aimed away or towards the stimulus [87, 89, 105].

Motivated by the Sensory Precision Integrative Model, Sedley et al (2019) used a roving intensity paradigm to elicit MMN in people with chronic tinnitus compared to age and hearing matched controls[95]. The roving paradigm is a type of oddball paradigm, but with two types of standard stimuli where deviants are defined as pseudo-random transitions between one standard type and the other. The high intensity (loud) standard was interrupted by a quieter (downward) deviant, while a low intensity (quiet) standard was interrupted by a louder (upward) deviant. The findings indicated that, in response to sounds of a frequency that was similar to their tinnitus, participants with tinnitus had larger MMN responses to upward deviants, but smaller MMN responses to downward deviants, compared to the control group. No relationship was seen between MMN, and Tinnitus Handicap Inventory (THI) [106] or visual analogue scale of tinnitus loudness score (subjective measure of tinnitus loudness); this finding was termed ‘Intensity Mismatch Asymmetry’ (IMA). The hypothesis was that downward deviant stimuli sounded more similar in intensity to the default prediction of tinnitus intensity, so people with tinnitus showed a reduced response to this change as it was a more expected sound. However, the upward deviant was further from the default prediction, thus making the MMN response larger. However, because only stimuli at or close to the tinnitus frequency (or frequency band) were tested, it is not known whether this asymmetry of intensity mismatch responses in people with tinnitus compared to controls is specific to the tinnitus frequency or generalised across frequencies.

The current study attempted to replicate the findings of the original roving intensity paradigm, with an addition of a control frequency that is far from the tinnitus frequency, to see whether

differences seen between tinnitus and control subjects were: 1) replicable, and 2) frequency-specific.

## 2.1 Materials & Methods

### *2.1.1 Participants*

Volunteers with tinnitus (N=14) were recruited from affiliated volunteer lists at Newcastle University. The sample size was small largely because this study was seeking the kind of large and invariant effects indicated in the previous study (1), and whether those findings could be helpful in another group. To be included, participants needed to be over 18 years of age, with chronic tinnitus for over 6 months that did not have a physical source and was not due to Meniere's disease, who could make an informed choice about volunteering. Exclusion criteria included using ongoing sedating or nerve-acting medications, and mental health conditions severe enough to interfere with everyday life activities. Non-tinnitus participants were recruited using the same mailing lists, and individually matched to tinnitus participants, based on an approximate match of their overall audiometric profiles, with particular attention to the vicinities of 1 kHz and the tinnitus frequency. It was also ensured that there were no significant group differences between tinnitus and control groups in age or sex.

Recruitment and data collection occurred between November 2019 and June 2021. Participant data was anonymised after data collection with the use of a participant number. Approval was given by the Newcastle University Research ethics committee, and all participants gave written informed consent according to the Declaration of Helsinki (reference number 5619/2020).

### *2.1.2 Psychophysical assessment*

All research activity took place within the Auditory Cognition Lab, Newcastle University. Subjects completed a short demographic questionnaire, with additional questions about any health conditions or medications, and the Hyperacusis Questionnaire (HQ)[107]. Participants with tinnitus also completed THI. All participants underwent pure tone audiometry at 0.25, 0.5, 1, 2, 4, 6, and 8 kHz.

Tinnitus participants underwent two computerised tasks, performed under supervision. In the first, they performed 5 rounds of tinnitus matching of a random sound generated by Matlab (The MathWorks). In each round of matching, they tuned an ongoing synthetic band pass noise stimulus with random starting parameters in real time in frequency, bandwidth,

intensity, and laterality balance. At the narrowest bandwidth, the stimulus became a pure tone. In cases of bilateral tinnitus, the participants heard the sound in both ears, and could adjust the ear balance. The intensity was based on an inverse Fourier transform of a Hanning spectrum noise, with peak amplitude at the centre frequency equal to 1. It was not a specific dB value, and it was always a relatively quiet stimulus which subjects needed to increase in intensity for the match.

Participants could discard any matches they felt were not close to their tinnitus. The average of the remaining matches was taken to be used in the second task as an indicative tinnitus match, to form the starting point for individual experimental stimulus determination. In this next task, tinnitus participants were presented with pure tones whose frequency was determined based on their average tinnitus match. Then, the experimental stimulus frequency for this experiment was determined using the same process as the edge frequency calculation (i.e., the lower spectral edge of the tinnitus match) in the original experiment, because the IMA effect was stronger when participants were presented with the edge frequency rather than the centre frequency of their tinnitus [95]. To achieve this edge frequency, participants were asked to ensure that it was slightly below the lower spectral edge of their tinnitus (i.e., that they could discern the tones and their tinnitus as two distinct non-overlapping sounds). They were able to adjust the frequency of these tones, if needed, until they were satisfied that they had found the edge frequency of their tinnitus. They were then asked to adjust this sound to a comfortable but loud volume. They then adjusted the intensity of 1 kHz pure tones until they matched the subjective loudness of their tinnitus edge frequency tones. These two stimulus intensities (one for tinnitus edge and one for control frequency) were designated the ‘high’ stimulus intensities for the main experiment, with ‘low’ intensities set 6 dB lower than this. A final check was performed to ensure that subjects could hear both ‘low’ intensity stimuli and distinguish them as subjectively quieter than the ‘high’ intensities. In cases where these criteria were not both met, subjects could increase or decrease the intensity of the ‘low’ intensity stimuli, to ensure that they were both audible and differentiable from the ‘high’ intensity stimuli. In other cases, the 6 dB intensity difference was maintained. Control participants were allocated the same experimental frequency as their matched tinnitus subject and had full control over stimulus intensities as for the tinnitus subjects.

### *2.1.3 Experimental design*

EEG was recorded in a soundproof room, using a 64 channel Active two system (Biosemi). No EOG (extra-oculogram) channel was applied as the standard EEG channels were sufficient

for removing ocular artifacts. Participants watched a silent subtitled movie of their choice, while the stimuli were played to them through headphones. Electrode offset was kept at manufacturer-recommended limits of +/- 10 mV.

The experimental design closely followed the paradigm used in the original study [95], with the additional inclusion of a 1 kHz tone as a control condition. The roving paradigm employed in this study is a type of oddball paradigm, but with two types of standard stimuli, and where deviants are defined as pseudo-random transitions between one standard type and the other every 4 to 8 stimuli. Stimuli were 300 ms tones, with 10 ms onset/offset ramps, followed by 300 ms inter-stimulus intervals. The tones were presented isochronously to the ear(s) that the tinnitus participant indicated as the tinnitus ear(s), or the same ear(s) for their matched control. For example, if the participant with tinnitus only had tinnitus in their right ear, the matched control would also only hear the tones in their right ear. The high intensity (loud) standard was interrupted by a quieter (downward) deviant, while a low intensity (quiet) standard was interrupted by a louder (upward) deviant. There was also a duration deviant condition, in which a duration deviant tone of 150 ms was followed by a 450 ms gap every 1 out of 10 stimuli. The purpose of duration deviants was to assess for the presence or absence of more general auditory mismatch detection differences associated with tinnitus.

### *2.1.4 EEG data processing*

Data analysis was performed in Matlab, using the EEGLAB toolbox [108]. Data were downsampled to 256 Hz from the original 1024 Hz, and re-referenced to combined P9/P10 channels, approximating to linked mastoids. Data were then filtered using a high-pass cut-off of 0.3 Hz and a low-pass cut-off of 25 Hz. Bad channels were removed using 0.8 as the minimum acceptable correlation with nearby channels. The removed channels were then reconstructed through interpolation. Data were then epoched between -0.1 and 0.5 s peristimulus time. Denoising Source Separation [109] was used as to remove artefacts. The first four components were retained for all subjects, based on prior inspection of all subjects' data in order to achieve an optimal balance between preserving signal and eliminating noise. The data were then put through EEGLAB automatic artefact rejection using probability of 5 and kurtosis of 8. The epochs were baseline corrected to -100-0 ms peristimulus time.

### *2.1.5 Statistical analysis*

Statistical analysis was performed using MATLAB. To compare the evoked responses in participants with tinnitus and controls, a three-way ANOVA was used, with subject group,

frequency, and intensity used as factors of interest, and including interaction terms. Additionally, two-way ANOVAs were used to look at each frequency separately due to differences between the two frequencies potentially overshadowing any differences between subjects and intensities. Additionally, two-way ANOVAs were carried out to look at the duration deviants.

## 2.2 Results

### *2.2.1 Demographic information*

Each volunteer sample comprised 14 right-handed participants (overall N=28), matched based on age, sex and hearing measured with pure-tone audiometry. The age and hyperacusis information for all participants can be found in Table 2.1. In the tinnitus group, 5 participants had hyperacusis, according to the HQ score, applying a more stringent but potentially more sensitive threshold (HQ score >16) [110].

Tinnitus matches, as well as general information about the condition in the tinnitus group can be found in Table 2.2. According to the THI, tinnitus was causing a slight problem to four participants (<16 THI score), a mild problem to seven participants (18-36), a moderate problem to two participants (38-56) and a severe problem to one participant (58-76). Tinnitus duration ranged from 4 to 60 years. The duration was skewed to the left, so the mean (21) was lesser than could be expected in a normal distribution. Seven participants described hearing their tinnitus centrally/equally between the ears; two participants had tinnitus more in the right ear than the left; one had it more in the left ear than the right; two had it mainly in the left ear; finally, two participants had it entirely in the left ear. Eleven participants described their tinnitus as whistling/ringing/pure tone. One person indicated having both ringing and hissing sounds. One person stated they had hissing/static. The last person described their tinnitus as ‘staccato sounds’. Half the sample said their tinnitus fluctuated over days/weeks. Nine participants indicated that their tinnitus became worse during/after being in loud environments. The average tinnitus edge frequency in Table 2.2 was the frequency chosen by the participant to represent the lowest frequency of their tinnitus.

| Demographics | Tinnitus Group Mean (Standard Deviation) | Control Group Mean (Standard Deviation) | Comparison (Mann-Whitney U) |
|---|---|---|---|
| **Age** | 52.64 (21.24) Range: 20-80 | 50.57 (18.34) Range: 22-70 | p=0.734 |
| **Hyperacusis** | 16.57 (7.83) | 6.64 (4.99) | p = 0.001* |

***Table 2.1. Age and hyperacusis results in both groups, which were non-normally distributed.*** *Significant differences between the groups highlighted with *.*

| Measure | Mean (Standard Deviation) |
|---|---|
| THI | 27.29 (16.69)* |
| Duration (years) | 21 (19.16) * |
| Loudness on average (0-10) | 5.14 (1.83) |
| Bothersomeness (0-10) | 4.57 (2.65) |
| Loudness on the day (0-10) | 5.57 (1.56) |
| Aware of tinnitus in the day (%) | 51.79 (33.43) |
| Average tinnitus edge frequency (Hz) | 5075.91 (1747.52) |

** Indicates non-normally distributed data, according to Shapiro-Wilk's test.*

***Table 2.2. Tinnitus questionnaire findings.*** *Loudness, and bothersomeness were measured using a scale 0 to 10, with 0 being not at all and 10 being the most possible. Tinnitus awareness was measured out of 100%, 100% representing the entire day.*

There were no significant differences in hearing thresholds between groups at any of the frequencies measured (p = 0.851; p = 0.138; p = 0.753; p = 0.627; p = 0. 216, p = 0.085; p = 0.087, for frequencies in ascending order) (Figure 2.1). Thresholds at each tinnitus frequency linearly interpolated for each individual were also not significantly different between groups (p=0.271).

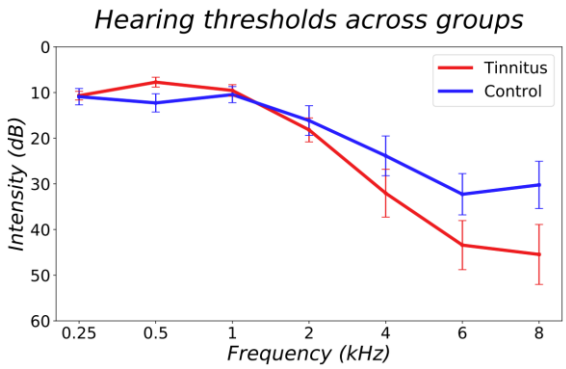



***Figure 2.1. Mean hearing threshold of the tinnitus (red) and control (blue) groups at 0.25, 0.5,1,2,4,6 and 8 kHz.*** *Tested using a pure tone audiometer. Data for each frequency, except 2 kHz, was not normally distributed.*

In the tinnitus group, 5 participants left the relative difference in intensity between loud and quiet stimuli at -6 dB, with the rest adjusting the difference in intensity. In the control sample, 8 participants left the difference in the volume of the stimuli as set by their counterpart with tinnitus; 4 control participants also left the difference at -6 dB. Nonetheless, the mean dB difference between the loud and quiet stimuli was not significant between the tinnitus and control groups (Man U test, p =0.376). To investigate any systematic differences in the stimulus intensities between the two groups, sensation levels were calculated, i.e. the difference between hearing threshold (dB HL) and stimulus intensity (db SPL) as measured by a sound level meter (Table 2.3).

| | Tinnitus Group Mean | Control Group Mean |
|---|---|---|
| 1 kHz (dB HL) | 9.55 (5.96) | 10.45 (8.30) |
| 1 kHz (dB SPL) | 56.01 (10.06) | 63.56 (9.93) |
| 1 kHz sensation level (dB) | 46.46 (10.03) | 53.11 (12.16) |
| Edge frequency (dB HL) | 34.35 (21.80) | 27.33 (19.88) |
| Edge frequency (dB SPL) | 68.31 (18.36) | 75.48 (17.32) |
| Edge frequency sensation level (dB SL) | 33.96 (15.45) | 48.15 (20.02) |

***Table 2.3 Audiometry table.*** *The edge frequency audiometric threshold was calculated linearly for each participant. dB HL represents the mean PTA thresholds for each group; dB SPL represents the mean intensity at which the stimuli were set to play through the headphones by the participants in each group; dB SL represents the mean perceived intensity by the participants (dB SPL – dB HL).*

In the right ear, there was no significant difference in the sensation levels between the tinnitus and control groups at 1 kHz (mean = 47.95 and 53.00, respectively; $t(34) = 0.038$, $p = 0.113$), but there was a significant difference at the tinnitus edge frequency (mean =31.40 and 52.85, respectively; $t(34) = 1.74$, $p < 0.001$).

In the left ear, there were significant differences in the sensation levels between tinnitus and control groups at both 1 kHz (mean = 45.43 and 53.18, respectively; $t(50) = -2.26$, $p = 0.029$) and tinnitus edge frequency (mean = 35.74 and 44.90, respectively; Mann-Whitney U, $p = 0.011$). Overall, the tinnitus group received stimuli with lower sensation levels than the control group. These findings were not related to HQ scores.

### *2.2.2 Spatiotemporal organisation of stimulus response*

Grand average ERP data for channel FCz across all stimulus conditions and subjects (Figure 2.2 a) was used to determine timeframes for quantifying P50, N100 and MMN responses (Table 2.4), based on visual inspection. Difference waveforms were calculated by subtracting standard responses from their equivalent deviant conditions.

| ERP | Timeframe (ms) |
|---|---|
| P50 | 40 – 75 |
| N100 | 93 – 114 |
| MMN | 140 – 243 |

***Table 2.4. ERP timeframes.*** *Timeframes for analysis of P50, N100 and MMN ERPs based on figure 2 a).*

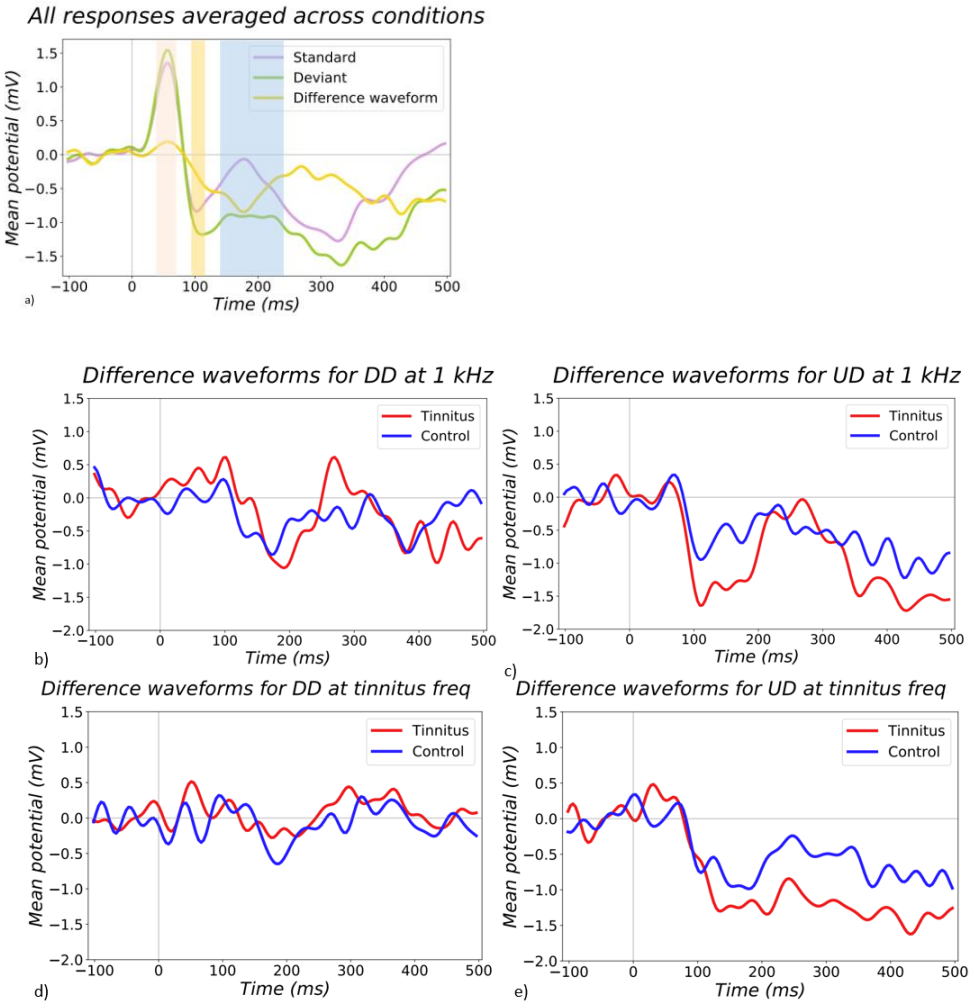



***Figure 2.2.*** *a) The plot shows the averaged standard, deviant, and difference waveforms to all intensity conditions. The mean potential in microvolts is on the y-axis, which is plotted against the timeline, where 0 ms is the stimulus onset (shown as a vertical grey line). These responses are averaged across tinnitus and control groups to avoid any bias towards timelines in either group. Graphs b),c),d) and e) show difference waveforms separated by subject group and stimulus condition. Tinnitus group is shown in red and control group is shown in blue. The MMN responses at the control frequency (1 kHz) to DD are shown on graph b) and to UD on graph c). The MMN responses at the tinnitus frequency to DD are shown on graph d) and to UD on graph e).*

*Key: Upward Deviant (UD), Downward Deviant (DD), Standard Quiet, Standard Loud.*

### *2.2.3 Early evoked potentials (P50, N100) are not affected by tinnitus*

#### *2.2.3.1 Standard and deviant stimuli are affected by frequency*

Figure 2.3 shows P50 responses to standard stimuli. A three-way ANOVA (subject group, stimulus frequency, stimulus intensity) showed a main effect of stimulus frequency in P50 responses to these stimuli (p=0.0003). No other significant effects were identified.

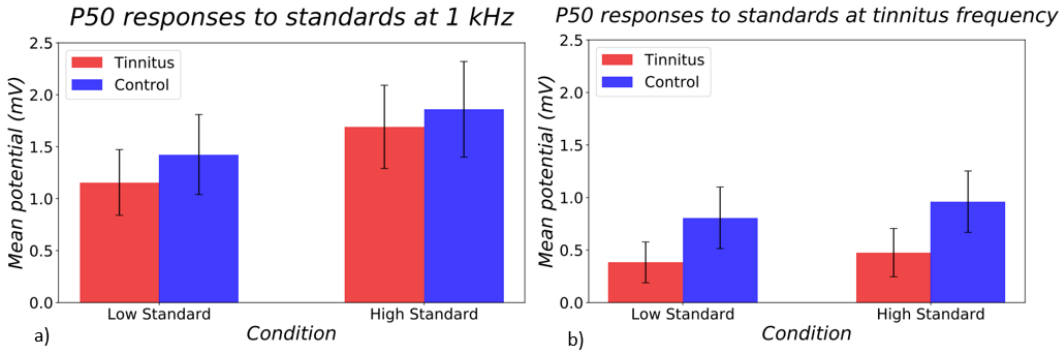



***Figure 2.3. P50 responses in tinnitus (red) and control (blue) groups to standard stimuli at the a) control frequency and at the b) tinnitus frequency.*** *On the left hand side of both bar graphs, responses to the quiet standard is shown, and on the right, responses to the loud standard is shown.*

N100 findings were similar to P50 findings in showing similar responses in both tinnitus and control groups (Figure 2.4). A main effect of frequency was found in a three-way ANOVA (p= 0.0005).

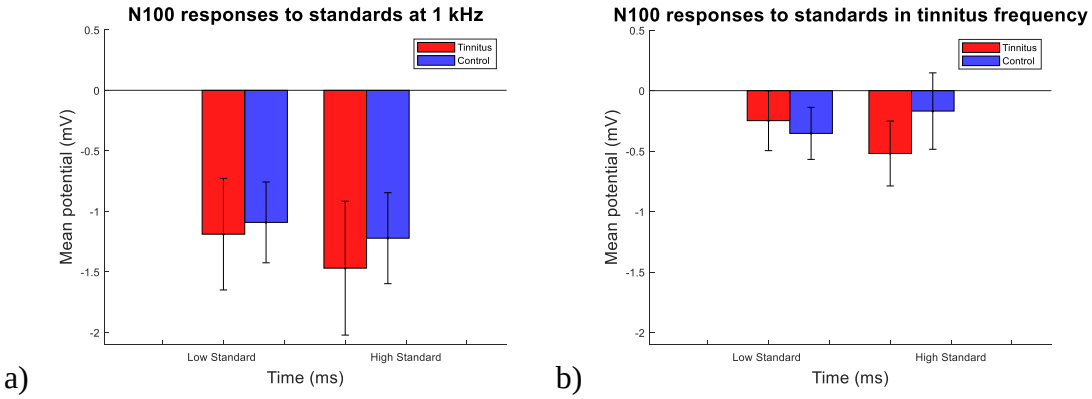



***Figure 2.4. N100 responses in tinnitus (red) and control (blue) groups to standard stimuli at the a) control frequency and at the b) tinnitus frequency.*** *On the left hand side of both bar graphs, responses to the quiet standard is shown, and on the right, responses to the loud standard is shown.*

### *2.2.3.2 Difference waveform*

The difference waveform between standard and deviant stimuli was also investigated in the P50 and N100 timeframes to ensure that any differences seen in the MMN timeframe were not dependent on differences in earlier stages of processing carried forward. No significant effects were found within the P50 timeframe. The N100 difference waveform showed a main effect of deviant direction ($p<0.0001$) (Figure 2.5). A stronger negative response was seen to the upward deviant.

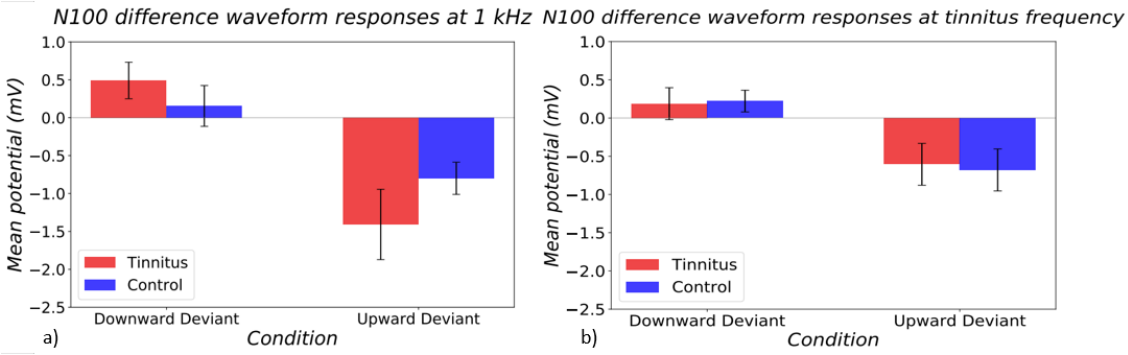



***Figure 2.5. Difference waveforms between deviant and standard responses at N100 timeframe at a) control frequency and b) tinnitus frequency in tinnitus (red) and control (blue) groups.*** *On the left side of both graphs, responses to the DD condition are shown, and on the right side, responses to the UD condition are shown.*

Additionally, a three-way ANOVA (subject group, stimulus frequency, stimulus intensity) indicated a main effect of directionality in a late negative potential (280 - 500 ms; figure 2.2), which was greater in upward deviants than downward deviants ($p=0.0002$) but was not influenced by tinnitus status.

### *2.2.5 Direction of the deviant affected responses in the MMN timeframe*

As we included duration deviants in this paradigm, a similar analysis was carried out for these as the intensity deviants.

#### *2.2.5.1 Standard stimulus responses*

Responses in the MMN timeframe to standard stimuli (Figure 2.6) in a three-way ANOVA (subject group x frequency x intensity) showed a non-significant trend towards a main effect of larger responses in tinnitus subjects ($p=0.061$).

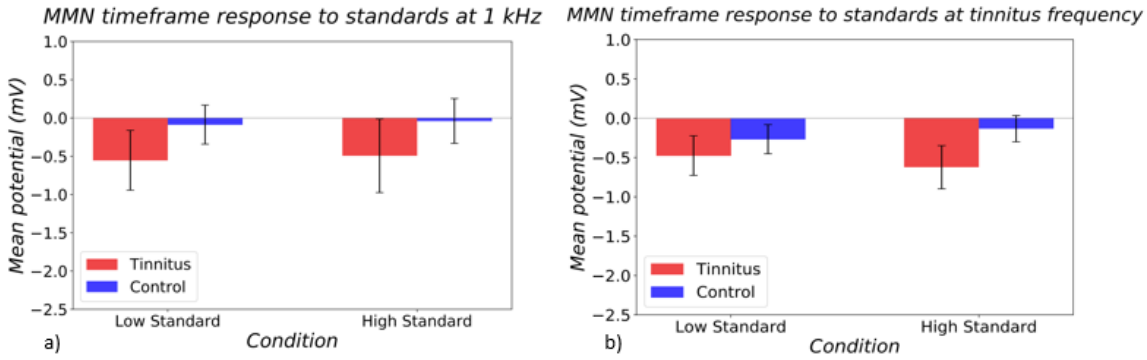



***Figure 2.6. MMN timeframe responses to standard stimuli at a) control frequency and b) tinnitus frequency in tinnitus (red) and control (blue) groups.*** *On the left hand side of both bar graphs, responses to the quiet standard is shown, and on the right, responses to the loud standard is shown.*

#### *2.2.5.2 MMN difference waveforms*

The main response of interest was the MMN difference waveform between deviants and standards, with the expectation being to see a group x direction interaction (if the previously observed effect generalised across frequencies), or a group x frequency x direction interaction (if the effect did not generalise). A three-way ANOVA (subject group, stimulus frequency, stimulus intensity) showed a main effect of deviant direction in the MMN responses of the participants (p=0.049) (Figure 2.7), with larger responses to upward intensity deviants. Unlike the original study, we did not observe a significant difference in asymmetry in deviant direction responses between tinnitus and control groups (group x direction interaction p = 0.239; group x direction x frequency interaction p=0.752). The pattern seen in the tinnitus group was similar to the pattern seen in the original study, in showing larger MMN responses to upward than downward intensity deviants, though this deviant direction effect did not quite reach significance even when analysed in tinnitus group only (p=0.058). However, the results from the control group in the present study were different to those from the original study; in the present study, responses from control subjects followed a similar pattern to the tinnitus group at the tinnitus frequency (Figure 2.7 b). The similar findings in the tinnitus and control groups were reflected in a two-way ANOVA (subject group and deviant direction as factors) showing a main effect of deviant direction at the tinnitus frequency (p = 0.015), but not at the control frequency (p=0.811). The group x direction interaction was not close to significance in either of these analyses (p = 0.305 for control frequency, and p= 0.534 for tinnitus edge frequency). In summary, MMN responses at the tinnitus edge frequency in the present study were similar to the previous study (Sedley et al, 2019) for the tinnitus group, but different in

the control group, who in this study appeared more similar to the tinnitus group. Conversely, the control stimulus frequency showed a very different intensity MMN pattern, with no clear directional asymmetry.

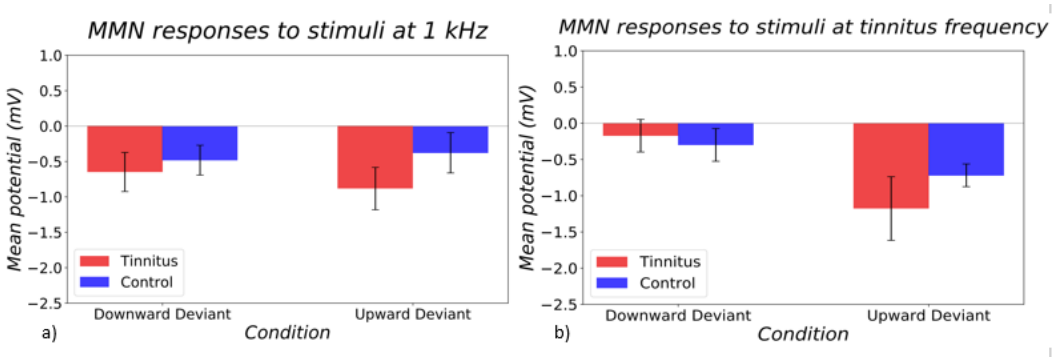



***Figure 2.7. Difference waveforms between deviant and standard responses at a) control frequency and b) tinnitus frequency in tinnitus (red) and control (blue) groups.*** *On the left side of both graphs, responses to the DD condition are shown, and on the right side, responses to the UD condition are shown.*

### *2.2.6 Duration deviants*

Similarly to the intensity MMN, timeframes for the duration MMN was chosen based on inspection of grand average ERP data across all stimulus conditions (Figure 2.8 a). The MMN timeframe chosen was 220-340 ms. Standard responses appeared similar across groups and conditions upon visual inspection (Figure 2.8 b), whereas difference responses appeared somewhat larger in the tinnitus group at the control frequency compared to the control group (Figure 2.8 c). This difference, however, was not significantly different based on an ANOVA (subject x frequency) ($p=0.099$). However, when looking specifically at deviant responses rather than difference waveforms, an ANOVA (subject x frequency) showed a main effect of frequency ($p=0.009$) (Figure 2.8 d,e).

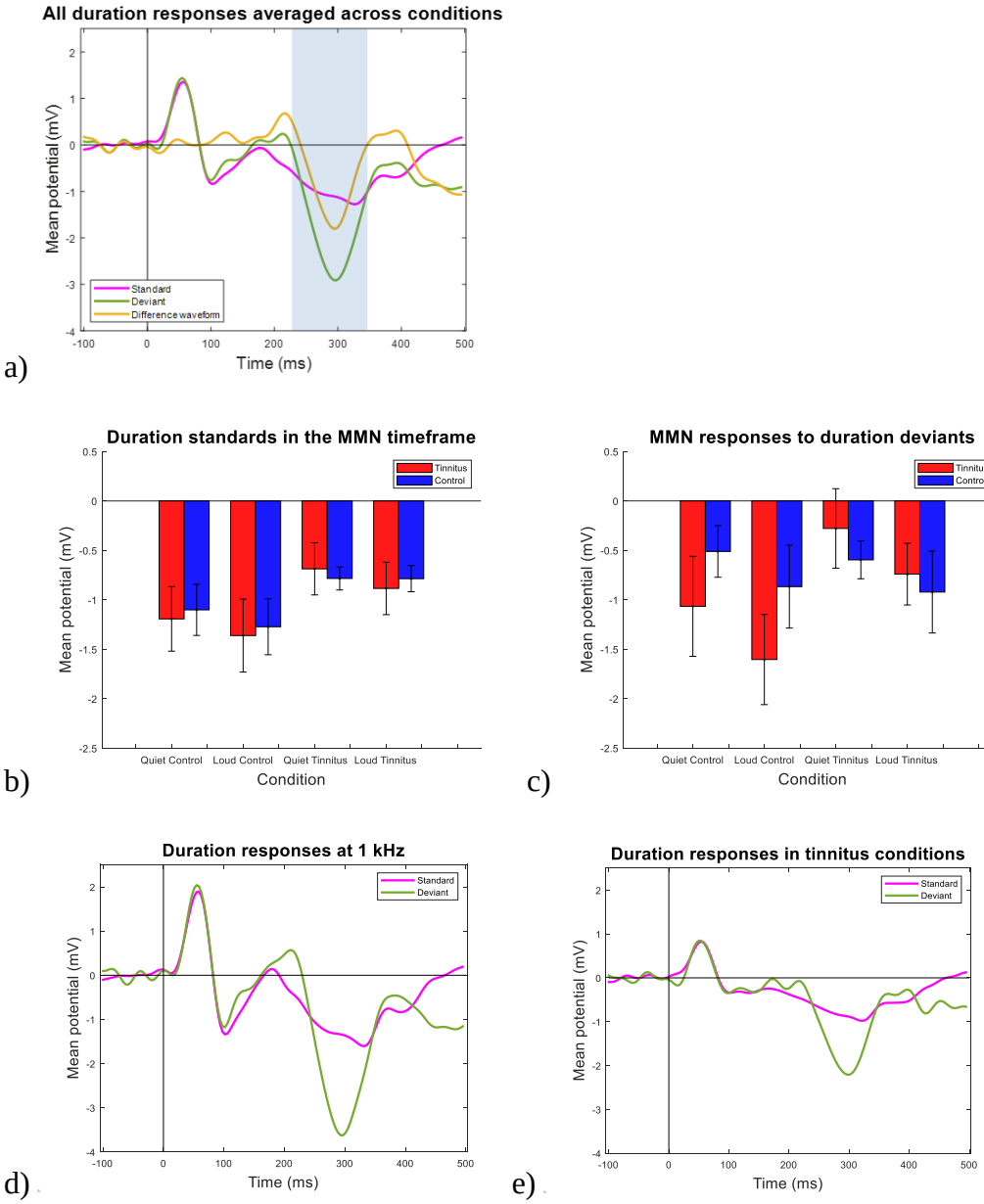



***Figure 2.8. Responses to standard and deviant duration stimuli.*** *a) The plot shows the averaged standard, deviant, and difference waveforms to all duration conditions (shorter duration, standard duration). The mean potential in microvolts is on the y-axis, which is plotted against the timeline, where 0 ms is the stimulus onset. Graph b) shows responses to the standard duration stimuli duration each of the intensity conditions (control = control frequency, tinnitus = tinnitus frequency). Tinnitus group is shown in red and control group is shown in blue. Graph c) shows the MMN responses to duration deviant stimuli during each of the intensity conditions. To look more closely at the pure deviant responses at each of the frequencies, graph d) shows responses to standard (purple) and deviant (green) stimuli at the control frequency and e) shows responses to standard and deviant stimuli at the tinnitus frequency.*

## 2.3 Discussion

### *2.3.1 Differences between current and original studies are likely due to the control sample*

This study was carried out to replicate previous findings [95]. The expectation was that there would be asymmetry in the MMN responses between tinnitus and control groups at the tinnitus frequency, but not at the control frequency, where the tinnitus group would have larger, more negative responses to upward deviants than downward deviants, and the control group would have the opposite pattern or lack of a deviant direction effect. There were no differences between the groups for the control frequency, as was anticipated, with similarly sized responses to upward and downward deviants. The pattern seen in the tinnitus group was similar to the pattern seen in the original study. However, the control group in the present study followed a similar pattern to the tinnitus group at the tinnitus frequency. A number of technical considerations could be involved in this finding, such as the smaller sample size and individual differences in the subjects (e.g. overall higher hearing loss levels at higher frequencies in this experiment), as well as inter-researcher differences in the implementation of methods. The current study also tightly controlled for sex. There were differences in the stimulus properties between the two groups, with the tinnitus group receiving perceptually quieter sounds. Nevertheless, this was unexpected and may require further investigation.

### *2.3.2 Upward intensity deviants may elicit stronger negative ERP components than downward deviants*

In the current study, the upward deviants caused a stronger N100 and a late negative response, when compared to the downward deviants in both groups and frequencies. This is in accordance with recent unpublished data from our group, where N100 was also significantly increased for upward but not the downward deviants, compared to their respective standards. Previous research showed similar patterns to sound intensity changes [111-113]. These researchers used 1 kHz tones that were either 50/60 dB or 80 dB, which is a much larger difference in intensities than used in the studies in our lab, however, the overall paradigm seems fairly similar so that comparisons could be drawn. Additionally, a stronger late negative potential has been previously shown in relation to upward deviants, and to metrically accented sounds along with the N100 [111, 114]. Therefore, stronger negative ERP components might be expected in response to the louder deviant sounds in such paradigms.

Additionally, there is a striking finding of stronger early components of responses to the standard stimuli in the control frequency compared to the tinnitus frequency. This may be an

intrinsic property of the brain response, or it could reflect the hearing loss both participant groups exhibited at higher frequencies.

### *2.3.3 Stimulus frequency differences may influence intensity mismatch asymmetry (IMA)*

As early ERP components were significantly affected by the frequency of the stimuli, it is possible that incorporating a control frequency that was on average 4 kHz lower than the tinnitus edge frequency affected the overall stimulus context of the whole experiment, and therefore shifted the response pattern at the tinnitus edge frequency even in blocks where control stimuli were not presented. It has been suggested that MMN is an indicator encompassing all dimensions of the stimuli presented, or combinations thereof, which could have different representations on the cortical surface depending on the paradigm features and would be advantageous in terms of survival in unexpected or improbable events [115-118]. While there is a plethora of research indicating the importance of immediate context preceding a deviant stimulus in terms of the ERP waveform shape, not much is known about any differences in evoked response waveforms in contexts of longer time periods, e.g. minutes [115, 119-121]. Whilst it is speculative at this stage, we wonder whether the different experimental context here (a wider difference between frequencies) had a relatively larger effect on control subjects than tinnitus subjects, making their responses at the tinnitus frequency more ‘tinnitus-like’. This could indicate that tinnitus-driven and context-driven effects could share a common mechanism. However, other potential reasons for the discrepant results in this study compared to the original study also exist.

### *2.3.4 Tinnitus subjects had higher scores on hyperacusis questionnaires*

Hyperacusis causes normal environmental sounds to be uncomfortably loud [122]. Tinnitus and hyperacusis are often comorbid, but there are some distinctions in the auditory pathway changes related to each condition [123, 124]. There are difficulties with finding participants with tinnitus who do not also have some sound sensitivity; higher THI scores have been found to associate with co-occurrence of hyperacusis [42, 125]. The addition of hyperacusis has been shown to affect resting state EEG activity, compared to participants with only tinnitus [42, 122, 126]. The presence of hyperacusis has also been found to enhance the average sound-evoked activity both to frequencies that are affected by hearing loss and those that are intact, in subcortical and cortical structures, while reducing the responses to tinnitus frequency specifically when compared to a group that did not report having hyperacusis [26, 125, 127-130]. Therefore, the findings in the current study may also be affected by this factor. The HQ scores were not related to the perceived loudness of stimuli in this study, however;

the relationship between uncomfortable loudness level (ULL) and HQ scores has previously shown to be weak (e.g. [131, 132]) and the differences in stimulus sensation level between groups is likely to have been compensatory for the hyperacusis and therefore to allow the stimuli to be perceptually similar to the control group. We specifically used a subjective method for stimulus intensity determination, to try and minimise the effect of hyperacusis on intensity MMN. However, it may be that it is not possible to fully account for the impact of hyperacusis in this way; in future studies, it may be important to further distinguish between hyperacusis and tinnitus and the combination of the two conditions, through careful subject group selection, and how brain responses to the current paradigm are affected by these [123].

### *2.3.5 Conclusion*

The current study failed to replicate the Intensity Mismatch Asymmetry as a marker of tinnitus status [95]. The new findings could potentially be due to the overall frequency context of the paradigm affecting the responses to deviant stimuli, particularly in the control group, or other subject factors or technical aspects. Another potential reason for the discrepant findings could simply be the smaller sample size, however it is important to note that this study shows that the strength of IMA is at least smaller than it previously appeared, and in its present form far from the intended biomarker reliable at the individual subject level. An interesting next step would be investigating the effects of varying study contexts (e.g. large, small, or no difference between frequencies used in different blocks of the paradigm). This, and systematic exploration of other contributory factors, and other paradigm variants, may help to improve the diagnostic accuracy of prediction violation-based tinnitus biomarkers in future.

# Chapter 3. Online sound therapy for chronic tinnitus using a novel cross-frequency covariance-cancelling stimulus.

## 3.0 Introduction

Tinnitus is a persistent sound heard by an individual without an environmental source, which affects 10-15% of the population [1]. Hearing loss has been established as the largest risk factor for tinnitus [11]. Tinnitus can be non-bothersome, but an estimated 20% of people with tinnitus seek treatment. However, patients with tinnitus tend to find treatments difficult to access and largely ineffective, or misaligned from their main concerns; patients mostly want to reduce tinnitus loudness, while many of the available treatments are instead focused on reactions to tinnitus [133, 134]. Because of the large mismatch between the very high prevalence of tinnitus and the much lower capacity of specialist audiology or tinnitus clinics, any treatment aiming to become a mainstay of management must be deliverable in either primary care, or outside of a clinical setting altogether.

The neurophysiological model of tinnitus (which has prevailed for several decades, and undergone numerous refinements and variants) can act as a way of categorising different types of sound therapies for tinnitus; the tinnitus signal is generated and processed in the auditory pathway, and then perceived, attended and reacted to in a wider network of brain areas interacting with auditory cortex (including limbic system and prefrontal cortex) that contributes to the patient reactions towards the percept [135-138]. Within the auditory pathway, there has been interest in numerous changes as potential underlying drivers for tinnitus, which have in common that they are all increased by hearing loss, but are affected by other factors also; such changes include neuronal firing rates, central gain, lateral inhibition, tonotopic map plasticity, neurotransmitter and neuromodulator changes, and neural synchrony (over various spatial and temporal scales). We have previously argued that most of these changes have in common that they act to increase the weighting (or *precision*) that the tinnitus signal is given in processes of perceptual inference, helping to determine how loud tinnitus is perceived, or even whether it is perceived at all to begin with.

### *3.0.1 Types of sound therapy*

Sound therapy is a non-invasive approach in which external sounds are used to provide relief from tinnitus symptoms that is easily accepted by tinnitus patients and can take various forms [139-141]. A simple binary categorisation can be made for types of tinnitus sound therapy in

terms of their presumed mechanism of action and their primary aims. 1) Working at a higher hierarchical level of neural processing, one category of sound therapy can promote 'habituation', the process by which distressing tinnitus is re-categorised as an irrelevant and non-threatening signal, thereby reducing its impact [136]. 2) Conversely, targeting at a lower hierarchical level of processing aims to quieten the tinnitus sound itself by disrupting key features of the underlying auditory pathway signal that are responsible for its intensity, or even its presence at all. One category aims to quieten tinnitus by interfering with the tinnitus signal itself, and the second category aims to reduce distress by changing reactions to or awareness of the tinnitus, and/or introducing a sense of control over the tinnitus.

Sound therapy types can be further divided into two groups: non-customised and customised [141]. While both types could be aimed at reducing tinnitus loudness itself or modify reactions to it, the customised sounds would be more likely to reduce tinnitus loudness as they tend to be aimed at the underlying processes of each specific case of tinnitus. Non-customised sounds involve hearing aids/cochlear implants, or unmodulated sounds that seek to reduce adverse reactions to tinnitus by masking or helping to habituate to their tinnitus. Sometimes, with the addition of counselling, various types of sound therapy aim to aid patients to understand tinnitus better [136, 142, 143]. Further examples of the non-customised sounds are masking [143], which employs an external sound to fully or partially cover the sound of tinnitus [144], and Tinnitus Retraining Therapy (TRT) [145], which is a combined version of therapy for tinnitus where the participant receives both counselling and sound therapy.

While earlier literature focused on unmodulated sounds, more recently modulations have been applied to different aspects of the sounds (e.g. amplitude, frequency), or by removing energy and creating a "notch" in specific frequencies close to tonal tinnitus frequency found through a process of precise frequency matching [146-148]. Customised sounds are based on the tinnitus features, particularly tinnitus frequency, of each individual, and aim to alter lateral inhibition, cortical reorganisation, reduce pathological central gain and/or disrupt neural synchrony, processes that are thought to be involved in tinnitus causation [97, 149]. Customised sounds have a stronger tinnitus suppression effect than non-customised sounds [141]. While some of these therapies involve concomitant psychological therapy or other forms of in-person treatment, other types focus purely on modulated sounds. Some examples of these are described below.

HNMT (Heidelberg neuro music therapy) involves a comprehensive psychological management plan with eight 50-minute sessions of music therapy along with counselling,

with the sound therapy comprising intonation training and resonance training, which involve somatosensory training to improve auditory attention control and aid neural reorganisation [150]. The main outcome in a controlled clinical parallel intervention trial where HNMT was compared to counselling alone was Tinnitus Questionnaire scores, which were 33% more reduced in the HNMT group compared to the reduction seen in the counselling only group [151]. Similarly to much of HNMT research, an MRI study quantified symptom improvement using the Tinnitus Questionnaire, and saw an increased in grey matter in treated patients and active controls compared to untreated patients, allowing the authors to suggest that these effects were part of a 'mental wellness' approach that influenced the distress network [152]. Neuromonics Tinnitus Therapy (NTT) [153] also combined counselling and sound therapy into a 6 month program, delivered through a clinic, costing up to $5,000. NTT uses a Class 2 medical device that is fully programmed to consider the hearing profile of each patient [154]. The sound therapy was intended to enrich the auditory regions affected by hearing loss and has been shown to be effective in reducing tinnitus reactions and changing magnetoencephalography (MEG) responses in a 30-week long single arm study, with the MEG results being compared to a control group [155]. It also utilised aspects of masking, as it allows tinnitus to be covered by sounds during particularly upsetting episodes. Participants were instructed not to use the sound therapy when their tinnitus was not disturbing. Tinnitus reaction scores were around 50 on average at baseline and went down to around 17 at the final appointment just after finishing the sound therapy. These NTT results persisted up to 36 months after treatment [154]. A limitation of this type of program may be that the clinics choose most suitable candidates, both based on tinnitus/comorbid symptoms and demographics such as a language barrier [154]. Auditory discrimination training (ADT) is another type of therapy, that works in an oddball paradigm-like fashion, where patients are trained to separate sound frequencies in more complex stimuli, and learn to re-categorize and ignore the tinnitus sound [156, 157]. This type of therapy is based on lateral inhibition, as the frequencies around but not the same as tinnitus are stimulated, which is thought to disrupt synchrony [157]. Prior to starting this training, a full ENT examination was needed for comprehensive tinnitus assessment. In a randomised clinical trial, after the examination, participants completed 20 minute discrimination tasks for 30 days [158]. Patient results were compared to a waiting list group. While the THI score of the ADT groups did decrease significantly after the treatment compared to the waiting list group, the visual analogue loudness score (VAS) intensity scores difference was not. Notably, this study only focused on pure tone tinnitus. However, a later double-blind randomised trial showed that training at any frequency, not just around hearing loss frequency, improved tinnitus handicap questionnaire

scores, so the ADT resulted in generic cognitive improvement but not tinnitus specific [159]. These three approaches were shown to be successful at decreasing tinnitus distress, but their effect on perceived loudness was not significantly improved or was not measured (which is the primary concern of a majority of tinnitus patients), and they require lengthy in-person visits which are not feasible for every person with tinnitus.

Tailor made notched music training (TMNMT) is a treatment for tonal tinnitus specifically (not being applicable to noise-type tinnitus), in which a specific frequency band is removed from an auditory stimulus. Therefore, it requires accurate tinnitus estimation of tinnitus frequency conducted by a specialist. The neurons around the tonotopic region of the notched frequency are thought to inhibit activity within the notched region, thus encouraging the brain to reorganise the tonotopic structures and reduce auditory activity at the tinnitus frequency through the process of lateral inhibition [147]. The lateral inhibition theory of tinnitus states that due to reduced activity in the auditory nerve fibres responsible for certain frequencies, lateral inhibition of neighbouring neurons is reduced and the neurons become more hypersensitive and hyperactive, thus causing the activity that is translated as the tinnitus signal [149]. This method has been shown to somewhat reduce tinnitus loudness in an RCT, compared to a control group [146, 160]. In the 12–month double-blind study, 16 participants with tinnitus and no severe hearing impairment completed either the TMNMT or a placebo arm of the study, where they kept weekly tinnitus loudness score diaries (0-100 with 100 being extremely loud), as well as providing a baseline before the experiment. Participants listened to their assigned sounds for 7-21 hours per week on average. The researchers did not provide exact numbers, but, based on displayed data, the tinnitus loudness score progressively and significantly decreased from baseline in the TMNMT group, by around 25% in months 7-12 after starting the study, while a slight non-significant increase was seen in the placebo group (around 10%) [146]. The more powered (with 50 participants per group) stratified double blind two-arm study, though shorter in duration, involved participants listening to either the TMNMT or placebo sounds for two hours per day for three months and returning for a follow-up 1 month post-listening [160]. The tinnitus loudness and tinnitus distress measures were taken pre-, post-, and during the follow-up of the treatment. After 3 months, there were no significant effects pre vs post treatment on either of the primary measurements, though a structural equation model showed that the loudness change of the TMNMT group was significantly different from the change in the placebo group. Additionally, the researchers reported a considerable number of participants experiencing adverse effects during the study, which were evenly distributed between the groups. Both studies were selective regarding the

frequency of tinnitus. The two studies were conducted at different time scales, and the comparison between them showed that 3 months was not enough to create an immediate effect on the VAS intensity scores, while after 12 months the effects were more apparent [146, 160].

Tinnitus pitch-matched therapy aims to prevent tonotopic reorganisation by playing high frequency stimuli soon after noise trauma, also with matching done under supervision of an audiologist/researcher [161]. Difficulty with this treatment is that tinnitus may appear gradually, and patients may not get treated until much later after tinnitus development [141]. Another study found that compensating for hearing loss with this treatment was not beneficial to patients, with potential worsening of the symptoms if the treatment stimulus overcompensated for the hearing loss [162].

In coordinated reset (CR) neuromodulation therapy, a specifically timed sequence of various short tones are played at frequencies around a precisely matched tinnitus frequency, in order to desynchronise neural networks around the tinnitus frequency [163]. This method also requires the participant to have only tonal tinnitus. While some randomised studies show positive effects on tinnitus perception after CR neuromodulation therapy, subsequent larger studies have not shown any significant effect on tinnitus loudness [164, 165]. For example, in a double-blind RCT (12 weeks) with long-term open-label extension (further 24 weeks), 100 participants underwent CR neuromodulation, and showed no significant tinnitus distress level or loudness differences at 12, 24, or 36 time-points.

Modulated wave therapy [148] was thought to be most consistent with the hyperactive spontaneous rates and heightened synchrony in the auditory pathway and was theorised to induce residual inhibition (RI), possibly working similarly to masking [140, 148]. RI is a long-lasting suppression of spontaneous neural firing after exposure to certain sounds [166]. Low-rate amplitude and frequency modulated stimuli around the frequency of tinnitus have shown to be more successful at reducing tinnitus loudness than unmodulated stimuli, as shown in repeated measures studies [148, 167]. In one study, twenty participants listened to 17 3-minute tones in random order, in-person, some of which were amplitude or frequency modulated, and some being white noise control tones [148]. The modulated sounds played around 4 different frequency ranges (75-750 Hz, 750-1500 Hz, 3-6 kHz, and 6-9 kHz). The amplitude modulation rate of 40 Hz and frequency modulation rate of 8 Hz in 75-750 Hz frequencies and 40 Hz for all other frequency groups, with modulation depth of 10%. The carrier frequency was individualised to the tinnitus of each participant (tested by a researcher

in lab settings), so when the tinnitus was within the frequency range, that frequency was the carrier frequency. In other frequency ranges, multiplication/division of the tinnitus frequency was made for the carrier frequency to be the closest geometric centre frequency (237, 1061, 4243, or 7348 Hz). During the testing, the participant needed to give a baseline tinnitus loudness score and set the sounds to be slightly quieter than their tinnitus loudness. During listening to a 3-minute tone, participants reported loudness of the tone and also of the tinnitus at 30 second intervals. In one third of the trials, tinnitus was supressed to some degree in 90% of the participants, with the average suppression being 39%. Modulations within the two higher frequency range tones showed significantly more tinnitus suppression than white noise, which was not the case for the lower frequency range tones, with amplitude modulation being particularly successful (up to around 35% suppression compared to 20% in response to control pure tones/narrow-band-noise, at 6-9 kHz frequency range tone). Interestingly, there was a group of good responders (>70% suppression) and poor responders (<50%). The latter group started with higher tinnitus loudness but lower tinnitus sensation levels, which potentially indicated presence of hyperacusis in the poor responder group, also indicated by steeper loudness growth and narrower dynamic ranges. However, the specific modulation details and control tones have not been consistent across studies. In another in-person study, 29 participants were asked to listen to 3 10 Hz amplitude modulated (one with frequency modulation of 10 Hz, one modulated at tinnitus frequency (tone of primary interest), one modulated at 108 Hz), 2 notch filter amplitude modulated by 10 Hz (one applied to pink noise, one applied to music) and 2 unmodulated tones (one tinnitus frequency pure tone, one pink noise) [167]. The tones were also each 3 minute long but 60 dB SL in volume, unlike the previous study where the sounds were slightly quieter than tinnitus loudness. Overall, the experiment results in significant reduction in tinnitus loudness score, which reduced from a mean of 54.46 to 48.25, however it is not known which of the tones was most responsible for loudness decrease. While there are some differences between the approaches used in the two studies, a recent repeated measures trial compared five sounds, including 10 and 40 Hz amplitude modulations played at 60 dB SL and pure tones. The 10 Hz modulation showed a non-significant trend toward producing more suppression and being more well-tolerated than 40 Hz modulation and unmodulated pure tones, though some of these results may have been due to order effects [168]. The authors, however, concluded that they could not see a significant difference between the 10 Hz amplitude modulated sounds and pure tones at tinnitus frequency despite a trend being present. These studies also only saw short-term suppression, with longest reports lasting 20 minutes to 2 days [169], rather than days or months which would be required to give patients clinically meaningful benefits.

Another version of customised sound therapy involves playing tinnitus pitch-like stimuli while a patient is asleep (Levo device) [170]. These sounds are played through a specialised earbud system created by the researchers. An RCT in which 60 participants were assigned to either the Levo system with a tinnitus-like stimulus, Levo with a noise stimulus (white noise) or a bedside generator device, was carried out to investigate the efficacy of this customised sound therapy. The first two groups had personalised earbuds made to listen to the sounds. All audiological testing was performed at a clinic. All participants also received some educational tinnitus counselling. The baseline tinnitus loudness score overall was 6.3 out of 10, with 6.4 being average in the tinnitus-like group and 5.8 in the noise stimulus group. After 3 months of listening to sounds during sleep, the tinnitus-like group showed the most reduction of mean loudness score, -1.0 on the 0-10 scale, while the white noise and the bedside generator device showed -0.2 and -0.4 reductions respectively. This sound therapy has now been commercialised as the Levo system by the Otoharmonics Corporation, costing around $4500 [171].

There are also bimodal stimulation therapies available for tinnitus, as somatosensory system may be involved in hearing loss-related tinnitus [172]. An example is trigeminal nerve stimulation through an electrode put on the tongue, paired with auditory stimuli particularly at frequencies affected by sensorineural hearing loss, which has shown to reduce symptoms according to THI and TFI scores in 10- and 12-week RCTs [172, 173]. Tinnitus loudness matching, closest measure to a VAS/NRS tinnitus loudness score, showed that compliant participant saw a decrease from 44.8 to 37.3 dB, which was a significant reduction [172]. However, there was no control group, and participants needed to visit the clinic every 2 weeks. The other double-blind parallel-arm RCT looked at 3 different stimulation parameters (with main differences being in synchrony and coordination of stimuli), which showed that higher synchronisation lead to better outcomes, also did not measure any tinnitus loudness-related outcomes [173]. Another bimodal stimulation treatment double-blind RCT, vagal nerve stimulation (VNS), was shown to be effective specifically for tonal and non-blast-induced tinnitus [174]. But, based on the confidence intervals, the treatment vs control group had a lot of overlap on the THI scores. Additionally, loudness match showed that on average, there was a 1.06 increase in the treatment group (and 0.36 increase in control group). A recent systematic review of VNS trials (both with and without accompanying sound therapy) concluded that there are limitations to many of these studies, including adverse effects from implantation, and therefore the effectiveness cannot yet be concluded [175]. Perhaps one of the most encouraging sound therapy methods, used for somatic tinnitus specifically, is the

combined sound therapy and occipital nerve stimulation [176]. In a double-blind RCT completed by 56 participants, subjects were randomly split into two groups (active: combined auditory and somatosensory followed auditory only, or control: the other way round), and used the first treatment type for 6 weeks for 30 minutes per day followed by a 6-week washout phase. Then, the same process was carried out with the second treatment. Both arms of the study produced reductions in TFI scores, however the combined treatment produced larger decrease than the auditory only treatment (by 6.8 points). The tinnitus loudness levels also decreased in both groups, however, the decrease was more significant in the combined group (-7.2 dB SL in combined compared to -3.1 dB SL in auditory only). Further, the TFI and loudness level continued to reduce in the combined group through the washout period.

Overall, while there are sound therapies available, none of them are yet showing large average clinical effects in terms of tinnitus loudness, and many of them require in-person accurate matching with the supervision of an audiologist or a researcher (furthermore, tinnitus frequency matching is notoriously unreliable even when performed by expert clinicians and researchers), work only for specific types of tinnitus, work only as part of a comprehensive treatment programme involving psychological intervention, are expensive, and/or are not fully accessible to the public for various reasons. Therefore, there remain needs both for more effective forms of sound therapy, and for further forms of sound therapy that have similar more modest benefits to existing therapies, but are deliverable cheaply, safely and effectively on a large scale.

### *3.0.2 Theory behind our novel sound therapy*

We describe here a novel type of sound modulation, based in reduction of covariance (i.e. synchrony) of neuronal activity in different frequency channels within and surrounding the tinnitus frequency (or frequency range). As tinnitus is thought to result in large part from excessive synchrony between neurons, the motivating principle is to reduce synchrony between neurons representing different frequencies [177, 178]. This neural synchrony theory was the basis for types of sound therapy such as Coordinated Reset (CR) neuromodulation. From a different point of view, the covariance matrix between frequencies can be interpreted as related to the *precision matrix*, which determines the relative importance and influence afforded to the tinnitus signal in perceptual inference. [82, 83, 179]. Disrupting these established inter-frequency relationships may therefore decrease the extent to which the tinnitus signal is interpreted as reliable or important by the brain, and therefore the extent to

which it is perceived, leading to quieting of tinnitus. Two theories converge on a similar approach, giving rise to the theory behind the covariance cancelling method.

### *3.0.3 Aims & hypotheses*

Our aim was to develop a type of sound therapy for tinnitus that is effective in reducing tinnitus loudness, does not require direct clinician or researcher input, can be delivered with ordinary smartphone or computer equipment and headphones, and can be applied to all subtypes of tinnitus without requiring precise tinnitus frequency matching.

Motivated by (but not contingent upon) neural synchrony and sensory precision tinnitus theories, a new type of sound was created in which broadband pitch stimuli were modulated within a one-octave frequency range approximately (but not needing to be exactly) centred on the tinnitus frequency. This applied modulation aimed to target the precision matrix of the tinnitus signal by reducing cross-frequency neural synchrony [177].

Specifically, the hypothesis for the use of this sound was that by reducing the covariance between the tinnitus frequency channel(s) and the other nearby channels, (or within the tinnitus frequency range), precision of the tinnitus signal will be reduced. Two alternative implementations of the modulation were applied: as an amplitude modulation (range 0-2) and a phase modulation (range 0 to 2pi, i.e. one full cycle of phase advancement), the latter being equivalent to a subtle frequency modulation.

A third condition, featuring a different kind of sound therapy, was also included, in which frequency-specific notch degradation sound was presented to participants, with the aim to encourage the brain to reduce estimated precision in tinnitus-related frequency channels by removing informational content (but preserving spectral energy). This approach differed from Pantev et al's notch filtering approach, which is thought to work by lateral inhibition [147].

To understand the tolerability and effectiveness of these new types of sound, we compared them to perceptually similar sham versions of the same stimuli (i.e. modulation applied at non-tinnitus frequency ranges) in a blinded randomised trial format. A recent review of sound therapies for tinnitus concluded that there is a need for better controlled, randomised trials in order to identify the most effective tinnitus management, and the current study design was constructed with this in mind [141].

Finally, the study aimed to establish the feasibility of running a fully automated online interventional study in which participants with tinnitus were asked to perform daily listening. If successful, such an approach would prove useful not just for testing, and delivering, this

form of sound therapy, but a potentially limitless range of other types of sound therapy with much greater numbers than in-lab studies. Because no direct clinician or researcher involvement was required in the setup or delivery process, any techniques used in this study could potentially be rapidly rolled out on an unlimited scale to both tinnitus patients, and to people living with tinnitus who are not patients within a healthcare system, and with little or no cost attached.

## 3.1 Methods & Materials

### *3.1.1 Participants*

This online randomised blinded study was fully completed by 77 participants. Volunteers were recruited from affiliated volunteer lists at Newcastle University, via the British Tinnitus Association, and via Tinnitus Talk online forum. To be included, participants needed to be over 18 years of age, with chronic tinnitus for over 6 months that did not have an objective physical source, who could make an informed choice about volunteering. Exclusion criteria included having pulsatile tinnitus, or profound hearing loss in the range of the higher frequencies, as it was unlikely that the sounds used in this study would have effect on their tinnitus. No eligibility criteria related to the subtype of subjective tinnitus. Participants were discouraged from participating if they had prior experience of their tinnitus being made worse by exposure to moderately loud sounds or environments.

Recruitment and data collection occurred between October 2020 and April 2022. Approval was given by the Newcastle University Research Ethics Committee, and all participants gave electronic informed consent according to the Declaration of Helsinki (reference number 11138/2018).

### *3.1.2 Assessment & Experimental Design*

#### *3.1.2.1 Questionnaires*

Participants accessed the information sheet online via Outlook Forms and were able to ask questions of the research team via e-mail if they had outstanding queries. They used an anonymised identifier code to preserve their identity. They could come back to the form again if they wished to think about their participation first. Once they decided to take part, participants signed an electronic consent form. After this, they answered the following questions: gender, whether they have difficulty hearing, whether they find it difficulty following a conversation when there is a background noise, whether they use a hearing aid or

a cochlear implant, age, tinnitus duration, laterality of their tinnitus, type of their tinnitus sound, numerically rated tinnitus loudness and distress scores, whether their tinnitus is affected by moderately loud sounds either during their time in that environment or soon after this time. Participants also completed the following standardised questionnaires: Tinnitus Handicap Inventory (THI) [106], Tinnitus Functional Index (TFI) [180] and Hyperacusis Questionnaire (HQ) [107].

#### *3.1.2.2 Hearing slope and tinnitus frequency estimation*

Before the start of the trial, each participant underwent approximation of their slope of hearing loss (Figure 3.1) and tinnitus frequency using a procedure involving listening to sound files provided by the researchers and reporting on the best fitting sound or file (Table 3.1). Specifically, each hearing loss estimation sound file comprised a series of pure tones in ascending frequency order. Files differed according to how much the intensity of tones increased with increasing frequency, with a flat frequency spectrum being the smallest slope. From the optimal file, participants then selected the specific tone most closely matching their tinnitus frequency (in the case of tonal tinnitus) or selected from an equivalent series of narrowband noise stimuli for non-tonal tinnitus (Table 3.1). This process did not need specialist software or researcher/audiologist input, thus was feasible in real world conditions.

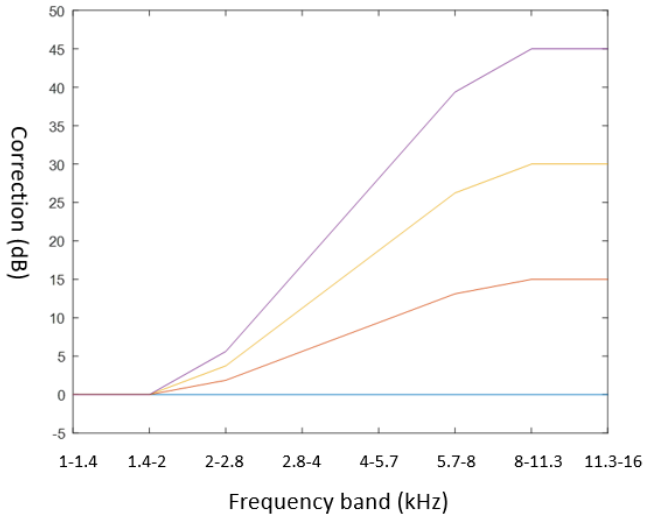



***Figure 3.1. Hearing correction profiles.*** *Audio files used for hearing estimation and tinnitus matching included 8 sounds at ascending frequencies, from 1 kHz to 16 kHz. The four different frequency profiles are shown here. The blue line represents a normal hearing slope, in which each frequency band was equally loud. The red line represents a mild hearing loss slope, in which volume of the sound increased incrementally up to 15 dB at the highest frequencies. The yellow line represents a moderate hearing loss slope, with incremental increases up to 30 dB at highest frequencies. The purple line represents a severe hearing loss slope, with incremental increases up to 45 dB at the highest frequencies. Each subject's own preferred frequency-intensity correction profile was also applied to their active and sham therapeutic sounds used in the study.*

| | ***1-2 kHz*** | ***1.4-2.8 kHz*** | ***2-4 kHz*** | ***2.8-5.7 kHz*** | ***4-8 kHz*** | ***5.7-11 kHz*** | ***8-16 kHz*** |
|---|---|---|---|---|---|---|---|
| ***1 kHz*** | *A1* | | *C1* | | | | |
| ***1.2 kHz*** | *A1* | | *C1* | | | | |
| ***1.4 kHz*** | *A1* | | *C1* | | | | |
| ***1.7 kHz*** | *A2* | *A1* | | *C1* | | | |
| ***2 kHz*** | | *A1* | | *C1* | | | |
| ***2.4 kHz*** | | *A2* | *A1* | | *C1* | | |
| ***2.8 kHz*** | | | *A1* | | *C1* | | |
| ***3.4 kHz*** | | | *A2* | *A1* | | *C1* | |
| ***4 kHz*** | | | | *A1* | | *C1* | |
| ***4.8 kHz*** | | *C2* | *C1* | *A2* | *A1* | | |
| ***5.7 kHz*** | | | ***C1*** | | ***A1*** | | |
| ***6.7 kHz*** | | | *C2* | *C1* | *A2* | *A1* | |
| ***8 kHz*** | | | | *C1* | | *A1* | |
| ***9.5 kHz*** | | | | *C1* | | *A1* | |
| ***11 kHz*** | | | | | *C1* | | *A1* |
| ***13 kHz*** | | | | | *C1* | | *A1* |
| ***16 kHz*** | | | | | *C1* | | *A1* |

***Table 3.1: Selection of active and sham modulation bands based on tinnitus frequency match.*** *Each participant selected the best match to their tinnitus frequency from 17 stimuli at the frequencies shown here (tones or narrowband noise stimuli, according to their stated tinnitus type) presented to them in a pre-recorded online file. This gave the researcher an estimation of the tinnitus frequency around which the sound therapy sound file should have the modulation applied. The 17 stimuli were played at the frequencies referred to in equivalent row titles. There were 7 possible frequency bands to which modulation could be applied (to serve as either active or sham). The frequency chosen by the participant would be near the middle of the frequency range presented below. The 7 frequency bands here refer to the equivalent column titles. The correct modulation type was chosen based on the correspondence between the best tinnitus match out of 17 stimuli and the 7 frequency bands for the modulation. Each tinnitus match had a pre-assigned modulation band, with A1 being the preferred active frequency band and C1 being the preferred sham frequency band. A contingency option was specified in case either the active or control frequency contained frequencies the participant could not hear at all (A2 and C2 respectively).*

#### *3.1.2.3 Trial organisation*

Participants were randomly assigned to receive either sham or active sound therapy first, and then crossed over to the other type subsequently (Table 3.3). Subjects were also randomised to receive one of three types of modulated sound: 1) Notch degradation (N=24), 2) Amplitude modulation (N=27) or 3) Phase modulation (N=26). Each participant received only one type

of modulated sound in the study, but both active and sham versions of each. Interaction with a researcher was by e-mail only, for the purposes of sending the appropriate sound files for the subject's tinnitus match, hearing profile, modulation type and active/sham phase, and answering questions from participants if applicable. Automated e-mail prompts to submit daily listening figures and tinnitus symptom data at appropriate times were sent to participants. Participants were blinded as to their group and active/sham stage, and sound files were cryptically named so as to not convey information about their contents. Whilst the researcher had access to information the groups to which participants were allocated, they did not refer to this when corresponding with participants. Overall, we believe the possibility of any degree of inadvertent un-blinding to be negligible. In the sham condition, the modulation was applied to a different range of frequencies, usually below their tinnitus frequency (Table 3.3). The modulation is perceptually subtle, with perception dominated by the carrier stimulus to which it is applied, and feedback from participants was that they could not knowingly differentiate active from sham sounds. Each participant completed the two listening periods (active and sham, 6 weeks each), with 3-week washout periods after each listening period (Figure 3.2). Before and after each listening period, participants were asked to fill in a Numerical Rating Scale (NRS) loudness score, THI, TFI, HQ, and NRS annoyance scores. Participants were asked to listen to the sounds each day of the listening period and complete a daily listening log where they recorded how long they listened to the sounds for. Participants were not instructed to listen to the sounds for a specific duration per day, though audio files were 60 minutes long, which provided an implicit cue for listening duration. As with the initial questionnaires and matching process, responses were submitted using Outlook Forms. Specific instructions were given in order to inform participants of safe listening practices. These included: 1) No more than 60% of device's volume; 2) No more than 60 minutes at a time; 3) Leave 60 minutes break between listening; 4) Can use any device, speakers or headphones; 5) Can listen while doing other activities as long as sounds are audible.

The primary outcome measure was the NRS loudness score, reflecting the intent being to quieten tinnitus itself rather than simply change participant reactions to it. A detailed map of the experimental design can be seen in Figure 3.2.

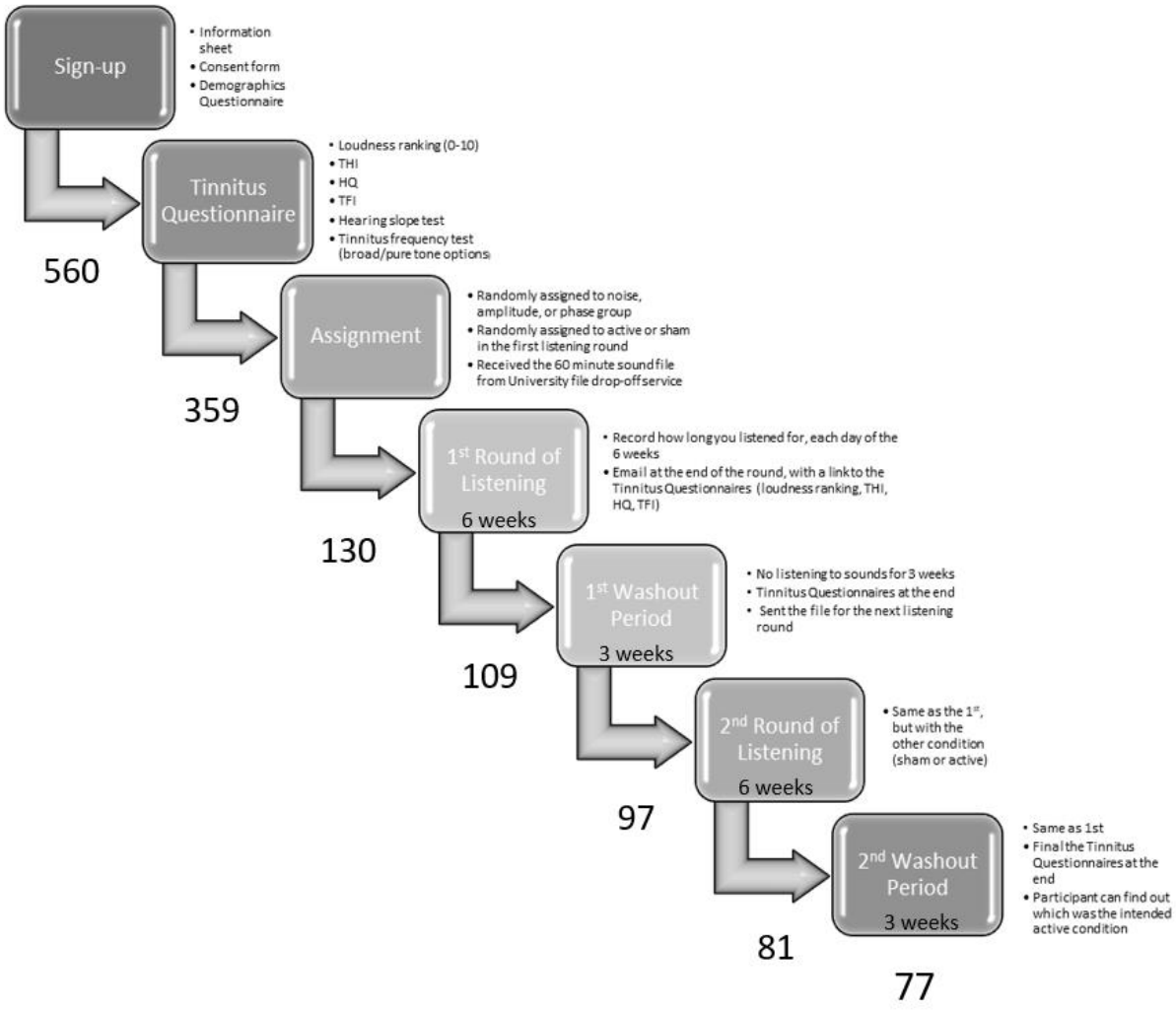



***Figure 3.2. Study procedure.*** *Under the boxes, the number of participants who completed that step is presented.*

As we focused primarily on the amplitude and phase modulation groups, we also looked at the drop-out rates in these two conditions (combined). Out of 130 that completed the first questionnaire (who were then assigned and began the first listening round), 77 completed the first round including the end questionnaire, using either amplitude or phase modulated sounds. 67 participants then completed the questionnaire after the washout period. 56 participants completed the second round of listening, and 53 participants completed the final questionnaire after the second washout period (3 participants had to be excluded due to inconsistent listening records).

#### *3.1.2.4 Therapeutic sound creation*

The carrier sounds, to which the active or sham modulations were applied, were hour-long sequences of non-overlapping 4 second broadband harmonic complexes, each with

randomised fundamental frequency selected from a specific range, with 1 second onset/offset cosine ramps. The modulation applied to the sounds was based on a down-sloping dynamic spectral ripple, modified in one additional novel way. Standard dynamic spectral ripples are sound stimuli containing regular temporal and spectral modulations with a limited number of parameters, which result in a spectral ripple drifting in time along the frequency axis. However, these still create high levels of off-diagonal covariance between frequencies, as the time-frequency drifting is constant, and therefore the activity in one frequency band has a highly predictable relationship to current, past and/or upcoming activity in other frequency bands. In our modulation, the spectral modulation rate of the dynamic ripple constantly doubled and halved over time (specifically, it was sinusoidally modulated over a one octave range), and through this it was constantly changing the relationships between spectral frequencies (Figure 3.4), nullifying any enduring correlations. Two alternative implementations of the modulation were applied (Figure 3.5): as an amplitude modulation (range 0-2, yielding mean 1 i.e. unchanged mean amplitude), and a phase modulation (range 0 to 2pi, constituting phase advancement to a maximum of 1 cycle), the latter being equivalent to a frequency modulation (e.g. phase advancement going from 0 to one cycle over one second would be equivalent to a frequency modulation of +1 Hz). In the amplitude implementation, the amplitude envelopes of any off-diagonal pair of frequency channels were temporally uncorrelated, but temporal fine structure was unaltered. In the phase implementation, amplitude envelopes were unaltered, but phase correlations between frequency pairs were eliminated. We tested both modulations, as we were not aware of any *a-priori* reason to favour either one of amplitude or temporal fine structure over the other as a determinant or (disrupting) the kind of cross-frequency neural synchrony relevant to tinnitus. To approximately compensate for sloping high frequency hearing loss, the spectra of the carrier stimuli were either flat, or contained additional energy in the higher frequencies, based on the hearing slope estimation of the individual participant. One of the four frequency correction profiles shown in Figure 3.1 was used for each subject. The modulations were perceptually subtle and had minimal effect on intelligibility or tolerability of the sounds.

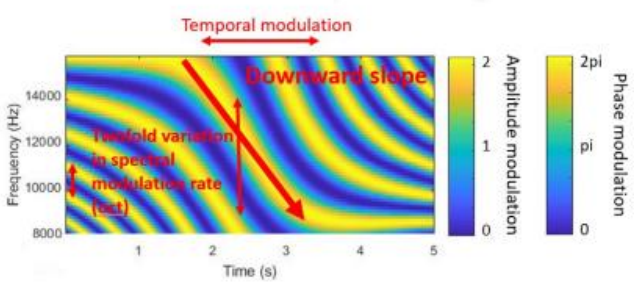



***Figure 3.4. Example of a section of the modulation.*** *All modulations were applied in a one-octave bandwidth, which was 8-16 kHz in this example. Activity outside this frequency range was left unaltered. The two colour scales indicate how the modulation would be applied to activity at each corresponding time-frequency point. Red arrows and annotations highlight the key features of the modulation.*

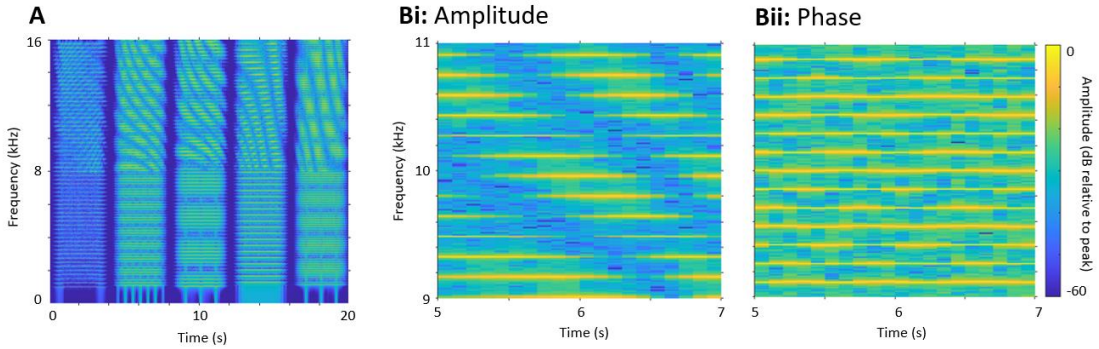



***Figure 3.5. Example application of the modulation to carrier sounds.*** *(**A**) Time-frequency spectrogram of the first 20 seconds of one of the therapeutic sound files listened to by participants. The first five 4s sounds can be seen, along with their different (randomly determined) fundamental frequencies, reflected in the spacing of harmonics (visible as horizontal lines). The modulation ripple structure (here an amplitude modulation) can be seen in the 8-16 kHz range. (**B**) Magnified section of representative sounds with each of the two ripple modulation types. (**Bi**) In the amplitude modulation, exact frequency spacing of harmonics is preserved but amplitude is altered. (**Bii**) In the phase modulation, amplitude remains constant, but the cycles of phase advancement and retraction can be seen as a subtle modulation of frequency (i.e. the frequency of each harmonic is not constant and flat, but resembles a very shallow sine wave).*

### *3.1.2.5 Outcome measures and statistical analysis*

The primary outcome measure was the NRS loudness score, however THI and TFI scores were also analysed. The questionnaire and NRS loudness scores at the beginning of a listening round were compared to the scores at the end of the listening round using paired t-

tests, in both active and sham. Then, the start score minus the end score value was calculated for each participant and active and in sham, and compared between the two conditions through paired t-tests; i.e. the value (active_pre_treatment – active_post_treatment) – (sham_pre_treatment – sham_post_treatment). This latter analysis, reflecting the difference in post- vs.pre-treatment scores between active and sham conditions, was used as the primary measure of treatment efficacy. The amplitude and frequency modulation conditions showed near-identical results in all outcome measures, so they were combined for further analysis.

### 3.2 Results

Demographic, tinnitus and hearing information for all three study groups is shown in Table 3.2.

| | **Amplitude (N=27)** | **Phase (N=26)** | **Noise (N=24)** |
|---|---|---|---|
| **Age** | 54.33 (31-83) | 57.35 (41-75) | 58.42 (33-75) |
| **Tinnitus frequency match** | 1.4-13 kHz,<br>mode: 9.5 kHz<br>(both 18.5%) | 2-17,<br>mode: 8 kHz<br>(19.2%) | 4-17,<br>mode: 8 kHz<br>(25%) |
| **Gender** | 8 f, 19 m | 10 f, 16 m | 9 f, 15 m |
| **Hearing slope match (Figure 3.1)** | 8 NH<br>9 mild HL<br>5 moderate HL<br>5 severe HL | 15 NH<br>6 mild HL<br>3 moderate HL<br>2 severe HL | 11 NH<br>5 mild HL<br>5 moderate HL<br>4 severe HL |
| **Self-perceived hearing difficulty** | 10 yes, 17 no | 10 yes, 16 no | 17 yes, 7 no |
| **Difficulty following conversations** | 4 yes, 12 no, 11 sometimes | 3 yes, 8 no, 15 sometimes | 9 yes, 6 no, 9 sometimes |
| **Hearing aids** | 3 use most of the time, 24 don't have | 2 use most of the time, 20 don't have,<br>4 have but don't use | 7 use most of the time,<br>14 don't have,<br>3 have but don't use |
| **Which ear is tinnitus in** | 2 entirely left<br>9 mostly left<br>11 roughly equal<br>2 mostly right<br>3 entirely right | 3 entirely left<br>8 mostly left<br>9 roughly equal<br>3 mostly right<br>3 entirely right | 1 entirely left<br>7 mostly left<br>12 roughly equal<br>2 mostly right<br>2 entirely right |
| **Is tinnitus affected during noise** | 17 quieter<br>8 unchanged<br>2 louder | 17 quieter<br>5 unchanged<br>4 louder | 13 quieter<br>10 unchanged<br>1 louder |
| **Is tinnitus affected after noise** | 6 quieter<br>17 unchanged<br>4 louder | 4 quieter<br>12 unchanged<br>10 louder | 2 quieter<br>17 unchanged<br>5 louder |
| **Related health conditions** | none | 1 acoustic neuroma, and 1 intracranial hypertension | 2 antibiotics or other meds, 1 blast injury, 1 acoustic neuroma |

***Table 3.2. Participant demographic information.***

### *3.2.1 Days missed & average listening times*

There were no significant differences between sham and active conditions within each group separately in terms of days of listening missed (notch degradation p=0.224; phase p=0.125; amplitude p=0.452), or between all 3 groups in either active (p=0.068) or sham (p=0.710).

While in notch degradation condition, the largest amount of listening to active was 85.14 minutes but 109.27 to sham, the durations of listening within groups were not significantly different (notch p=0.599; phase p=0.734; amplitude p=0.730). There were also no significant differences between groups in either the active (p=0.118) or sham condition (Kruskall-Wallis p=0.092). The amplitude modulation showed the highest amount of listening compared to other types, with one participant who listened to 422 minutes of the sound in active and 368 minutes in the sham condition. Without the numbers from this participant, the average listening times in amplitude condition are still higher than the other two conditions but by a smaller margin. In all conditions, the data for average listening amounts was skewed to the left, showing that most people listened for around 1 hour a day (note, the sound files sent to participants were 60 minutes long).

### *3.2.2 Drop-out rates*

Across the 3 conditions, out of 130 participants who started the first 6-week listening round, 109 participants completed it (Figure 3.2). The washout period, where no listening was done, lasted 3 weeks and then participants were asked to fill in the tinnitus questionnaires again. 97 people completed this. After the washout period they started the second round of listening, which also ended with questionnaires. 81 participants completed this stage. Finally, the second washout period questionnaires were completed by 77 participants. In summary, out of the 130 people that started this largely unsupervised online longitudinal study, 77 finished it.

| | **Notch degradation** | | **Phase modulation** | | **Amplitude modulation** | |
|---|---|---|---|---|---|---|
| | **Active** | **Sham** | **Active** | **Sham** | **Active** | **Sham** |
| **Days missed** | 4.54 (3.51) | 5.75 (5.05) | 3.50 (4.13) | 4.85 (4.51) | 5.56 (5.91) | 6.22 (5.61) |
| **Average listening times** | 54.01 (17.60) | 54.91 (21.38) | 66.47 (36.51) | 65.23 (33.46) | 87.70 (82.44) | 88.65 (74.93) |

***Table 3.3. Means (and SD) of days missed & daily listening times.***

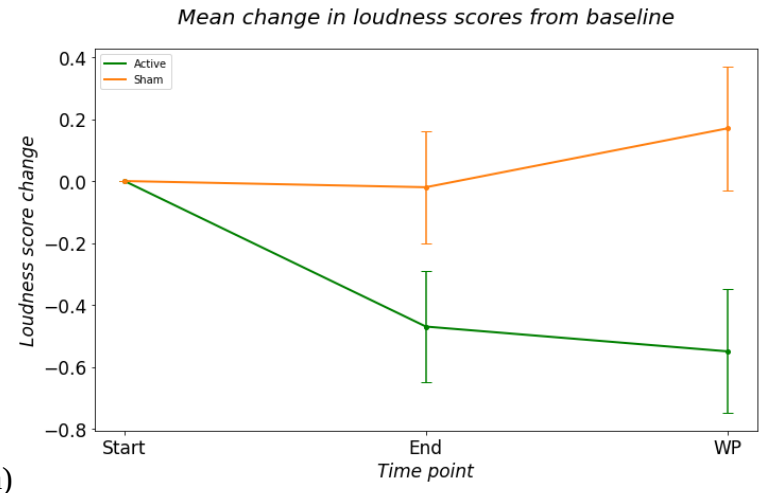
### *3.2.3 Combined Amplitude & Phase Modulation Results*

The two novel ripple modulation conditions showed very similar results, so they were pooled for analysis (Table 3.4).

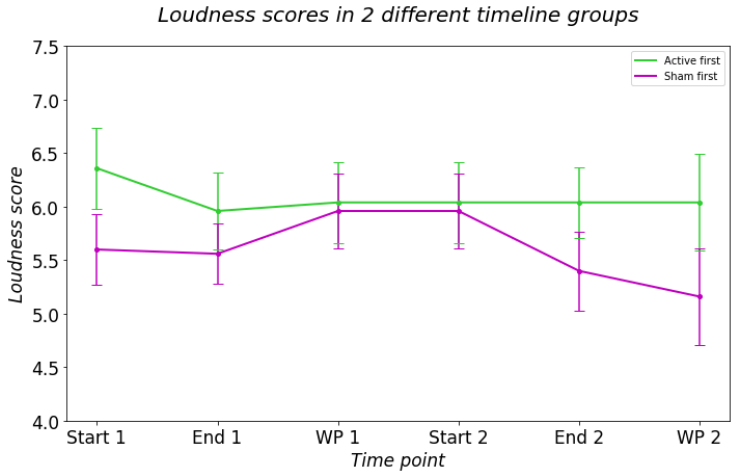
| | **NRS loudness** | THI | HQ | TFI |
|---|---|---|---|---|
| **A_Start** | 6.17 (1.89) | 42.42 (24.04) | 14.23 (7.41) | 45.68 (21.69) |
| **A_End** | 5.70 (1.88) | 37.09 (22.28) | 14.25 (8.00) | 40.82 (19.97) |
| **A_WP** | 5.62 (2.16) | 38.98 (24.25) | 14.58 (6.57) | 42.05 (21.10) |
| **S_Start** | 5.83 (1.84) | 43.66 (22.76) | 14.25 (6.83) | 46.71 (18.61) |
| **S_End** | 5.81 (1.59) | 38.91 (21.77) | 13.81 (7.70) | 42.20 (20.29) |
| **S_WP** | 6.00 (2.09) | 38.68 (22.87) | 14.72 (8.08) | 42.24 (21.79) |

***Table 3.4. Mean and SD of each measure at every time point of the experiment in the combined sample.*** *In the time point titles, 'Start' refers to the beginning of a listening period, 'End' refers to the end of a listening period, and 'WP' refers to the end of the washout period in which participants did not have any daily listening. The prefix 'A' indicates that the title refers to the active condition, whereas the prefix 'S' refers to the sham condition. For example, S_WP means the end of the washout period after the sham condition.*

The primary measure, the NRS loudness score, was significantly reduced in the active condition ($t(52) = 2.59$, $p = 0.012$) but not in the sham ($t(52) = 0.106$, $p = 0.916$) (Figure 3.5 a)). Moreover, the difference between start and end scores (start score – end score = difference) in active were significantly different compared to sham ($t(52) = 2.26$, $p = 0.028$), which was even more evident for the difference between start and end-of-washout-period scores in active compared to sham ($t(52) = 2.61$, $p = 0.012$); i.e. there was a trend towards a further reduction between the start and end of the washout period following the active condition. These differences were also significant in the amplitude group by itself, and trends towards significance were seen in the phase group. Note that the half of each group receiving that treatment second was affected by potentially having the after-effect of the other group (Figure 3.5 b), e.g. the group receiving sham treatment second started from a baseline corresponding to the enduring effect of active treatment. THI and TFI scores significantly reduced in both active (THI: $t(52) = 3.40$, $p = 0.001$; TFI: $t(52) = 2.99$, $p = 0.004$) and sham conditions (THI: $t(52) = 2.77$, $p = 0.008$; TFI: $t(52) = 2.45$, $p = 0.018$), but there was no significant difference between the reduction following active or sham listening. HQ scores did not significantly change. None of the results were not dependent on hearing profile or whether the participants indicated having pure tone or broadband tinnitus.




a)




b)

***Figure 3.5. NRS loudness score change at different time points.*** *a) shows change in loudness scores from a baseline at the start of each type of condition. b) shows the average scores collected from participants who either started with the active condition, or those who started with the sham condition.*

### *3.2.4 Notch Degradation Results*

The average outcome measure scores at each time point are shown in Table 3.5.

| | **NRS Loudness** | THI | HQ | TFI |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **A_Start** | 5.50 (2.21) | 29.17 (14.65) | 15.83 (8.89) | 39.35 (20.72) |
| **A_End** | 5.50 (2.49) | 30.67 (16.11) | 16.50 (8.77) | 39.77 (21.97) |
| **WP_A** | 5.38 (2.57) | 30.17 (16.39) | 16.29 (9.41) | 40.85 (24.86) |
| **S_Start** | 5.33 (2.43) | 36.42 (20.29) | 16.54 (7.71) | 43.73 (24.13) |
| **S_End** | 5.08 (2.67) | 30.83 (18.67) | 16.17 (8.64) | 38.23 (24.51) |
| **WP_S** | 5.46 (2.64) | 29.17 (17.42) | 16.13 (8.91) | 39.95 (22.53) |

***Table 3.5. Mean and SD of each measure at every time point of the experiment in the notch degradation sample.*** *In the time point titles, 'Start' refers to the beginning of a listening period, 'End' refers to the end of a listening period, and 'WP' refers to the end of the washout period in which participants did not have any daily listening. The prefix 'A' indicates that the title refers to the active condition, whereas the prefix 'S' refers to the sham condition.*

Unexpectedly, there was a significant difference between start and end NRS loudness scores in the sham condition ($t(23) = 2.135$, $p = 0.044$), which was not seen in the active condition ($t(23) = -0.89$, $p = 0.381$). However, this was not sustained throughout the washout period. THI scores reduced by 6 points in the sham condition, and were unchanged in the active condition. The difference between the start - end scores in active compared to sham was also significant ($t(23) = -2.14$, $p = 0.043$). No other measure showed significant differences between start and end, or in the differences scores between active and sham.

## 3.3 Discussion

Overall, our novel covariance cancelling stimulus had a significant effect in reducing tinnitus loudness over 6 weeks of regular listening, and this reduction persisted for at least a further 3 weeks of no listening. This was not seen in the sham condition, and thus indicates a specific effect of the novel modulation. On the other hand, our results also indicate feasibility and good tolerability of this stimulus, as shown through the mean reduction in THI and TFI during both active and sham conditions, and through dropout rates between starting and completing the regular listening phases of the trial being favourable for an online study.

### *3.3.1 Comparison to previous studies*

The method in the current study allows for a more inclusive treatment than existing sound therapy approaches. Many previously mentioned methods, including ADT, HMNMT, vagal nerve stimulation, and CR neuromodulation have been aimed only at people with tonal tinnitus, whereas in this study, the effects spread both to tonal and noise tinnitus types regardless of initial reason behind the condition. Additionally, many approaches require regular visits and possibly extensive explanation, which sometimes excludes those who do not

fluently speak the language of the researcher [154], and limit availability of treatment to only specialist centres. This also excludes people who cannot easily travel/ take time away from their daily activities. For example, trigeminal nerve stimulation required clinic visits every 2 weeks while others required extensive initial testing and weekly counselling meetings. Therefore, the pure online therapy would be much more accessible to adults from a variety of working and nationality backgrounds, geographical locations, and mobility restrictions.

The majority of existing sound therapies need input of specialists to carry out precise tinnitus matching, including NTT, ADT, CR neuromodulation, and the Levo system. In the current study, the tinnitus tone and hearing slope matching was entirely unsupervised, and therefore any resulting inaccuracies might have, if anything, reduced the effect sizes seen in the data. However, the match needed only to be approximate, unlike some other treatments discussed in the introduction. One discussed study also only required approximate tinnitus matching, the modulated wave therapy [167]. Interestingly, this is the most similar method to the one used in the current study, though the significant tinnitus suppression (equivalent of 0.62 reduction on a 10-point scale) of modulated wave therapy only lasted a maximum of 2 days but usually minutes. Our approximate matching could have been a potential weakness, however, as part of the focus was on investigating the feasibility of a fully unsupervised online sound therapy, to ensure accessibility of the treatment, its significant findings show that the approximate, online process can be successful despite any inherent inaccuracies in tinnitus matching, and therefore proved to be a strength of the procedure. Subsequent improvements in the unsupervised tinnitus frequency matching process might help increase the efficacy of the method.

Similarly to our findings, previous studies have found that a positive effect on tinnitus distress can occur even when the sound therapy is not aimed at the tinnitus frequency of the individual, thus potentially pointing towards the overall “cognitive benefit” of tinnitus therapy [151, 157]. The authors suggested that their therapy influenced the distress network, thus helping the participants cope with tinnitus. It is uncertain whether other mentioned forms of sound therapy also reduce tinnitus distress regardless of the precise tinnitus frequency, as experimenters may choose different forms of control conditions that do not include a sham that is similar to this study, e.g. instead opting for a waiting list group. While it is not possible to assess why the THI and TFI scores reduced both in sham and in active conditions in our study, by similar margins (around 5 points in each questionnaire in each condition), the THI and TFI improvement may perhaps indicate an overall positive effect of the act of participating in sound therapy. This is important for the feasibility of automated online

studies, as it indicates that there is a net beneficial placebo effect, even without direct communication with a researcher or clinician.

While the overall experiment in the current study was 18 weeks, the active condition only lasted 6 weeks and the washout period lasted 3 weeks, making the overall active part of the study 9 weeks. This is one of the shorter time periods in which a longitudinal sound therapy experiment took place, except for ADT study that lasted 30 days [157]. Other studies lasted from 10 weeks to 12 months. The ADT study showed a smaller, non-significant effect on loudness (0.5 on a 10-point scale), following a daily 20-minute active auditory discrimination task. While the current study lasted longer, the effects were somewhat stronger and the listening could be done alongside other activities, thus possibly being less taxing. This ADT experiment, however, only required one visit prior to the start of the therapy, so it may have been more agreeable for some participants compared to other cited forms of tinnitus treatment. The next shortest study was the 10-week trigeminal nerve stimulation experiment, in which an equivalent of 0.62 on a 10-point scale loudness reduction occurred. This is a somewhat larger effect on loudness than in the current study, however the procedure requires frequent visits and placements of electrodes on the tongue, so some participants may prefer to avoid it if there are similarly effective at-home/more readily available/less invasive treatments. The 12-week CR neuromodulation study in which participants listened to sounds for a similar amount of time a week to the current study resulted in a non-significant reduction of 0.45 on a 10-point scale, which is less effective than our novel stimulus. The Levo system study also lasted 12 weeks, with many hours of listening per night, and did lead to a reduction of 1.00 (absolute reduction, not compared against sham treatment), however this system is largely unavailable to people due to its high price. The TMNMT study lasted 12 months and showed an impressive reduction of 25% by the end of the experiment. However, it is not certain whether this is reliable as the study had 8 participants per group. Additionally, a larger cohort study lasting 3 months did not see a significant reduction in loudness, therefore it would be preferable to carry out further longitudinal experiments into this method. Overall, at this stage of testing, our covariance cancelling stimulus seems to present similar or more efficacious results than the previous studies, is based on larger numbers and more rigorous control groups than most comparable methods, and is unique in its complete non-reliance on clinician or researcher-involved methods or bespoke hardware, thus giving instant and almost unlimited scale of delivery.

### *3.3.2 Strengths & Limitations*

A minor limitation of the study is that the length of listening each day was not controlled. The amplitude modulation showed the highest amount of listening compared to other types. However, potentially higher amounts of average listening may indicate better tolerability of amplitude modulation compared to phase modulation. Nevertheless, the data indicated that the majority of participants listened for around 1 hour a day. Additionally, it is potentially important for the sounds to be beneficial at a realistic range of daily listening amounts as this would allow for greater world applicability, due to varying everyday responsibilities and lifestyles.

There was a reasonable retention rate for online studies of sound therapy. This study took 18 weeks overall, with 12 weeks of involving listening for 60 minutes on average. In previous studies where 6-12 weeks were dedicated to tinnitus therapy, the dropout rate was between 14% and 28% [160, 173, 176]. The current study has a slightly higher dropout rate, but the study was completely online whereas in the other 3 examples, participants needed to come in for initial assessments, which potentially motivated them to be more determined to finish the study. In the current study, the majority of dropouts occurred soon after receiving the first sound file. After this, retention rate was very high.

### *3.3.3 Future Directions*

The overall reduction in tinnitus loudness in the active condition of the combined sample was by around 5.5% in absolute terms, or 9% in relative terms. Our next steps should be to improve tolerability and pleasantness of the sounds, and investigate how or whether we can maximise the tinnitus loudness reduction. For example, further research is needed to understand how long the effects can last after finishing the listening periods, and what is the optimal length of listening both daily and for the overall treatment round that would maximise the reduction in loudness score. There is presently no evidence that tinnitus loudness reduction reaches a plateau within the 6 week listening period we tested, whilst there is evidence that the effect endures for at least 3 weeks after that. Additionally, there are many free parameters of both the carrier sounds as well as the modulation itself that can be modified to potentially increase efficacy, and we have tested only one parameter combination so far. These include the range, and rate of change of modulation rates for either type of modulation (amplitude and phase), and also the bandwidth in which the modulation is applied. For instance, as well as tonotopy (organisation by frequency), the central auditory pathway is organised by periodotopy (temporal modulation rate), in which it is sensitive to modulations up to at least 256 Hz [181], contrasting with maximal rates of less than 10 Hz we used.

Therefore, large proportions of neuronal assemblies in the auditory pathway might be targeted, which our present implementation was not able to recruit. Additionally, if the phase modulation is considered a frequency modulation, then the limit of modulation need not stop at 2pi, which constitutes a very small frequency modulation, but could go orders of magnitude higher. Furthermore, amplitude and phase modulations are not mutually exclusive, but could be combined, with either the same modulation ripple, or separate orthogonal ones. Therefore, by systematically exploring these parameters, we may be able to greatly increase the efficacy of our novel stimulus.

Further, while specific sequences of sounds were used for this experiment, they do not necessarily need to be the carrier for this modulation. The modulation could be applied across a range of different natural or synthetic sounds that have sufficient high-frequency content. Importantly, the reaction and potential benefit from the sound sequences used in sound therapy can be individualistic, depending on the emotions elicited by the sounds [182]. Therefore, a potential next step could be to apply the modulation to already well-tolerated music/sound sequences that the participants enjoy, thus allowing for an even more individualistic approach.

#### *3.3.4 Conclusion*

Automated online sound therapy is feasible for longitudinal tinnitus treatment studies. Moreover, our novel covariance cancelling amplitude and phase-modulated sounds show promise in reducing perceived tinnitus loudness. Once optimal parameters and efficacy are determined, the treatment can be made freely available on an unlimited scale to the tinnitus community, and iteratively improved by the low-cost-low-burden running of large automated online randomised controlled trials.

# Chapter 4. Distinct profiles of tinnitus and hyperacusis in intensity deviant responses and auditory evoked potentials

## 4.0 Introduction

### *4.0.1 Tinnitus & Hyperacusis*

Tinnitus is a persistent sound heard by an individual without an environmental source [1]. It is a common condition, yet the search for a human biomarker of tinnitus is still on-going, as discussed in the introduction to this thesis. There is a high correlation between presence of tinnitus and hyperacusis, which increases with severity of tinnitus [72, 99]. Hyperacusis is an auditory condition that causes normal environmental sounds to be uncomfortably loud [9]. It can strongly impact quality of life [124]. Hyperacusis is likely to occur with hearing loss and in the ageing population, though it also occurs in people with normal audiograms (possibly with hidden hearing loss such as asymmetric and notched audiometric results) [26, 183, 184]. In general, hyperacusis occurs due central gain changes, possibly from weakened inhibition from the auditory cortex to the inferior colliculus, and likely involves the limbic system [185, 186]. A previous fMRI study on adults with vs. without hyperacusis showed a global effect of enhanced sound-evoked activation across all frequencies [129], while another study showed reduced habituation (less N100 suppression) in patients with Fragile X syndrome who also had hyperacusis [187].

Four categories of hyperacusis have been described: loudness, annoyance/avoidance, fear, and pain [188]. Annoyance/avoidance and fear types are accompanied by strong emotions, while the last type is accompanied by pain around the face and the ear/s. Usually, the latter three types accompany the loudness hyperacusis, but have their own unique mechanisms [188]. Hyperacusis is highly prevalent in people with a variety of neurological conditions such as autism, ADHD, chronic pain, head traumas, depression, PTSD, etc [188], with some of these conditions being particularly related to fear and annoyance/avoidance hyperacusis (e.g. autism) while others are more related to pain hyperacusis (e.g. patients with chronic pain). Hyperacusis of an emotion-inducing subtype has also been related to chronic stress due to a connection between the amygdala and the autonomic nervous system. For example, rats did not adapt to prolonged exposure to noise and their levels of corticosterone were increased the longer they were exposed to this noise [189]. Another study showed a heightened connectivity between the orbitofrontal cortex/dorsal anterior cingulate cortex and the auditory cortex. Orbitofrontal cortex and dorsal anterior cingulate cortex have been previously implicated in pain perception and anticipation of noxious stimuli [188, 190]. People with hyperacusis also reported higher stress levels from their work environment compared to people without hyperacusis [191].

Much of the brain activity changes may be related to levels of hearing loss as well as distress caused by the tinnitus/hyperacusis [123]. Both tinnitus and hyperacusis may stem from abnormal central gain in the auditory cortex and related connections due to overcompensation

by the brain for reduced environmental input, but in different ways [45, 123]. It has been suggested that tinnitus is the addition of central noise that compensates for reduced input by maintaining usual levels of activity, possibly through prediction errors, by increased spontaneous firing rates and/or heightened neural synchrony. On the other hand, hyperacusis stems from multiplicative central gain [14, 192]. Another theory states that a larger extent of peripheral pathology/deafferentation may lead to a failure to compensate sufficiently by increasing central gain, which is related to tinnitus, while hyperacusis may relate to the over-amplified compensating central gain increase [26]. Both processes could occur in one person, from which an interaction between the two conditions could be seen that could in turn alter the shape of studied activity. Further discussion of potential hyperacusis and tinnitus mechanisms can be seen in the introduction chapter of this thesis.

However, despite so much overlap between tinnitus and hyperacusis prevalences and aetiologies, much of earlier research did not account for or focus on hyperacusis as a potential confounding factor. Hyperacusis presence may affect patterns and regions implicated in tinnitus research, though the existence of a connection between the two conditions has been long-known [192-194]. As an example of a problem with such conflations in previous research, an fMRI study showed that activation in inferior colliculus and medial geniculate body, previously correlated with tinnitus, was more directly related to reactivity to external sounds, which was not accounted for in other studies [45]. This oversight has come to light in the recent years, along with the realisation that many hyperacusis-focused studies mainly test subjects with minimal hearing loss [129]. Some more recent studies focus on comparisons of activity in tinnitus with and without hyperacusis [129], for example, an fMRI study demonstrated that responses to auditory stimuli in subcortical auditory structures and cortices (such as inferior colliculus and medial geniculate body) are increased when participants have hyperacusis (as well as overt moderate hearing loss and tinnitus). This finding was not due to reduced cortical frequency specificity that could be seen in the presence of hearing loss. Interestingly, they also established that the group with both tinnitus and hyperacusis had significantly smaller activation in response to tinnitus-like frequency stimuli compared to the group without hyperacusis but with tinnitus. There are two potential explanations for this phenomenon. The hyperacusis group may have more central noise at the tinnitus frequency, meaning that less gain is needed. Alternatively, this could occur due to ceiling effect of the fMRI-related background noise: tinnitus frequencies are already hyper-stimulated, so they have less room to increase their activity even further when played a matched tone [195].

### *4.0.2 Event-related potentials*

Auditory event-related potentials, such as P50, N100, and Mismatch Negativity (MMN) have been used to investigate mechanisms and as potential biomarkers for a number of conditions, including tinnitus, schizophrenia, autism, mild cognitive impairment, Alzheimer's disease and psychosis [95, 179, 196-199].

#### *4.0.2.1 Standard responses*

Generally, P50, N100 and P200 components are related to an inhibitory function called sensory gating, such as reducing responses to repetitive stimuli (filtering out irrelevant stimuli), or detecting novel stimuli [200]. It is a multistage mechanism, with each of the three ERPs having their own function [201]. P50 and N100 usually represent a pre-attentional and an attention-triggering filter mechanism, occurring around 50 ms and 100 ms after stimulus onset, respectively [202]. P200 may be related to allocation of attention, and can be an independent component from the N100 despite earlier research possibly suggesting otherwise [203]. These ERPs decrease in amplitude as a result of repeated stimuli. A reduction in P50 and N100 amplitudes reflect sensory gating-out of a repeating stimulus. One study showed that while change in frequency did not cause significant increase in P50 or N100, whereas intensity change did [200]. P200 has also been found to be intensity dependent [204].

Attenuated reduction in amplitudes to a second stimulus compared to an identical first stimulus have been found to be indicative of altered information processing in conditions such as schizophrenia and even in children with cochlear implants [201, 205]. Further, in a study using 1 kHz tone bursts, a bilateral tinnitus group were more sensitive to an increase in intensity from 70 dB to 90 dB SPL in terms of increasing N100 and P200 amplitudes, compared to unilateral tinnitus and control groups. However, inhibitory mechanisms are also diminished in hearing loss and thus any conclusions must be reached with caution [206].

N100 has also been implicated in the stimulus-specific adaption theory [207]. One study used trains of tones with a number of potential deviants. A standard repeating pattern included two tones (A B), one was a lower frequency tone (800 Hz) (A) which was then followed by a tone of a higher (1600 Hz) (B) frequency. Two of the possible deviants from this pattern had the first frequency repeat as the second tone (A A or B B), instead of the usual higher frequency tone. N100 suppression was larger to the deviants AA and AB compared to the standard AB, showing that N100 adapts to the physical attributes of the stimuli rather than more complex irregularities. On the other hand, MMN responses were larger to the deviants AA and AB compared to standard AB, thus showing the more complex processing underlying MMN.

In a study with 15 mild-tinnitus participants with normal hearing in normal and extended frequency audiometry testing, paired stimuli were presented to test sensory gating function [208]. Compared to the age and hearing-matched control group, the tinnitus group showed no significant differences in the gating processes, however, within the tinnitus group, there were 'high suppressors' and 'low suppressors'. The two suppressor groups were defined based on the Pa amplitude, which is a small positive peak that occurs around 30 ms, just before the P50 response [209, 210]. The high suppressors showed similar activity to the control group, but low suppressors increased early P50 amplitude to the second tone, as well as overall reduced N100 to both tones. This group also had an overall higher P200. Notably, hyperacusis was not measured in this study, therefore it is not possible to assess whether any differences between the tinnitus groups were due to hyperacusis presence or a different unmeasured factor. A review showed that N100 finding have been inconsistent so far both in the matching of hearing loss of tinnitus and control group, in the findings themselves both at frequencies below or within the tinnitus frequency of a participant [30]. Studies investigating solely hyperacusis are scarce [211], however, MEG studies on children with autism showed that M100 latencies were longer in children with auditory sensitivity compared to children without auditory sensitivity, and M50 dipoles were larger [212, 213]. In a group of children with Williams syndrome and hyperacusis, amplitudes in the P50-N100-P200 complex and MMN were increased compared to controls [214]. A study that used pairs of identical stimuli, found that P50 amplitude could predict acceptable noise level, which is associated with sensory gating functioning [215].

#### *4.0.2.2 Mismatch Negativity*

MMN has been discussed in the introduction of this thesis. Briefly, MMN is a neural correlate of change detection [207]. Within the predictive coding framework, MMN occurs when a comparison between an expected stimulus and the sensory input do not match, thus producing a prediction error. The larger the difference, the larger the prediction error. Another theory of MMN is that it reflects stimulus-specific adaptation based on sensory memory, where repetition suppression occurs after a stimulus is repeatedly presented. In an fMRI study, it was found that MMN contains a number of subcomponents that stem from different cortical regions, which differentiate the responsibility for simpler feature detection (similarly to N100) and more complex processes such as prediction violation and uncertainty [207].

### *4.0.3 Previous MMN research in tinnitus*

An MMN-based biomarker in humans, called Intensity Mismatch Asymmetry (IMA), was identified as a potential tool for identifying a tinnitus biomarker when measured in response to intensity deviants at tinnitus frequency. IMA was based in predictive coding, aimed to relate to mechanisms forming part of a 'final common pathway' for tinnitus, irrespective of specific contributory mechanisms [83, 95]. Such a biomarker would help to better understand tinnitus mechanisms and allow treatment studies to determine the effectiveness of their treatment across tinnitus groups [14, 98]. IMA showed that participants with tinnitus had larger MMN responses to upward deviants (UD), but smaller MMN responses to downward deviants (DD), compared to the control group [95]. This first study focused on frequencies close to the tinnitus frequency only, and therefore a second study was conducted in which the tinnitus frequency responses were compared to responses to a 1 kHz control frequency [179]. However, the second study did not find the same pattern of activity. Potential reasons could have been higher levels of hearing loss overall, the overall paradigm context (the addition of much lower frequency), but also presence of hyperacusis. It is not known whether participants had hyperacusis in the original study, however in the second study, the majority of the tinnitus group had hyperacusis based on the updated cut-off score of 16 on the Khalfa Hyperacusis Questionnaire [107, 110].

Both tinnitus and/or hyperacusis have different effects on brain function, including MMN amplitude [95, 183, 192, 216]. For example, when comparing MMN responses in a multi-deviant paradigm, tinnitus participants with normal extended frequency hearing had weaker MMNs for frequency, intensity, duration, location and silent gap deviants, compared to an age and gender matched control group [217]. The tones involved in this study were 500-1500 Hz, therefore far away from the usual tinnitus frequencies. Hyperacusis was not measured. In another example of a study that utilised a non-standard multi-feature MMN paradigm that included noise, pitch, location, intensity, laterality and rhythm deviants, with standards being chords that could often be heard in Western music, researchers found that central auditory mechanisms are altered in subjects depending on whether they had low/medium/high noise sensitivity (but no tinnitus) [216]. As tinnitus and hyperacusis can affect similar processes in different (or similar) ways, disentangling the two conditions is an important step in furthering our understanding of both conditions. Therefore, it would be useful to understand how each condition affects the auditory event-related components such as P50, N100, P200 and MMN, especially when these components are often used as a measure of tinnitus.

There are numerous motivating factors for the present study, which is a rigorous and systematic exploration of hyperacusis, tinnitus, and their combination, on early and late

evoked responses and intensity deviant-related MMN. To my knowledge, this is the first study to assess ERPs between all four combinations of tinnitus and hyperacusis (both conditions, one of the conditions, neither condition), as well as the first study to investigate differences between responses to deviant stimuli overall, and specifically intensity deviant stimuli, between these groups. The four groups in this study are controlled for hearing profile, eliminating hearing loss as a potential confound. This should allow us to reflect on whether any of the previous tinnitus research tells us more about the involvement of hyperacusis than tinnitus specifically.

## 4.1 Materials & Methods:

### *4.1.1 Participants*

Four groups, each of 21 participants, were studied: 1) tinnitus without hyperacusis (T+H-), 2) tinnitus and hyperacusis (T+H+), 3) no tinnitus and no hyperacusis (C) and 4) hyperacusis without tinnitus (T-H+). Participants were recruited from affiliated volunteer lists at Newcastle University and via Google Ads. General inclusion criteria included being over 18 years old, and able to make an informed choice about volunteering. General exclusion criteria included using ongoing sedating or nerve-acting medications, and mental health conditions severe enough to interfere with everyday life activities. All potential participants needed to complete the Hyperacusis Questionnaire (HQ) [107] to understand which groups of participants they belonged to and whether they were eligible for the study. Those with an HQ score under 16 were in the H+ groups [110]. Those who scored above this, and also scored above 56 on the Inventory of Hyperacusis Symptoms (IHS) [218] were included in the H+ groups. To be included in one of the T+ groups, inclusion criteria also involved having chronic tinnitus for over 6 months, without an objective physical source, and which was not due to Meniere's disease (this was an exclusion criterion for T- groups). T- participants were individually matched to T+ participants, based on an approximate match of their overall audiometric profiles, with particular attention to the vicinities of 1 kHz and the tinnitus frequency. It was also ensured that there were no significant group differences between equivalent tinnitus and control groups in age or sex.

Approval was given by the Newcastle University Research Ethics Committee, and all participants gave written informed consent according to the Declaration of Helsinki (reference number 5619/2020).

### *4.1.2 Common methods: tinnitus psychophysics and EEG*

The psychophysical assessment in which the tinnitus frequency and the intensity of sounds played during the EEG recording were determined, and the experimental design, followed the procedure in Chapter 2 of this thesis. EEG data processing followed the procedure in Chapter 2, with the exception that rather than Denoising Source Separation used in Chapter 2, Independent Component Analysis was used to remove ocular artefacts.

### *4.1.3 Statistical analysis*

Statistical analysis was performed using MATLAB. To compare the evoked responses in participants with and without tinnitus and hyperacusis, three-way ANOVAs were used, with subject group (T+H-, T+H+, C, T-H+), frequency, and intensity used as factors of interest, and including interaction terms. Post-hoc analysis included Tukey Honest Significance Tests to determine any significant differences between the ERP amplitudes of the four groups, as this test is powerful when testing multiple numbers of means, and uses a similar parameter to the ANOVA tests [219]. Then, visual inspection of error bars created for the results section was carried out, based on which potential paired t-tests were run to see any patterns within-group, e.g. to see significant differences between downward deviant and upward deviant conditions.

## 4.2 Results

### *4.2.1 Demographic information*

Table 4.1 shows means and standard errors (SE) of the demographic information of the 4 groups, their HQ scores, and THI scores in the two groups with tinnitus. One-way ANOVA showed that age was not significantly different between the four groups ($p=0.892$). HQ, on the other hand, was significantly different between the 4 groups ($p<0.001$). T+H+ and T-H+ had no significant differences in their HQ scores ($p=0.089$), whereas both H+ groups had significantly higher HQ scores than both C and T+H- groups (all $p<0.001$). This was expected as this was one of the bases for allocating groups. The cut-off score for HQ was 16 therefore all 4 groups were comfortably within their hyperacusis presence requirements. Following previous literature, the sample with tinnitus and hyperacusis had higher THI scores than those with tinnitus only. Chi-square test was carried out to establish that there was no significant difference in sex across the four groups ($Chi\ (3) =2.61$, $p=0.456$). As the pure tone audiometry results were not normally distributed at the majority of frequencies/ears Kruskall-Wallis tests were performed and showed that across all 4 groups, there were no significant

differences in the hearing ability at 0.25, 0.5, 1, 2, 4, 6 or 8 kHz in right or left ears, with the smallest p-value being p=0.163.

| | T+H- | T+H+ | C | T-H+ |
|---|---|---|---|---|
| **Age** | 53.71 (3.29) | 55.24 (3.17) | 54.33 (3.40) | 56.95 (2.47) |
| **Sex** | 13 f, 8 m | 13 f, 8 m | 11 f, 10 m | 16 f, 5 m |
| **HQ scores** | 10.24 (0.74) | 25.76 (1.35) | 5.29 (0.91) | 30.71 (1.61) |
| **THI scores** | 20.29 (3.13) | 45.52 (5.43) | N/A | N/A |

***Table 4.1. Descriptive statistics of the four study groups.*** *Means and standard errors are given for every group for their age, HQ scores and THI scores. The sex split is also indicated for each group.*

### *4.2.2 Time course of the stimulus response*

Grand average ERP data for channel FCz (with P9/P10 reference) across standard and deviant responses for all stimulus conditions and in each group was used to determine timeframes for quantifying P50, N100, P200 and MMN responses, based on visual inspection (Figure 4.1). To calculate the MMN difference waveform, standard responses were subtracted from their equivalent deviant conditions (Figure 4.1).

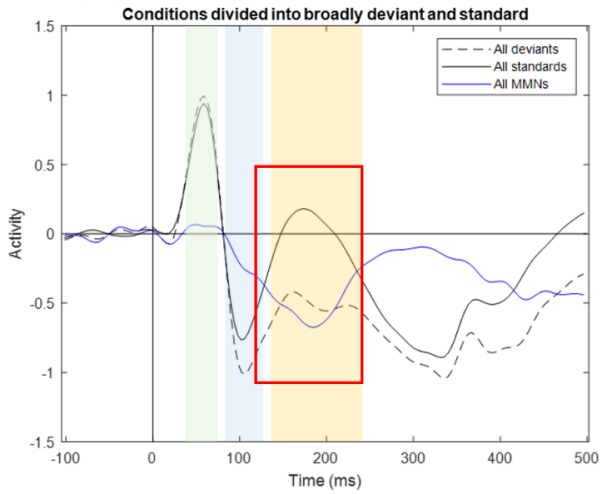
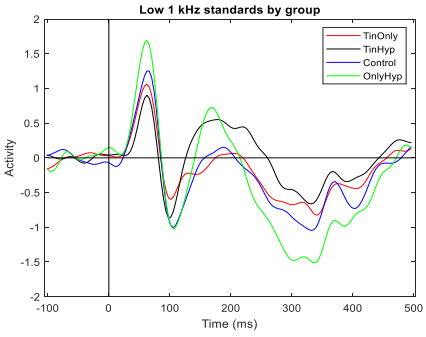



***Figure 4.1. Standard, deviant and MMN waveforms broadly combined across groups and conditions.*** *The chosen P50 timeframe was 45-75 ms (green), N100 was 85-120 ms (blue), P200 was 140-240 ms (orange). The MMN timeframe was 115-240 ms (red square). As the MMN and P200 overlap, P200 was only used in standards (and MMN in the difference waveform).*

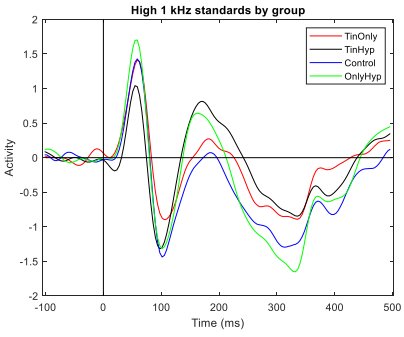
### *4.2.3 Standard and pure deviant waveforms*

The average waveforms to each condition are shown in Figure 4.2.

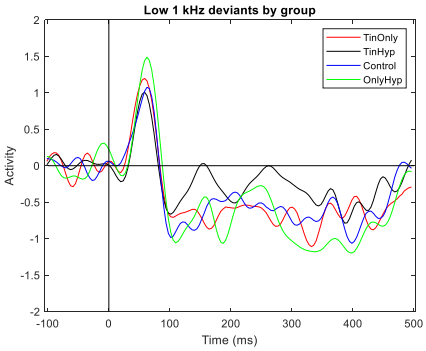
a)



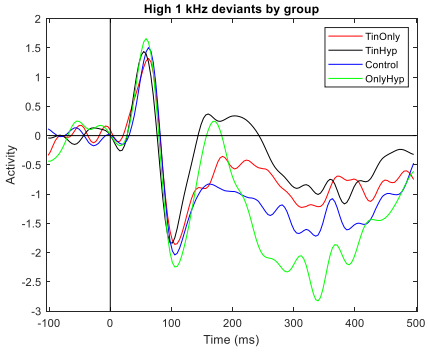

b)




c)




d)



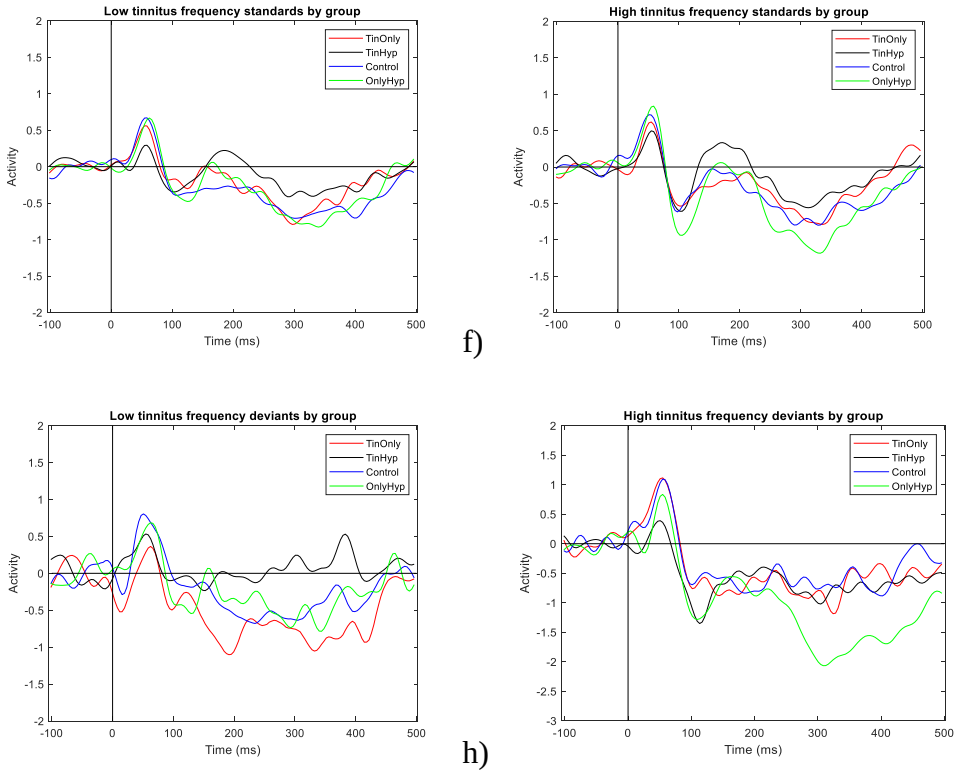



***Figure 4.2. Average waveforms to standard and deviant conditions.*** *Graphs a-d show responses at 1 kHz frequency, and e-h show responses to the tinnitus frequency. On the left are the quieter stimulus conditions and on the right are the louder stimulus conditions. The groups are colour-coded as follows: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

### *4.2.3.1 P50 responses*

*Standards*

Figure 4.3 shows P50 responses to standard stimuli in the four conditions (two intensities at two frequencies). A three-way ANOVA (group, frequency, intensity) showed significant main effects of group ($p<0.001$) and frequency ($p<0.001$), as well as an interaction between these two factors ($p=0.011$). Post-hoc test on group effects was carried out using Tukey test.

At low intensity stimuli at 1 kHz frequency, T-H+ group had a significantly higher amplitude than all other groups (all $p<0.001$); C group also showed significantly stronger response than T+H+ group ($p=0.002$); there were no significant differences between T+H- and T+H+ ($p=0.146$) or T+H- and C ($p=0.266$) groups. At high intensity at 1 kHz frequency, T+H+ group had significantly smaller amplitude compared to all other groups (all $p<0.001$); no other significant differences were seen.

At low intensity at tinnitus frequency, T+H+ group had significantly lower P50 amplitude compared to T+H- (p=0.031), control (p=0.039) and T-H+ (p=0.002) groups. There were no significant differences between T+H- and C groups (p=0.999), or C and T-H+ groups (p=0.613). At high intensity at tinnitus frequency, T+H+ group had significantly lower amplitude than T-H+ group (p=0.020), no other significant differences were seen.

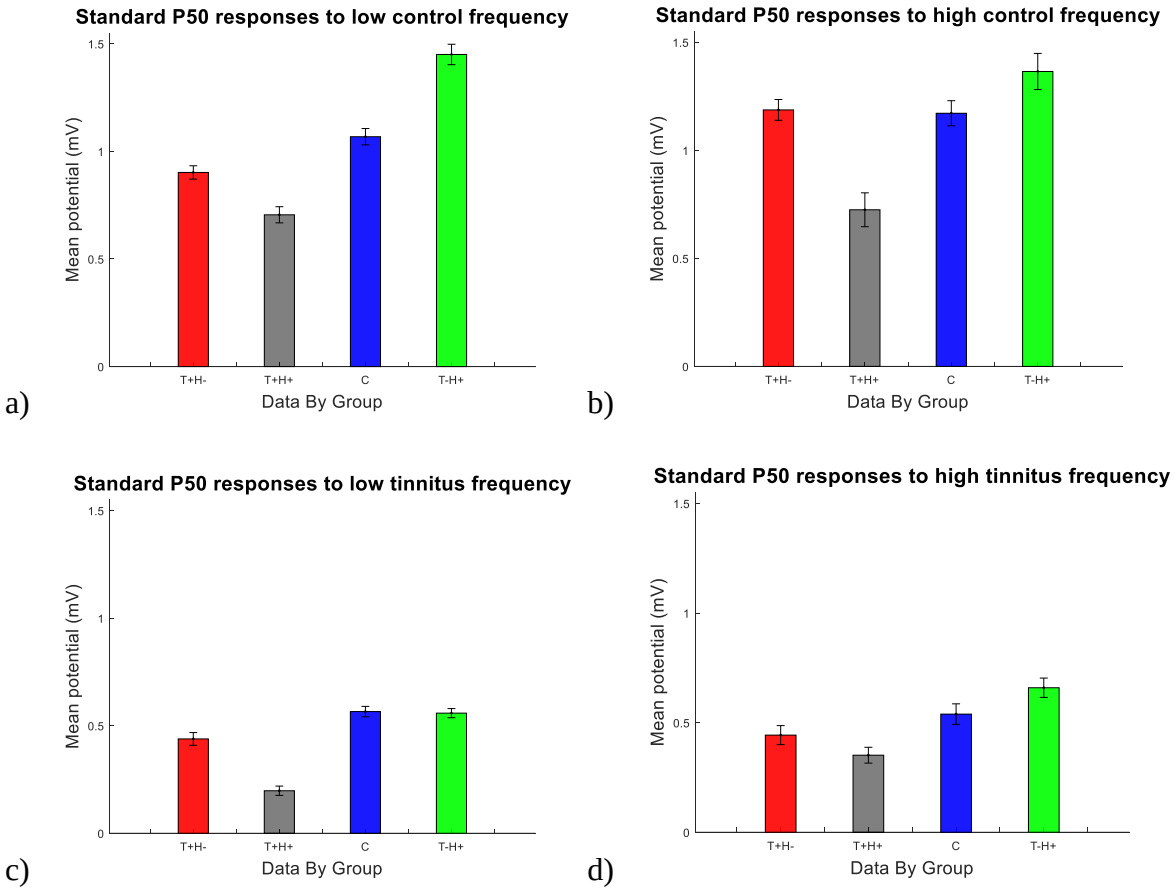



***Figure 4.3. Standard responses in the P50 timeframe.*** *Top two charts represent averaged responses to stimuli at 1 kHz frequency and bottom charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

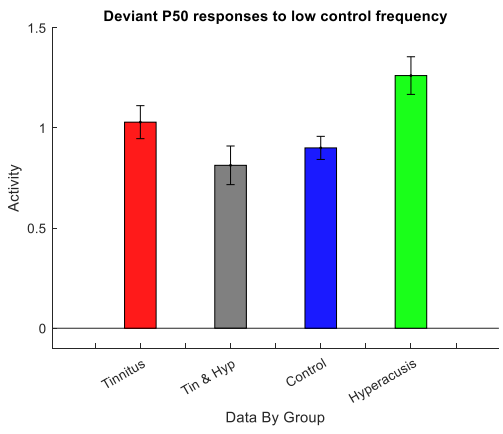
*Deviants*

Figure 4.4 shows P50 responses to raw deviant (not difference waveform) stimuli in the four conditions. A three-way ANOVA (group, frequency, intensity) was carried out. All factors showed a significant main effect (all at p <.001). There was a significant interaction between intensity and group (p=0.004) affecting p50 deviant amplitudes. There was also a significant interaction effect of group and frequency (p<0.001), as well as an intensity, group, frequency interaction effect (p<0.001). Post-hoc Tukey tests were carried.

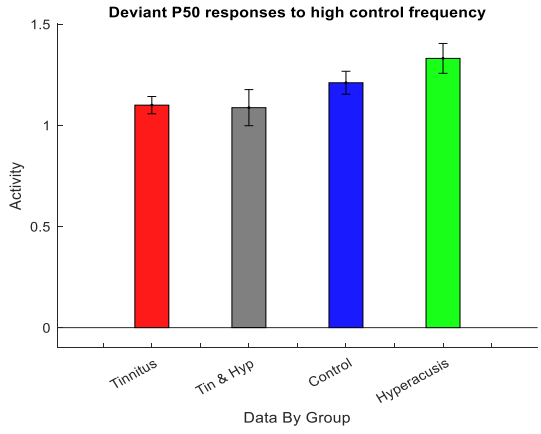
At low intensity 1 kHz deviant, T-H+ group had significantly higher amplitude compared to C group (p=0.003) and T+H+ group (p<0.001); there were no other significant differences. At high intensity 1 kHz deviant, there were no significant differences.

At low intensity tinnitus deviant, T+H- group had significantly lower amplitude compared to C and T-H+ groups (both p<0.001) as well as T+H+ group (p=0.024); T+H+ group also had significant lower amplitude compared to C group (p<0.001) and T-H+ group (p=0.004); there was no significant difference between C and T-H+ group (p=0.275). At high intensity tinnitus deviant, T+H+ group had significantly lower amplitude compared to T+H- and C groups (both p<0.001) as well as T-H+ group (p=0.012).

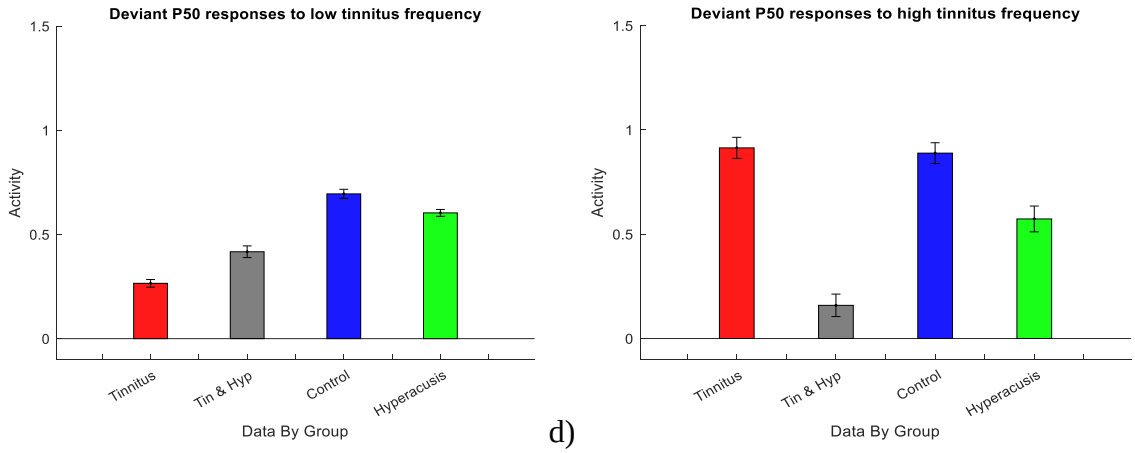
Based on observation of Figure 4.4 and ANOVA results, post-hoc within-subject tests were carried out, to investigate whether intensity at tinnitus frequency significantly affects the amplitude of p50 responses in the two T+ groups. When responses to low intensity were compared to high intensity in the tinnitus frequency, both T+ groups showed significant differences but in opposite directions. The T+H- group had significantly higher amplitudes in response to a high intensity deviant (t (7) =-8.45, p<0.001), while T+H+ group had lower amplitudes in response to a high intensity deviant (t (7) =5.59, p<0.001).




a)




b)




c) d)

***Figure 4.4. Deviant responses in the P50 timeframe.*** *Top two charts represent averaged responses to stimuli at 1 kHz frequency and bottom charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

#### *4.2.3.2 N100 responses*

*Standards*

Figure 4. 5 shows the mean amplitudes of N100 responses to standard tones in the four conditions. A three-way ANOVA (group, stimulus frequency, stimulus intensity) showed main effects of the three factors (all $p<0.001$). There were interaction effects of group and frequency ($p=0.004$), and a non-significant trend of group, frequency and intensity interaction ($p=0.054$). Tukey post-hoc analysis was carried out at the two frequencies.

At low intensity 1 kHz frequency, there were no significant differences between groups. At high intensity 1 kHz frequency, significantly weaker amplitude was seen in T+H- groups compared to T+H+ ($p=0.013$) and C ($p<0.001$) and T-H+ ($p=0.008$) groups; no other significant differences were present.

At low intensity tinnitus frequency, there were no significant differences between the groups. At high intensity tinnitus frequency, T-H+ group had significantly stronger amplitude compared to all other groups (all $p<0.001$); no other significant differences were present.

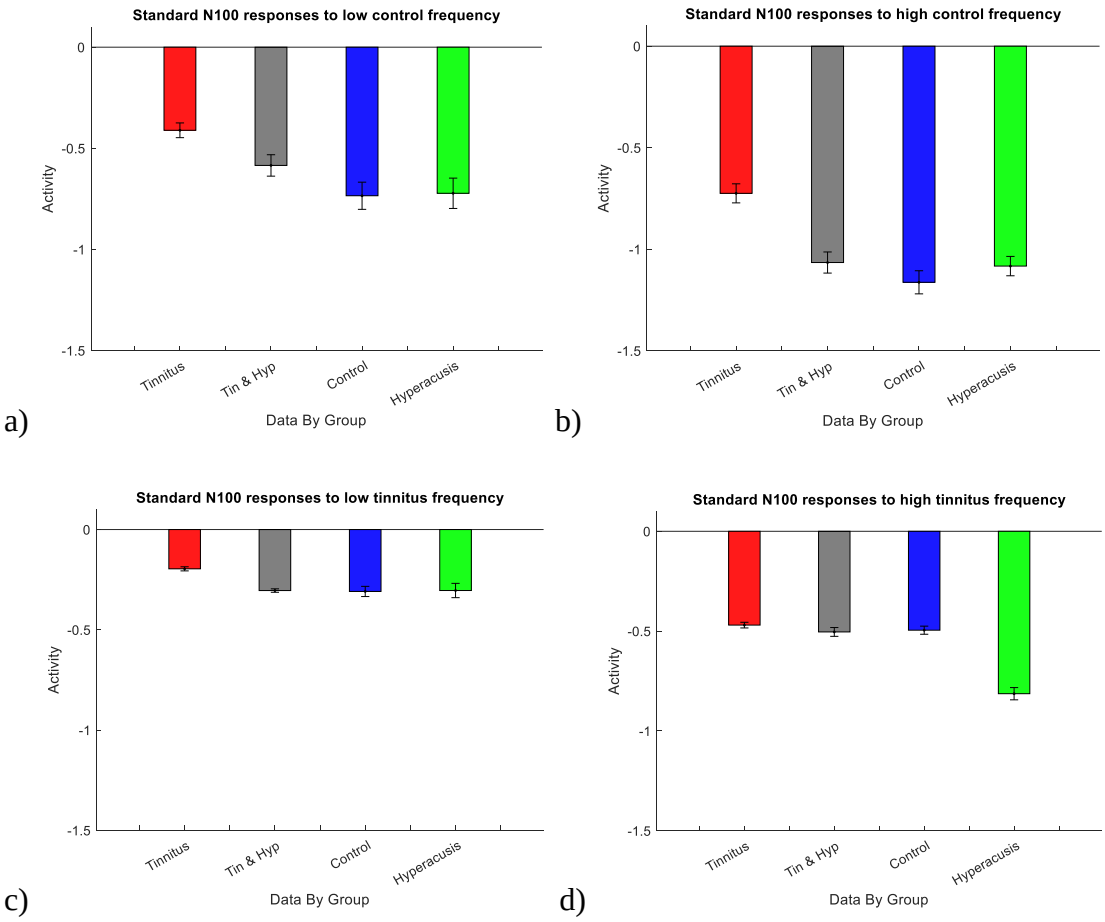



***Figure 4.5. Standard responses in the N100 timeframe.*** *Top two charts represent averaged responses to stimuli at 1 kHz frequency and bottom charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

*Deviants*

Figure 4.6 shows the mean amplitudes of N100 responses to deviant tones in the four conditions. A three-way ANOVA (group, frequency, intensity) showed main effects of all factors ($p<0.001$ for all), as well as interaction effects between group and frequency ($p=0.001$), group and intensity ($p=0.001$), and frequency and intensity ($p<0.001$). There was also a significant interaction effect between group, frequency and intensity ($p=0.022$). Tukey post-hoc analysis was carried out at the two frequencies.

At both low and high intensity 1 kHz deviants, no significant differences were seen between the groups.

At low intensity tinnitus deviant, significantly stronger responses were seen in T+H- group compared to T+H+ and control groups (both $p<0.001$); also, both T+H+ and C groups had significantly weaker responses compared to T-H+ group (both $p<0.001$). At high intensity tinnitus deviant, however, T+H- group had weaker responses than T+H+ and hyperacusis only groups (both $p<0.001$); T+H+ group had significantly higher responses than C group ($p<0.001$); no significant differences were seen between T+H- and C groups ($p=0.976$) or between T+H+ and T-H+ groups ($p=0.970$).

Post-hoc, paired t-tests were carried, that showed significant differences in responses to the two intensities within each group (all $p<0.001$).

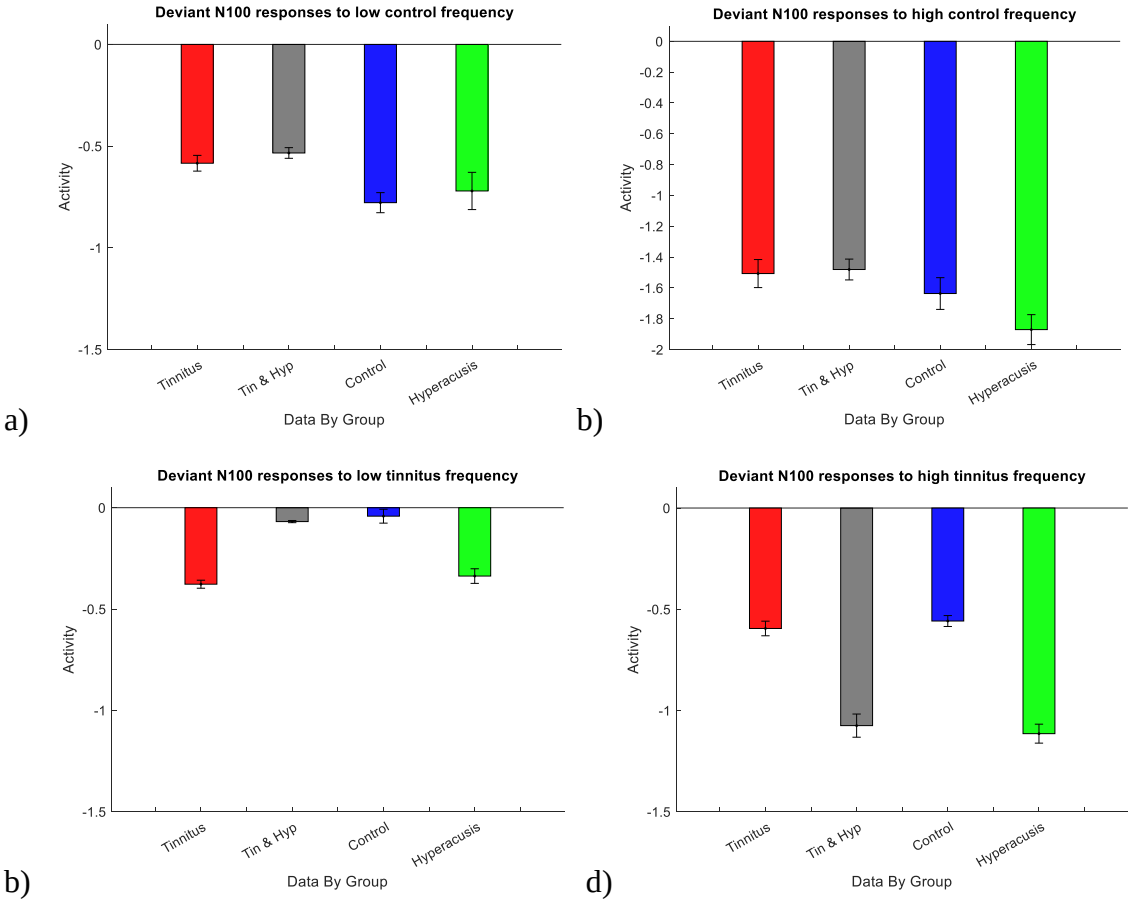



***Figure 4.6. Deviant responses in the N100 timeframe.*** *Top two charts represent averaged responses to stimuli at 1 kHz frequency and bottom charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

#### *4.2.3.3 P200 responses*

*Standards*

Figure 4.7 shows the mean amplitudes of P200 responses to standard tones in the four conditions. A three-way ANOVA (group, frequency, intensity) showed main effects of group and frequency ($p<0.001$) but not intensity ($p=0.173$). There were interaction effects between group and frequency, and group, intensity and frequency (both $p<0.001$). Post-hoc Tukey tests were carried out.

At the low intensity 1 kHz frequency, T+H+ group had significantly stronger p200 amplitude compared to all other groups (all $p<0.001$); T-H+ group had significantly stronger responses than T+H- and C groups (both $p<0.001$); no differences were seen between T+H- and C groups ($p=0.948$). At high intensity 1 kHz frequency, T+H+ group once again had significantly stronger responses than all other groups ($p<0.001$); C group had significantly lower responses than T+H- ($p=0.002$) and T-H+ ($p<0.001$) groups.

At the low intensity tinnitus frequency, T+H+ group had significantly higher responses than all other groups (all $p<0.001$) while C group had significantly lower responses than all other groups (all $p<0.001$). At the high intensity tinnitus frequency, again, T+H+ group had strongest responses (all $p<0.001$).

Overall, P200 responses to both high and low intensity standards were evident only in T-H+ and T+H+ groups at 1 kHz, and in the T+H+ group at the tinnitus frequency.

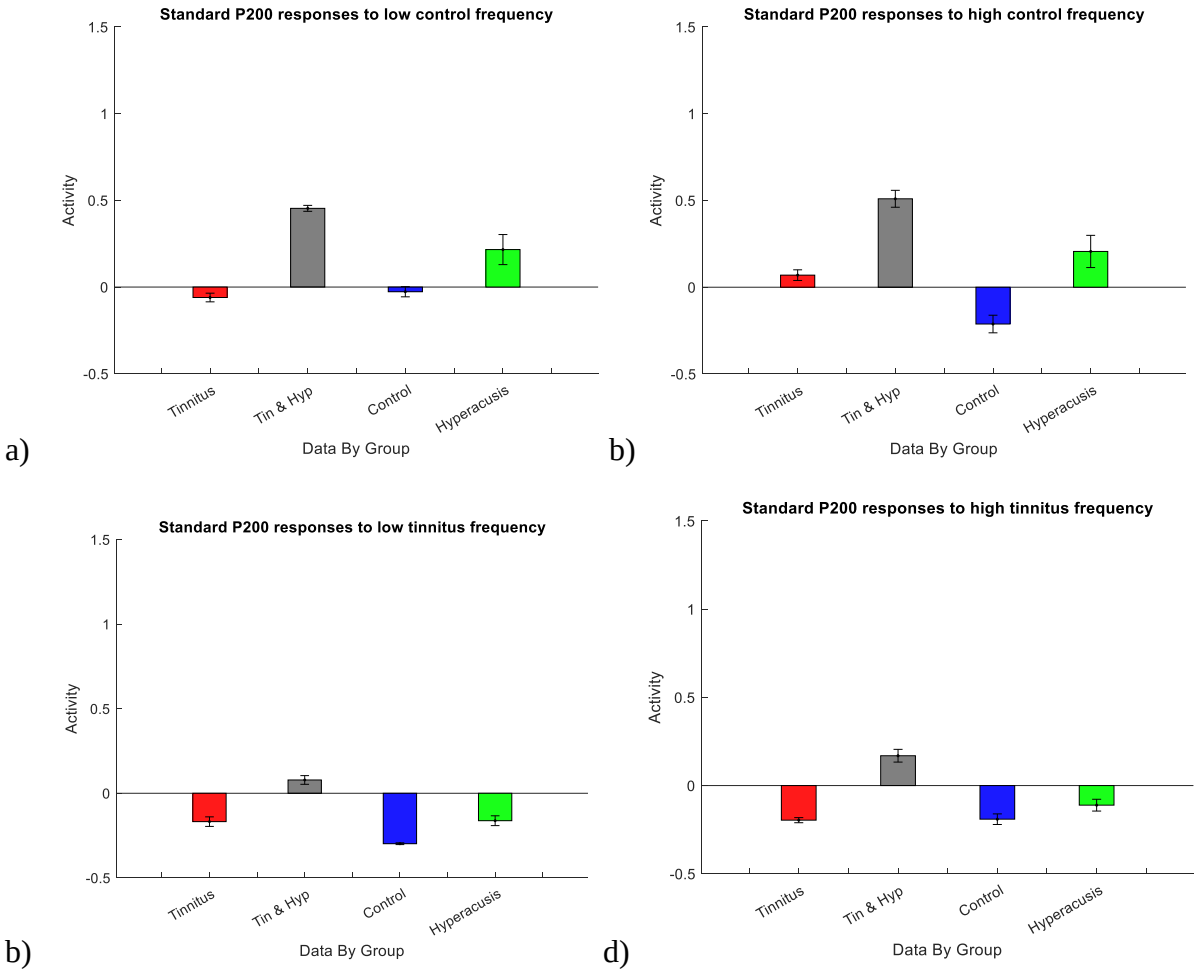



***Figure 4.7. Standard responses in the P200 timeframe.*** *Top two charts represent averaged responses to stimuli at 1 kHz frequency and bottom charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

### *4.2.4 Mismatch Negativity (MMN)*

A difference waveform was calculated by subtracting standard waveforms from deviant waveforms, which was used for MMN analysis. Figure 4.8 shows MMNs in the 4 conditions. A three-way ANOVA (group, frequency, intensity) showed main effects of all factors, as well as significant interactions of all possible variations (all $p<0.001$). Post-hoc tests were split by frequency (Figure 4.9).

At the 1 kHz frequency, T-H+ group had significantly stronger negative amplitude than the C group in response to DD ($p=0.003$), though there was also a trend towards a difference between T+H+ and C groups ($p=0.064$). In response to 1 kHz frequency UD, T+H+ group had significantly less negative amplitude compared to all other groups (all $p<0.001$).

At tinnitus frequency, responses to DD were significantly more negative in the T+H- group compared to all other groups (all p<0.001). In response to UD, T+H+ group had significantly more negative amplitudes compared to T+H- and C groups (p<0.001) and T-H+ group (p=0.024). T-H+ group also had significantly more negative amplitude compared to C group (p=0.002). There were no significant differences between T+H- group and C group (p=0.844).

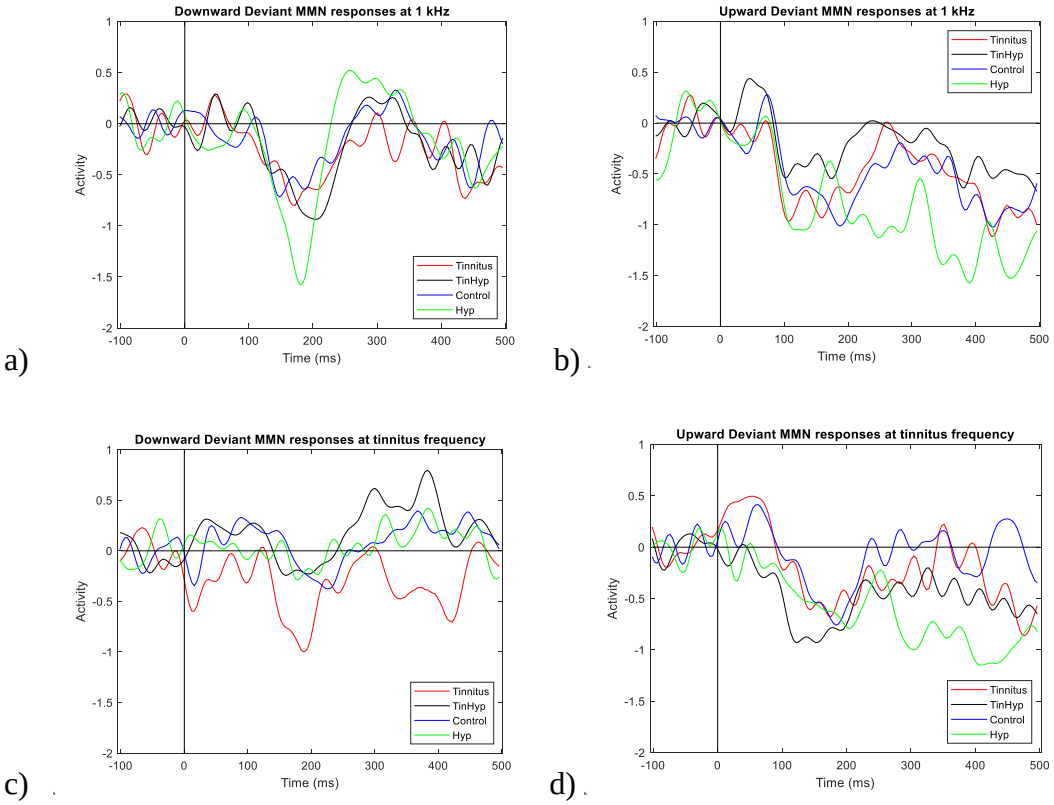



***Figure 4.8. Average MMN waveforms for each subject group, in each condition.*** *Graphs a-b show responses at 1 kHz frequency, and c-d show responses to the tinnitus frequency. On the left, are the downward deviants and on the right are the upward deviants.*

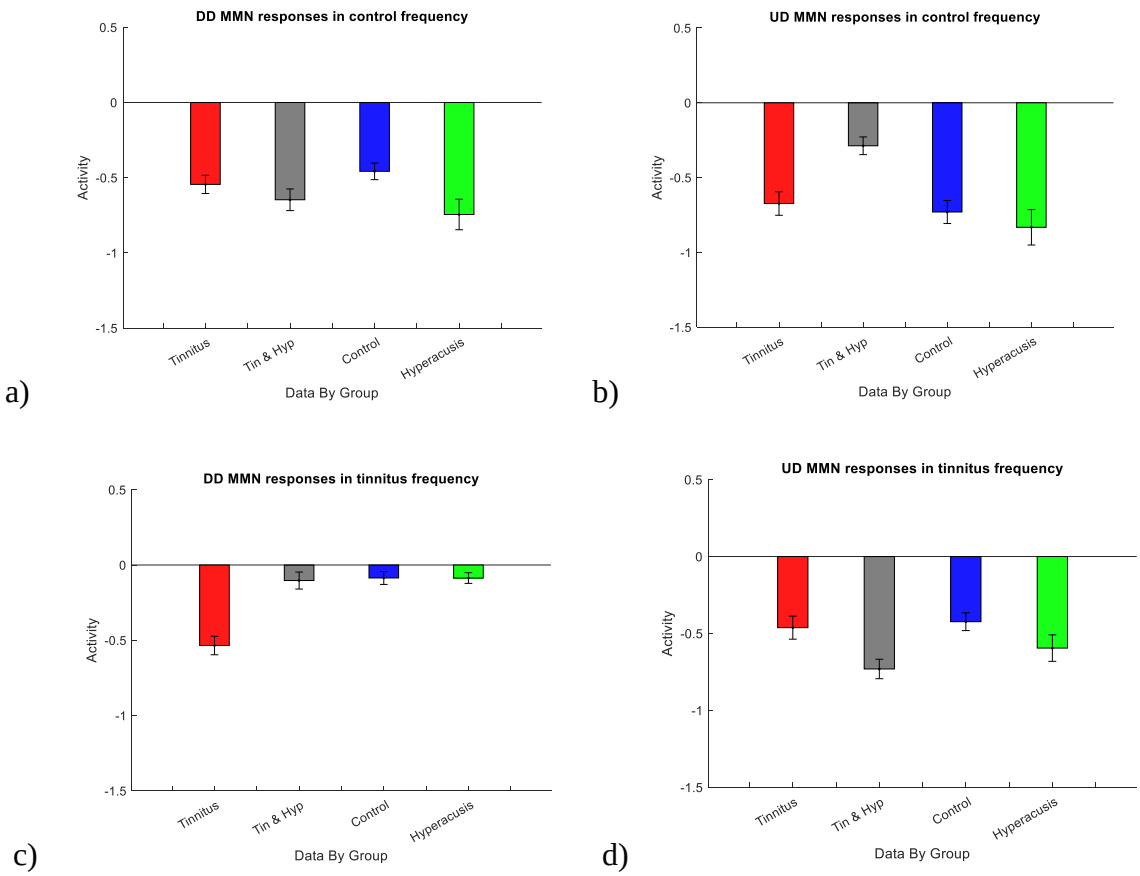
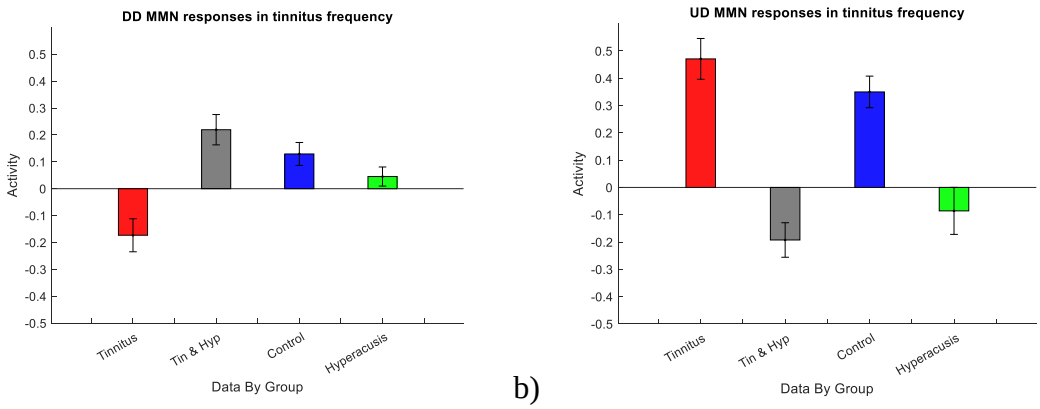



***Figure 4.9. MMN responses.*** *Top two charts represent averaged responses to stimuli at 1 kHz frequency and bottom charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

#### 4.2.4.1 P50 timeframe difference waveform

The trend seen in the pure deviant p50 response (Figure 4.2 g), where T+H- group had significantly lower amplitude than the C group, may be present in the difference waveform as well, based on observation of Figure 4.8. There, a two-way ANOVA (group, intensity) was carried out to see whether T+H- group would be significantly different from the other groups at tinnitus frequency specifically (Figure 4.10). The two-way ANOVA showed that both group and intensity had significant main effects (both $p<0.001$), and there was a significant interaction between group and intensity ($p<0.001$). Post-hoc Tukey tests showed that at DD difference waveform, T+H- had significantly lower amplitude than all other groups (all $p<0.001$); control group had no significant differences with either of the other groups (T+H+ $p= 0.068$, T-H+ $p=0.098$); however, there was also a significant difference between T+H+ and T-H+ groups ($p<0.001$).

At UD difference waveform, T+H- group had significantly higher amplitude than all other groups (p=0.033 compared to C, p<0.001 compared to either of the H+ groups); T+H+ group also had significantly lower amplitude than the C group (p<0.001) but no significant difference compared to T-H+ group (p=0.071); C group also had significantly higher amplitude compared to T-H+ group (p<0.001).




**a)** b)

***Figure 4.10. P50-timed deviant responses expressed as difference waveforms.*** *The charts represent averaged responses to stimuli at tinnitus frequency. The order of the groups in all charts is: T+H- (red), T+H+ (grey). C (blue), T-H+ (green).*

### *4.2.5 Results summary*

Below are summary tables of each response type, comparing the 4 groups at each stage of the response (Table 4.2-4.4). The C group was likely representing the typical healthy response pattern. Based on these graphs, it appears that the MMN responses in T+H- group likely occurred due to the responses to the deviant tones. T+H+ group had the largest P200 responses in the standards and the deviants but also somewhat smaller P50s and larger N100 responses to the louder deviant tones, so it is possible that a mix of both tone types may be responsible for the altered MMN responses in this group. T-H+ group had some enhanced standard and deviant responses, so it is also possible that both tone types contributed to the MMN response.

| | | T+H- | T+H+ | T-H+ |
|---|---|---|---|---|
| Standard p50 | 1 kHz L.I. | | ↓ | ↑ |
| | 1 kHz H.I. | | ↓ | ↑ |
| | Tin freq L.I. | | ↓ | |
| | Tin freq H.I. | | ↓ | |
| Standard n100 | 1 kHz L.I. | | | |
| | 1 kHz H.I. | ↓ | | |
| | Tin freq L.I. | | | |
| | Tin freq H.I. | | | ↑ |
| Standard p200 | 1 kHz L.I. | | ↑ | ↑ |
| | 1 kHz H.I. | ↑ | ↑ | ↑ |
| | Tin freq L.I. | ↑ | ↑ | ↑ |
| | Tin freq H.I. | | ↑ | |

***Table 4.2. Summary of standard responses in groups with tinnitus and/or hyperacusis, compared to control group.*** *Green indicates similar response amplitudes to C group; light blue indicates somewhat decreased amplitudes and darker blue with the thicker arrow represents most significant decrease compared to C group based on p-values. Light red represents increase compared to C group whereas bright red represent most significant increase. Tin freq = tinnitus frequency; L.I. = low intensity; H.I. = high intensity*

| | | T+H- | T+H+ | T-H+ |
|---|---|---|---|---|
| Deviant p50 | 1 kHz L.I. | | | ↑ |
| | 1 kHz H.I. | | | |
| | Tin freq L.I. | ↓ | ↓ | |
| | Tin freq H.I. | | ↓ | ↓ |
| Deviant n100 | 1 kHz L.I. | | | |
| | 1 kHz H.I. | | | |
| | Tin freq L.I. | ↑ | | ↑ |
| | Tin freq H.I. | | ↑ | ↑ |

***Table 4.3. Summary of deviant responses in groups with tinnitus and/or hyperacusis, compared to control group.*** *Green indicates similar response amplitudes to C group; light blue indicates somewhat decreased amplitudes and darker blue with the thicker arrow represents most significant decrease compared to C group. Light red represents increase compared to C group whereas bright red represent most significant increase. Tin freq = tinnitus frequency; L.I. = low intensity; H.I. = high intensity*

| | | T+H- | T+H+ | T-H+ |
|---|---|---|---|---|
| MMN | 1 kHz DD | | | ↑ |
| | 1 kHz UD | | ↓ | |
| | Tin freq DD | ↑ | | |
| | Tin freq UD | | ↑ | ↑ |

***Table 4.4. Summary of MMN responses in groups with tinnitus and/or hyperacusis, compared to control group.*** *Green indicates similar response amplitudes to C group; light blue indicates somewhat decreased amplitudes and darker blue with the thicker arrow represents most significant decrease compared to C group. Light red represents increase compared to C group whereas bright red represents the most significant increases. Tin freq = tinnitus frequency.*

## 4.3 Discussion

The current study shows distinct profiles for tinnitus and hyperacusis, as well as additional more nuanced interactions, which not only moves our understanding of each condition, but also speaks directly to possible mechanistic subtypes of tinnitus (and of hyperacusis) which might be disentangled through the cheap and available technique that is single-channel EEG. The current findings may explain some discrepant findings in past literature.

### *4.3.1 Correlates of tinnitus with/without hyperacusis*

There were some distinct differences seen between the response patterns of T+H+ and T+H- groups. In the presence of hyperacusis in the T+ groups, p50 amplitudes were smaller in response to standard tone of 1 kHz at high intensity and in response to standard tone of tinnitus frequency at low intensity. Additionally, though both T+ groups had weaker P50 responses to raw deviant tones at tinnitus frequency, the two groups had slightly different patterns. T+H+ participants had stronger responses to low intensity stimuli but weaker responses to high intensity stimuli compared to T+H- group (T+H+ group seemed to involve both differences seen in T+H- and T-H+, therefore potentially showing a non-direction-specific reduction in pre-attentive change detection at tinnitus frequency [91]). Usually, P50 is suppressed in response to a repeating stimulus. Therefore, the smaller P50 amplitude may indicate a greater degree of sensory gating in tinnitus groups, particularly around the tinnitus frequency. Notably, in T+H+ even the P50 to standards are decreased compared to C group, in all conditions.

The difference in P50 amplitude remained significant in the difference waveform. There was an opposing pattern between the two T+ groups, as well as a difference with the C group. This could not be explained by higher tinnitus severity as previously suggested, as the decreased p50 (and following more negative N100) occurred in both T+ groups just in different conditions, though heightened cognitive awareness may be playing a part [46]. In the T+H- group, P50 for UD is similar to C group responses, however, P50 is significantly diminished for DD. It is possible that this occurs due to suppression of ascending input when the sensory input first decreases, but it could also indicate a lower reliance on sensory input and a greater reliance on higher predictions compared to healthy participants. In a previous paper, it was suggested that participants with tinnitus could be less able to attend to relevant stimuli due to underactive feedback inhibition in the auditory microcircuits specifically in the tinnitus frequency regions [220], which may lead tinnitus subjects to over-rely on higher predictions in response to diminished sensory input. However, this also means that they cannot ignore

UDs due to hyper-excitability and therefore respond with higher alertness to UDs. On the other hand P50 in T+H+ are greatly suppressed in response to UDs, but not to DD, in a similar fashion to T-H+, therefore this may be an effect of hyperacusis.

N100 had almost an opposite pattern to raw p50 responses. The presence of hyperacusis was related to stronger response to high intensity standard tones at 1 kHz, weaker response to low intensity deviant tones at tinnitus frequency, as well as stronger response to high intensity deviant tones at tinnitus frequency. Potentially, this process allowed the two groups to even out sensory gating in the pre-attentive phase prior to MMN/P200 timeframes. MMN responses somewhat followed the N100 deviant response pattern at the tinnitus frequency: DDs elicited larger responses in T+H- group, whereas T+H+ group had stronger response to UDs. Additionally, T+H+ group had weaker responses to upward deviants at 1 kHz tone. In tandem to the potential theory about P50s, it is possible that for T+H-, stronger predictions would have greater prediction error responses when not completely fulfilled (i.e. in DDs, which can be thought of as incomplete omission responses). However, for the H+ groups the stronger MMN response to tinnitus frequency UD likely occurred due to heightened perception of changes in intensity.

#### *4.3.1.1 Comparison to previous studies*

A previous study mentioned in the introduction found stronger subcortical sound-evoked responses in T+H+ participants compared to T+H- [129]. This finding was not frequency-specific, but it is corroborated by our data: there was increased sensory gating at P50 (reduced amplitude) but a heightened P200. Additionally, the previous research showed that at 1 kHz, an UD increased N100 and P200 amplitudes in bilateral T+ group compared to C group. In our study, while P200 was increased in both groups in response to a 1 kHz UD, N100 remained similar to C group. This could be due to a 20 dB SPL rise in the previous study, compared to average of 6 dB in the current study, but the trend of larger responses can still be seen, especially in T+H+ group. However, the current study found that in response to stimuli at tinnitus-like frequency, the T+H+ group showed significantly smaller activation than T+H- group [129]. Importantly, in the aforementioned previous study, tones used were loudness-matched tones at frequencies ranging between 250 Hz to 8 kHz, with the most tinnitus-like tone used for this comparison, therefore the previous findings may not have been truly be representative of a response to a tinnitus-like frequency. This is an interesting divergence compared to the current findings, which could be due to design and more precise tinnitus frequency matching.

A multi-feature paradigm study mentioned in the introduction [216] concluded that people with high noise sensitivity showed significantly diminished P50s to all deviants and significantly less negative MMNs to noise deviants (when corrected for multiple testing) than the low noise sensitivity group. The pattern of the T-H+ group in the current study did not follow the previous finding, but T+H+ pattern of P50 responses did. However, the reason for this could be the more complex nature of the presented sounds (chords) as well as potentially different causes of hyperacusis in different groups. Unfortunately, it is not possible to make frequency comparisons as these researchers did not explicitly state the frequencies at which tones were played (though from figures, base tone seemed to be around 2 kHz). While not directly related the deviant used in the current study, this paper was one of the few studies investigating MMN and noise sensitivity specifically. Further, a number of multi-feature deviant paradigm papers reported that generally, MMN responses in tinnitus participants for all deviant types used tended to be smaller (e.g. [91, 93, 221]), both at higher and lower frequencies. Studies that focused on 0.5, 1 and 1.5 kHz frequencies saw that tinnitus presence was related to smaller amplitudes for a number of deviant types (though intensity deviants were inconsistent in showing any difference in amplitudes in tinnitus compared to control groups, and some studies not controlling for hyperacusis, while studies that did not show a difference being similar in their finding to our T+H- and C group MMN responses to 1 kHz frequency deviants in the current study) [91, 217, 222]. A number of studies have also looked at higher frequencies, e.g. 5 kHz and 8 kHz ([93, 222]). While at 5 kHz, once again, tinnitus presence was related to weaker MMN amplitudes [222], at 8 kHz a study found that when a participant was habituated to their tinnitus, responses were similar to controls, but when the participants were not habituated to their tinnitus, MMN amplitudes were weaker (for higher frequency deviant) [93]. No such findings were seen in the current study, rather an amplitude increase was seen at the tinnitus frequency. It is possible that the differences are due to a different deviant type (intensity vs. frequency) or because not all participants with tinnitus would find 8 kHz to be near the tinnitus frequency. Here, it was found that at 1 kHz, T+H- group had similar responses to C group, while T+H+ group had a weaker MMN response to UD at 1 kHz. T+H+ group also had higher THI scores so possibly overall the group would be less habituated to their tinnitus than T+H- group. On the other hand, it is possible that diminished auditory responses in such paradigms may reflect an auditory dysfunction more generally, or may reflect presence of noise sensitivity rather than tinnitus, or any mixture of the two conditions. The current study did not use a multi-feature paradigm which may be the reason why the current study did not always see similar patterns. Rather than seeing the general effect of auditory dysfunction, we were able to disentangle alterations related to

different conditions as well as their combined effects. The current study was rigorously controlled for hearing loss and hyperacusis presence, which may contribute to the disparities with previous findings.

### *4.3.2 Correlates of hyperacusis with/without tinnitus*

There were significant distinctions between T+H+ and T-H+ groups, in relation to each other as well as in comparison to C group. Presence of tinnitus in people with hyperacusis seems to have the opposite effect on ERP amplitudes compared to people only with hyperacusis at P50 and standard N100. T-H+ group had highest amplitudes to all P50 standards (though only significantly higher at 1 kHz), while T+H+ responses were weaker than both H- group responses at all frequencies, which may indicate opposite sensory gating alterations between the groups. T-H+ also had the highest P50 amplitude to low intensity deviant at 1 kHz, and strongest N100 in response to both deviant conditions at tinnitus frequencies, potentially indicating lack of habituation. N100 has previously been shown to be suppressed during repetitive stimulation, through a process called repetition suppression (RS) [223]. The N100 deviant responses at tinnitus frequency in T+H+ were lower than both H- group responses, though not significantly. T-H+ also had stronger N100 response to high intensity at tinnitus frequency standards, also indicating lack of habituation to a high intensity, potentially uncomfortable tone. However, this opposing trend between the groups changed in the later ERPs. P200 response at both frequencies was higher in T+H+ than T-H+, though T-H+ was still stronger than both H- groups at 1 kHz. The result was difficult to interpret at high intensity tinnitus frequency standard, as T-H+ P200 response followed a significantly lower N100 and still visually had a peak around 200 ms but the inferential statistics saw this as non-significant. At 1 kHz, MMN responses were strongest to DD in T-H+ and weakest to UD in T+H+. At the tinnitus frequency, the strongest response to UD was in T+H+ group, followed by T-H+. So, presence of hyperacusis reduced P50 in T+ participants but increased P50 in T- participants, however, later in the timeframe, both groups show an enhanced P200 response to both standard and deviant tones at tinnitus frequency, particularly in the T+ group (despite T+H- group showing amplitudes closer to controls).

Comparison to the original IMA study is slightly limited, as the the earlier paradigm included an edge frequency of tinnitus (sound just below the tinnitus frequency of a participant) , but not a 1 kHz control frequency [95]. However, the later follow-up study included a 1 kHz condition. The tinnitus group in the previous studies had non-significantly smaller amplitudes at tinnitus frequency. This is similar to the findings in the current study, where the two T+

groups were mostly similar to C group, but the T+H+ group had lower p50 amplitudes especially at tinnitus frequency while T+H- group had less strong n100 at 1 kHz in response to high intensity standards. If the two groups were mixed, it likely would reduce their differences with C group and would corroborate the previous findings. Overall, T+H- participants did not follow the original study pattern. However, the T+H+ group of the current study had a very similar pattern to the original study and the later follow-up study in the edge tinnitus frequency. The current results strongly indicate that differences seen previously were driven by hyperacusis rather than tinnitus [179]. Additionally, C group patterns seem similar in the current study and the earlier follow-up study, but different in the original study, which may be due to overall frequency differences in the paradigm.

#### *4.3.2.1 Comparison to previous findings*

In a paper comparing responses of controls and subjects with low vs high tinnitus-related distress to three tone bursts (1 kHz, 1.3 kHz and 1.6 kHz at 90 dB HL), participants either had to press a button when they heard a particular tone, or ignore the tones [224]. Findings showed that unattended stimuli elicited weaker N100 amplitudes than attended stimuli, which was only significant in the low tinnitus distress group, but not high distress. Additionally, the high distress tinnitus group had larger overall N100 amplitudes than either low distress or (especially) controls, indicating potentially reduced ability to habituate to the repetitive stimuli. Researchers concluded that high tinnitus distress is related to more attention paid to the tinnitus; the mean threshold of uncomfortable loudness was also similar between the groups in both ears, so presence of hyperacusis was unlikely to explain the results. Interestingly, in the current study, a consistent trend for weaker N100 (significant for high intensity, non-significant trend for low intensity) was seen in T+H- group at 1 kHz compared to C and T+H+ group. This was also the case at tinnitus frequency low intensity, but not high intensity standards. So, the T+H- group either exhibited the most habituation, out of the four groups, shown by ability to suppress the N100 response, in all tones except for the sound of their tinnitus becomes louder. This could also be potentially explained by an overall tendency of T+H- participants for higher reliance on higher predictions in the auditory system. While the previous study did not test T-H+ participants specifically, in the current study it seems that the T-H+ were the least able to suppress the N100 to repeating tones in all conditions.

### *4.3.3 Repetition positivity & habituation*

Repetition Positivity (RP) is an ERP that has been established as a function of memory trace formation and potentially suppression of prediction errors (opposite from MMN), which

presents as a positive wave around 200 ms that is enhanced by stimulus repetition [223, 225]. A systematic review of the P200 ERP has suggested that P200 amplitude is linearly related to increase in intensity of an auditory stimulus, and may even reach a saturation level at high intensities [203]. Additionally, P200 presence may relate to working memory and interference control but prolonged P200 latency may reflect early conscious attention towards a certain stimulus [226]. This may be interesting as the T+H+ group had longer P200 latencies than T-H+ group in response to 1 kHz stimuli, while T-H+ group tended to have an earlier peak within the P200 timeframe. Therefore, it is possible that H+ participants generally either perceive the stimulus as louder than H- participants and therefore have higher amplitudes of P200, or they use more interference control. T+H+ in particular may also pay more conscious attention to the stimuli as their P200 lasts longer than in T-H+ group. Perceiving the higher intensity as louder than H- groups may also in part explain the increased MMN response to UD in both H+ groups at the tinnitus frequency, and the increased conscious attention could explain why MMN response to UD was particularly high in T+H+. However, it could not explain the MMN response pattern at 1 kHz (increased amplitude in T-H+ to 1 kHz DD and decreased amplitude in T+H+ to 1 kHz UD). However, there is also a possibility that these differences were simply due to lower alertness in the H- groups, as lower P200 responses have previously been seen in healthy participants in 'mind wandering' condition during an oddball task compared to 'focused on breathing' condition [227].

#### *4.3.4 Future directions*

Taken together, the findings indicate that tinnitus researchers need to account for hyperacusis when utilising ERP-based paradigms, and research into correlates of hyperacusis must not focus solely on tinnitus-associated hyperacusis, especially because tinnitus and hyperacusis combined produces different patterns compared to people with only one of the conditions. Additionally, there may be a need to understand whether the frequencies used in the paradigm affect the patterns that are seen among groups, including controls. For example, the pattern of MMN responses was different in the original study compared to the replication study. An MEG study investigated repetition positivity alongside repetition suppression, in which they established that the two processes are separate but complementary. N100 was suppressed during repetitive stimulation, while an increasing later field was seen following repetition of a stimulus [223]. An interesting suggestion was made regarding two separate RP generators, one located more frontally (Fz) that is dependent on longer-term paradigm contexts (one tone vs roving paradigm) and another located towards the mastoids and dependent on short-term changes [228], which potentially affects overall presentation of RPs [229]. This further calls

for investigation of ERP patterns in different global contexts, as well as potential further study of standards in tinnitus and/or hyperacusis, as these conditions seem to affect RS and RP processes in different ways. Determining the expected patterns in varying overall paradigm contexts would allow researchers to better understand how activity is altered in the presence of tinnitus and/or hyperacusis.

### *4.3.5 Limitations*

A potential limitation of this study is that there are a number of types of hyperacusis (e.g. loudness vs pain subtypes), as well as prevalence of autism-related symptoms or comorbid pain disorders in people with hyperacusis, which may all have differing effects on brain activity [124, 230, 231]. Additionally, it may be useful in the future to conduct a source analyses-based study in order to investigate how the presence of hyperacusis and tinnitus work together, compared to presence of one of the conditions only.

### *4.3.6 Conclusion*

Hyperacusis has quite a broad influence on evoked responses, including early and late, and standard and deviant, across frequencies. For example, it is related to larger P50s and P200s, and particularly increased MMN responses to UDs. Overall, this indicates a hypersensitivity to unexpected intensity increases. With presence of tinnitus and hyperacusis, it seems that the later responses align with, and are potentially enhanced by, the patterns seen with hyperacusis alone. However, the earlier (P50) responses are suppressed, possibly exaggerating the effect of tinnitus alone on the evoked responses. Additionally, hyperacusis seen in tinnitus seems to have effects that are more limited to the tinnitus (hearing loss) frequency, unlike hyperacusis without tinnitus. This could be due to slightly different mechanisms between the two types. Tinnitus itself has a more specific pattern, almost limited to the tinnitus frequency only, with most of these evoked responses being normal (though, a few differences such as smaller P50 responses to standards, and a distinct pattern of P50 changes in deviants: smaller to downward and larger to upward), but a strikingly different (larger) MMN to downward intensity deviants at the tinnitus frequency only. This is potentially indicative of stronger formations of auditory predictions or memory traces around the tinnitus frequency (the area affected by hearing loss).

# Chapter 5. Effects of the overall paradigm context on intensity deviant responses in healthy subjects.

## 5.0 Introduction

Three experiments have been carried out to explore the differences between control and tinnitus group responses to intensity deviants. The original study involved two frequencies, which were centre frequency of tinnitus and just outside of tinnitus [95]. These two frequencies were 0.37 of an octave apart, so fairly close together. The other two studies (Chapter 2 and 4 in this thesis) involved a tinnitus frequency (average = 5.075 kHz) and a 1 kHz frequency, which were usually quite far apart from each other (on average, 2.3 octaves) [179]. Additionally, most participants had some level of hearing loss at the tinnitus frequency but not at the 1 kHz frequency. There were some differences in the responses seen in the paradigm with only tinnitus-like tones compared to the paradigm that included 1 kHz, despite no other differences being present.

The crucial differences between the two 1 kHz-inclusive studies were seen in the MMN response patterns to intensity deviants at the tinnitus frequency stimuli in the control group. In the original study, responses to the edge tinnitus frequency were larger in magnitude to the centre tinnitus frequency but otherwise showed the same pattern, so the edge frequency was used in the subsequent studies, and therefore the focus of the comparison for this introduction will be on high (i.e. tinnitus-related) frequency responses (Table 5.1). The MMN pattern was also similar between the two frequencies in the two later studies, though possibly somewhat underpowered in the second study compared to third. The paradigms in the three studies were identical, except for the addition of the 1 kHz tone instead of a second tinnitus-like tone. However, this seemed to affect the pattern of responses in the control group, with the amplitudes inverting with the addition of the more distant other frequency (i.e. 1 kHz). Additionally, unpublished data from our lab, where intensity deviant responses in healthy participants were elicited in paradigms with frequency deviants only or paradigms including both frequency and intensity deviants, showed different patterns of response to downward deviant (DDs) and upward deviants (UDs) for intensity.

| Frequency | Group | Original | Follow-up | Hyperacusis |
|---|---|---|---|---|
| **1 kHz** | **C** | N/A | DD = UD | ↓DD↑UD |
| | **T+** | N/A | ↓DD↑UD | H- ↓DD↑UD |
| | | | | H+ ↑DD↓UD |
| **Tinnitus** | **C** | ↑DD↓UD | ↓DD↑UD | ↓DD↑UD |
| | **T+** | ↓DD↑UD | ↓DD↑UD | H- ↑DD↓UD |
| | | | | H+ ↓DD↑UD |

***Table 5.1. Pattern of responses in each group to downward deviants and upward deviants.*** *C = controls, T+ = tinnitus group. The hyperacusis study also includes H- (without hyperacusis) and H+ (with hyperacusis).↑ = stronger response, ↓ = weaker response. The arrows are smaller when the directionality was not as pronounced and thicker when the differences were driven by a particular deviant. Green colour shows the major similarities in responses between the three studies, while red indicates the main difference in findings between the paradigms.*

The impression emerging from the differences between these similar studies, mostly in the control group, is that the inclusion of frequency changes elsewhere in the experiment may affect intensity mismatch asymmetry within a single frequency. Potential mechanisms responsible could be adaptive processes in which neural responses adapt to stimuli in a particular environment through mechanisms such as frequency-specific adaptation or contrast gain control (a mechanism in which neuronal responsiveness to intensity is dynamically adjusted based on the global context/statistics of recent stimulation) [232-235]. For example, bat auditory neurons react differently to a target sound in the context of being preceded by certain sound sequences [236]. Similar processes have been studied in the visual and somatosensory systems, where they help, for example, to adjust to a new context of a changing light intensity through enhancement or suppression [237, 238]. Gain control may be an important part of ensuring efficiency of the auditory neurons, e.g. they may have a redundancy-reducing effect within a local sensory environment [232, 239]. However, the mechanisms of auditory gain control more generally or its effects on perception are not fully understood [233].

A previous experiment was conducted to see whether different variability levels between auditory stimuli would affect neuronal responsiveness [232]. To achieve this, the experimenters played tone sequences with three contrasts (low: -/+5 dB deviant (relatively constant), medium: -/+ 10 dB and high: -/+15 dB (high variability)). They found that

responses to a standard sound depended on the overall context of the paradigm in two ways. Firstly, gain non-linearly increased as stimulus contrast decreased, thus partially compensating for the smaller changes through a more sensitive neural firing rate. Contrast gain is a form of statistical adaptation in the auditory cortex that creates representations of the complex statistical environment and the change in this environment over time [240]. It involves dynamic range adaptation of neurons within the auditory system and, based on the representation of contrast in the environment that has been created over time, predicts the way in which neurons will need to adjust their gain [240]. Secondly, decreases in neural gain occurred faster (in ms) than increases in neural gain. Therefore, adaptation to a high-contrast context was faster than to a low-contrast context. Additionally, neural gain was best modulated within their responsive frequency range, though further frequencies also had an effect on gain, and if the mean level of the stimuli was low, the overall effectiveness of this mechanism was reduced. Similarly, in experiments where extracellular single unit recordings were made from A1 neurons to study contextual interactions in a two-tone paradigm [238] when 6 kHz tones were presented after a 7 kHz tone, responses to the 6 kHz tone were suppressed. However, suppression did not occur if the 6 kHz tone was presented after a 2 kHz tone, outside the 6 kHz receptive field. Interestingly, when the researchers compared the suppression of response to a louder second tone (80 dB) versus a quieter second tone (30 dB), the region of suppression was larger for the quieter second tone than the loud one. Additionally, the firing rate evoked by the louder second sounds was higher than for quieter second tones. Therefore, suppression was proportional to intensity of the first tone and inversely proportional to intensity of the second tone, as well as being related to the frequency difference between the first and second stimuli.

It has been previously concluded, in support of the aforementioned studies, that through the process of forward masking, frequency tuning of auditory cortical neurons could be dynamically modulated by preceding stimuli, particularly by neurons with similar characteristic frequencies and particularly at higher stimulus levels [9]. These studies taken together may indicate that both frequency and intensity of the tones played in roving paradigms may interact to affect response to intensity deviants, and inter-frequency effects may be stronger in paradigms with small frequency contrast contexts than large frequency contrast contexts.

Therefore, the present experiment was carried out in order to establish how difference in intensity mismatch asymmetry (IMA) to the roving intensity paradigm result from frequency differences present between blocks within the whole experimental paradigm. Specifically, to

compare either a single frequency, two similar frequencies, or two far-apart frequencies. Based on the previous results, hypotheses were:

- The paradigm with a small frequency difference will create an opposite IMA pattern to the paradigm with a large frequency difference: larger downward and smaller upward deviants for small frequency differences, and smaller downward and larger upwards deviants for large frequency differences;
- Based on the unpublished data from our group, the single-frequency paradigm may show a prolonged P50 and an attenuated N100 unlike the paradigms that include two frequencies.

## 5.1 Materials & Methods:

### *5.1.1 Participants*

Participants were randomly assigned to one of three groups, who were presented a roving oddball intensity paradigm featuring either: 1) all stimuli at a single frequency (N=15), 2) blocks alternating between two frequencies, with a small frequency difference (1/3 of an octave) (N=15), or 3) blocks alternating between two frequencies, with a large frequency difference (1 kHz and 6 kHz) (N=13). Participants only did one version of the experiment because 1) all three paradigms in one session would have blurred any specific context effects, and 2) on separate days, there might have still been some carryover or familiarity effects from previous session/s. Participants were recruited from affiliated volunteer lists at Newcastle University. Inclusion criteria included being over 18 years old, and being able to make an informed choice about volunteering. Exclusion criteria included presence of any type of tinnitus (subjective, objective or intermittent), using ongoing sedating or nerve-acting medications, and mental health conditions severe enough to interfere with everyday life activities. It was also ensured that there were no significant group differences in age or pure-tone audiometry results. Approval was given by the Newcastle University Research Ethics Committee, and all participants gave written informed consent according to the Declaration of Helsinki (reference number 5619/2020).

### *5.1.2 Common methods: psychophysics and EEG*

The psychophysical assessment in which sound intensity played during the EEG recording were determined, and the experimental design, closely followed the procedure in Chapters 2 and 4 of the thesis. The main exception was the lack of tinnitus frequency due to the nature of

participants. Instead, all participants listened to 6 kHz tones. In the small frequency difference group, participants also listened to tones 1/3 of an octave lower than 6 kHz (4.643 kHz) as, on average, the edge tinnitus frequency in the original study was 1/3 of an octave lower than tinnitus centre frequency. In the large frequency difference group, the other tone was 1 kHz, to replicate the paradigm context of the two later IMA studies.

### *5.1.3 Statistical analysis*

Statistical analysis was performed using MATLAB. To compare evoked responses between the three groups at the 'tinnitus' frequency (6kHz), a two-way ANOVA was used, with subject group and intensity as the main factors of interest, along with the interaction terms. Post-hoc analysis included Tukey Honest Significant Tests to determine any significant differences between the ERP amplitudes of the three groups.

## 5.2 Results

Table 5.2 shows means and standard errors (SE) of the demographic information of the 3 groups. One-way ANOVAs were carried out to see whether age or HQ scores were different between the three groups. Age and HQ scores were not significantly different between the groups ($p=0.843$ and $p=0.790$, respectively). Average HQ scores were also below the cut-off score for hyperacusis presence. Chi-square test showed that there were significant differences in sex between groups ($p=0.01$). As the pure tone audiometry results were not normally distributed at the majority of frequencies/ears Kruskall-Wallis tests were performed and showed that across all three groups, there were no significant differences in the hearing ability at 0.5, 1, 2, 4, 6 or 8 kHz in right or left ears. At 0.25 kHz, there was also no difference between the groups in the right ear, but there was a significant difference in the left ear ($p=0.035$). This was acceptable as this frequency was not used as a stimulus. Importantly, at 6 kHz there were no significant differences between the three groups: $p=0.560$ for the left ear and $p=0.300$ for the right ear.

| | Single Frequency | Small Difference | Large Difference |
|---|---|---|---|
| **Age** | 48.40 (4.90) | 48.87 (4.51) | 52.15 (5.03) |
| **Sex** | 11 f, 4 m | 12 f, 3 m | 7 f, 6 m |
| **HQ Scores** | 9.87 (1.66) | 8.87 (1.33) | 10.38 (1.75) |

***Table 5.2. Descriptive statistics of the three study groups.*** *Means are standard errors are given to every group for their age and HQ scores, as well as the sex split.*

### *5.2.1 Time course of the stimulus response*

Grand average ERP data for channel FCz (with P9/P10 reference) across standard and deviant responses for all stimulus conditions and in each group was used to attempt to determine timeframes for quantifying P50, N100, and MMN responses, based on visual inspection (Figure 5.1). To calculate the MMN difference waveform, standard responses were subtracted from their equivalent deviant conditions.

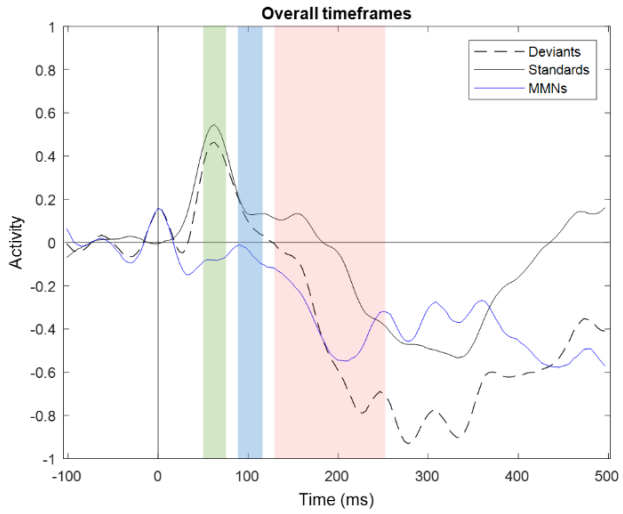



***Figure 5.1. Standard, deviant and MMN waveforms broadly combined across all groups at 6 kHz.*** *The chosen P50 timeframe was 50-75 ms (green), N100 was 90-110 ms (blue). The MMN timeframe was 140-250 ms (red).*

### *5.2.2 Standard and pure deviant waveforms*

The average waveforms of responses to 6 kHz tones of each group are shown on Figure 5.2.

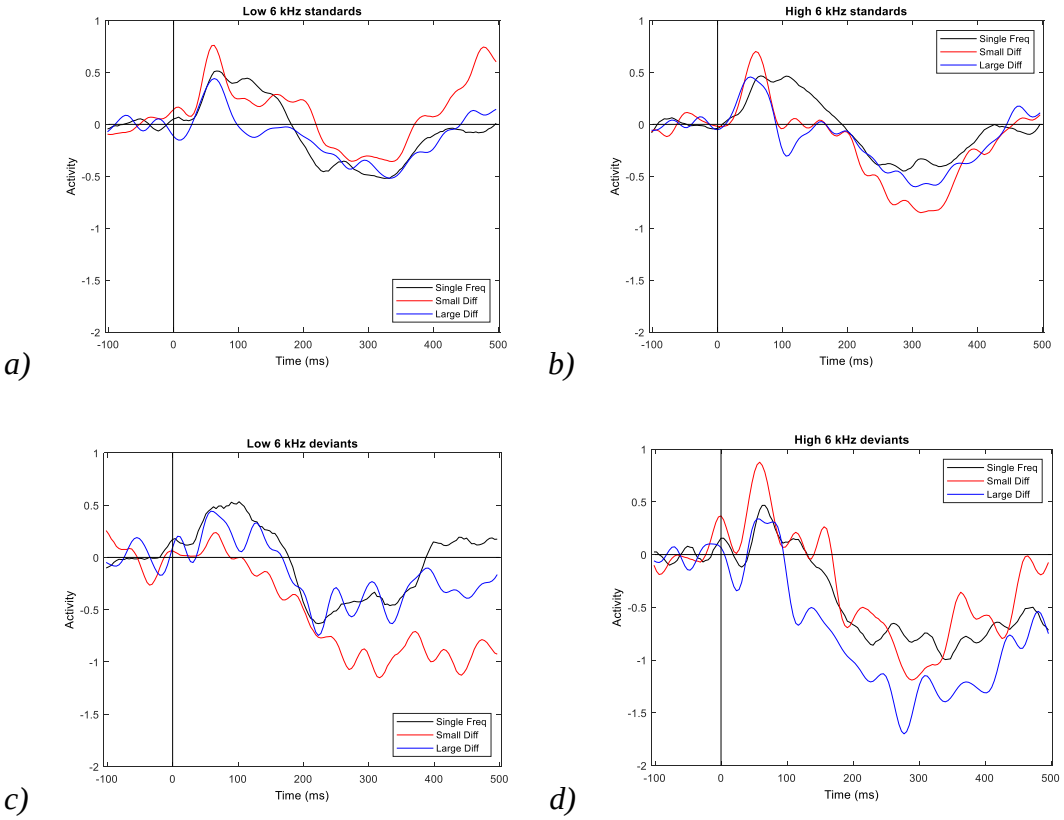



***Figure 5.2. Average waveforms to standard and deviant conditions at 6 kHz.*** *On the left, are the quieter stimuli conditions and on the right are the louder stimuli conditions. Responses to standard tones are shown on a) and b), while responses to raw deviant stimuli are shown on c) and d). Single frequency group is shown in black, small difference group is shown in red, and large difference group is shown in blue.*

#### *5.2.2.1 P50 responses*

Figure 5.3 shows P50 responses to the two standard and two raw stimuli. A two-way ANOVA (group, intensity) showed a main group effect on P50 amplitudes in response to standard stimuli. The small difference group had significantly larger responses than both of the other groups at both intensities (all $p<0.001$).

A two-way ANOVA (group, intensity) showed main effects of both factors on P50 response amplitudes to deviant tones, as well as a significant interaction between group and intensity. Post-hoc Tukey test showed that the single frequency group had larger amplitudes compared particularly to small difference group ($p<0.001$), but also large difference group ($p=0.025$). In response to high deviant stimuli, the small difference group had significantly larger

amplitudes than both other groups ($p<0.001$), but no differences were seen between single frequency and large frequency groups. This was a striking finding, as while the single frequency and large difference groups had relatively similar responses to both deviants, small difference group had a very clear pattern. The small difference group had weaker P50 amplitude to low deviants than the other two groups, and stronger P50 responses to high deviants than the other two groups.

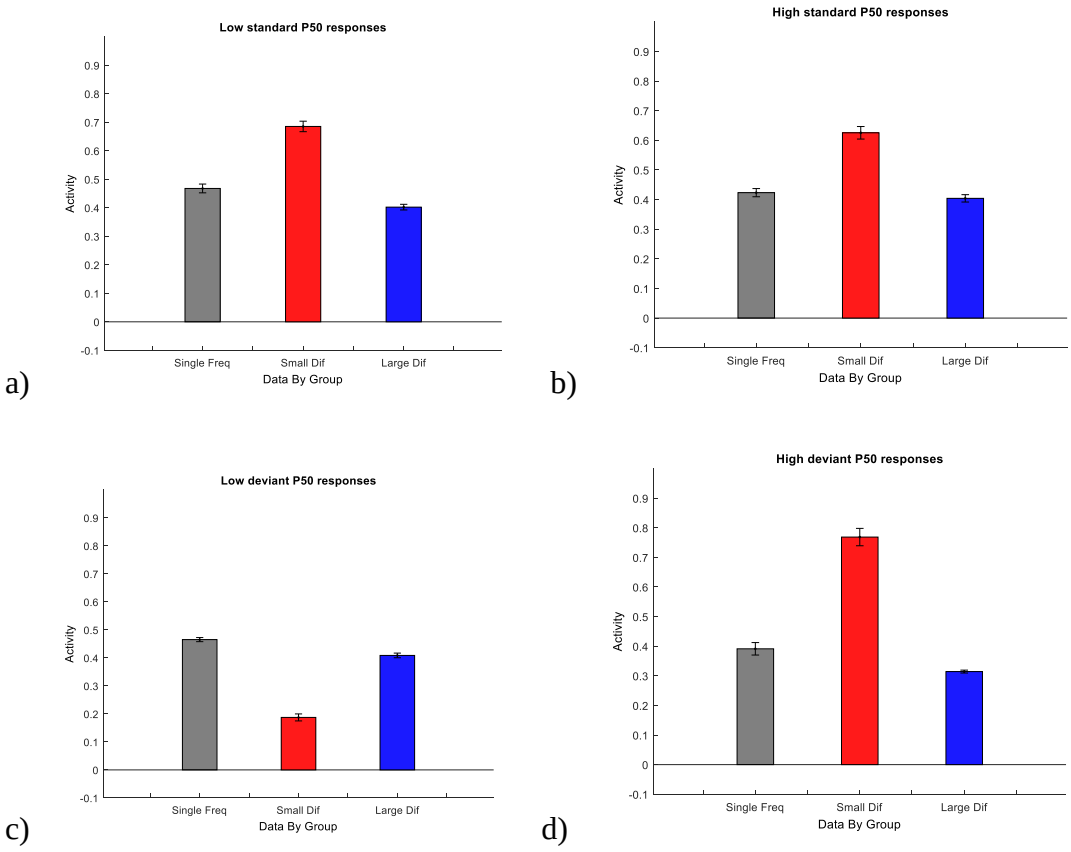



***Figure 5.3. Standard and deviant responses in the P50 timeframe.*** *Top two charts represent averaged responses to standard stimuli bottom charts represent averaged responses to raw deviant stimuli. The order of the groups in all charts is: single frequency (grey), small frequency difference (red), and large frequency difference (blue).*

### *5.2.2.2 N100 responses*

Figure 5.4 shows N100 responses to two standard and two raw stimuli. A two-way ANOVA (group, intensity) showed main effects of both main factors on N100 amplitude in response to standard tones ($p<0.001$). This is not surprising, as the single frequency group did not seem to display an N100 (Figure 5.1). The N100 amplitudes were significantly weaker in response to low standard tones than high standard tones both in the small difference group ($p<0.001$) and

large difference group (p=0.006), though the large difference group showed stronger N100 responses to standard stimuli overall.

A two-way ANOVA (group, intensity) also showed main effects of both main factors on N100 amplitude in response to deviant tones, as well as a significant interact between the factors (all p<0.001). Tukey post hoc tests showed that in response low deviant tones, the single frequency group had significantly more positive amplitudes during the N100 timeframe as expected (p<0.001), but the small difference group had significantly lower amplitude than the large difference group (p<0.001). In response to high deviant tones, the large difference group had a significantly stronger N100 compared to the other two groups (p=0.011 compared to single frequency and p=0.015 compared to small difference).

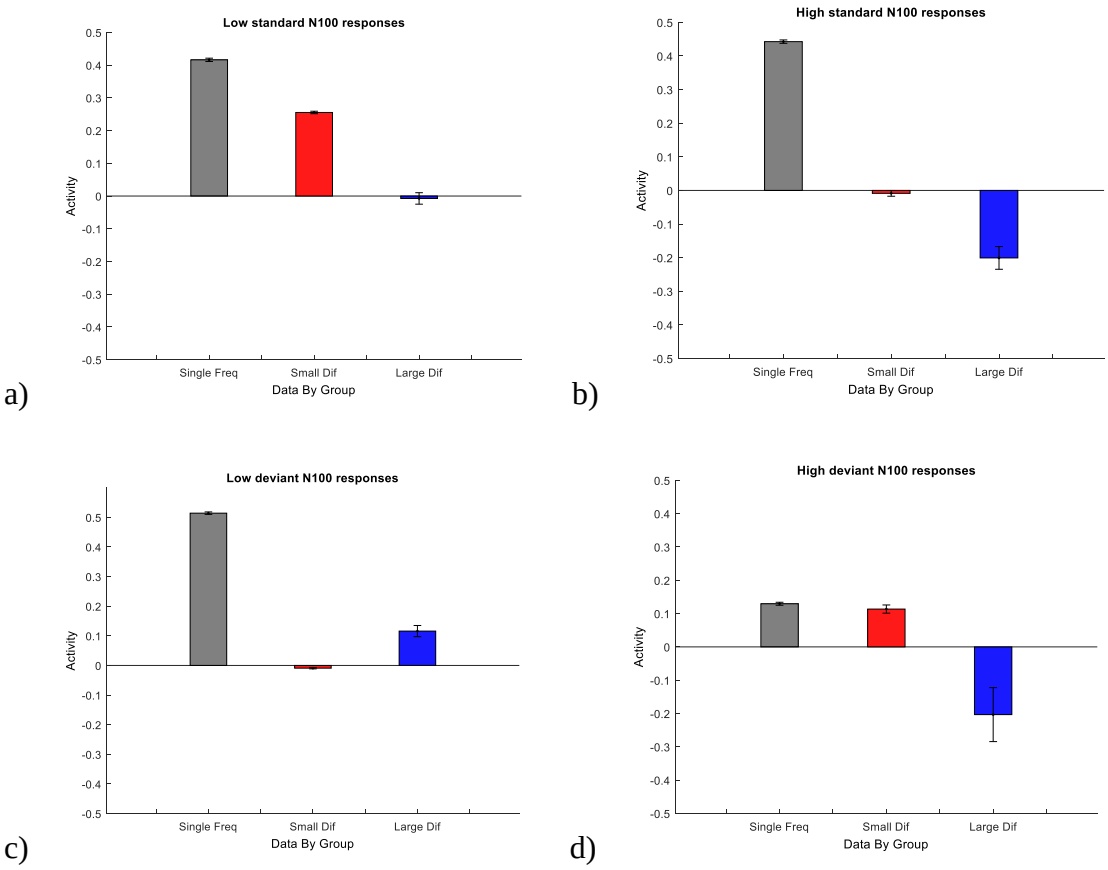



***Figure 5.4. Standard and deviant responses in the N100 timeframe.*** *Top two charts represent averaged responses to standard stimuli bottom charts represent averaged responses to raw deviant stimuli. The order of the groups in all charts is: single frequency (grey), small frequency difference (red), and large frequency difference (blue).*

### *5.2.3 MMN responses*

The average waveforms of responses to 6 kHz tones of each group are shown on Figure 5.5. Figure 5.5 also shows MMN responses to the two deviant conditions. A two-way ANOVA (group, intensity) showed that both main factors have significant effects on the amplitude of MMN responses, with a significant interaction between the factors (all $p<0.001$). Post hoc Tukey tests showed that in response to DDs, single frequency and large difference groups produce responses that are not significantly different from each other ($p=0.890$), however both of these are significantly weaker than the response produced by the small difference group (both $p<0.001$). In response to UDs, the large difference group produced an MMN response that was significantly stronger than both single frequency and small difference groups ($p<0.001$). However, the single frequency group still produced a significantly stronger response than the small difference group ($p<0.001$).

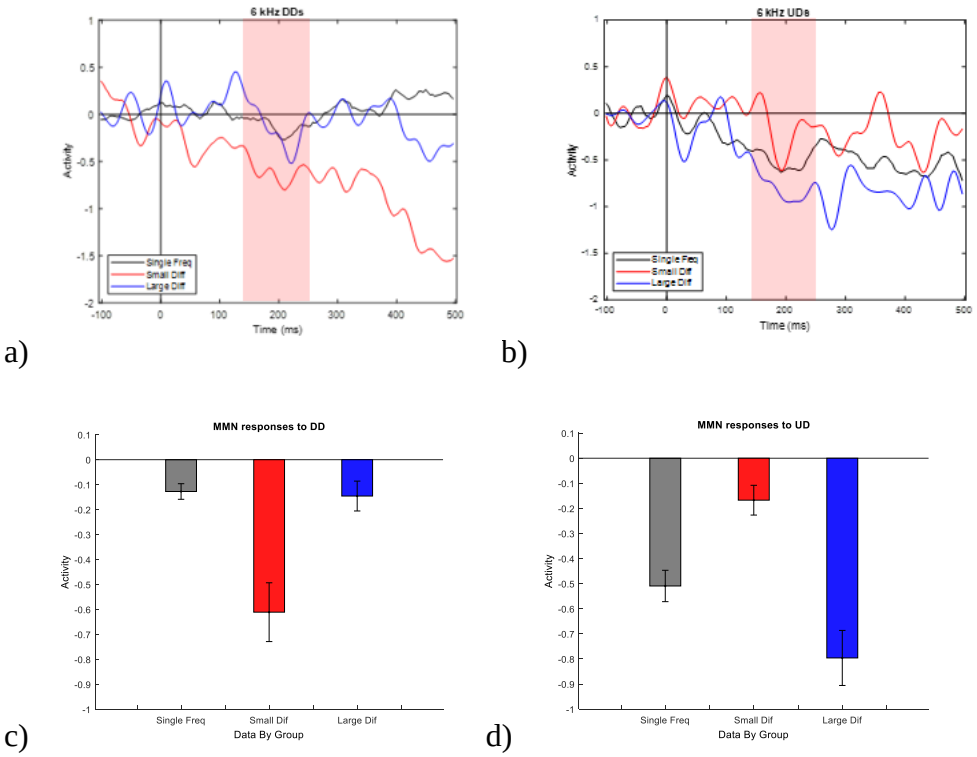



***Figure 5.5. MMN responses.*** *Top two charts represent Average MMN waveforms for each subject group, in each condition at 6 kHz. The MMN timeframe was 140-250 ms (red). On the left, is the downward deviant and on the right is the upward deviant. Single frequency group is shown in black, small difference group is shown in red, and large difference group is shown in blue. Bottom two charts represent averaged responses to downward deviants and upward deviants. The order of the groups in all charts is: single frequency (grey), small frequency difference (red), and large frequency difference (blue).*

## 5.3 Discussion

### *5.3.1 Summary*

Overall, the deviant/MMN responses in the small difference group were often opposite to the large difference group. The large difference group somewhat followed the waveform patterns seen in the single frequency group. The small frequency difference group MMN was stronger in response to DDs than UDs, while the other two groups show the opposite pattern of stronger MMN to upward intensity deviants.

P50 amplitudes were the most evident in the small difference paradigm, with an interesting pattern coming through in response to raw deviant stimuli. The low deviant responses in the small difference paradigm were lower than the other two groups, while the high deviant responses were larger than the other two groups. Single frequency and large difference paradigm responses remained similar within the two intensity conditions. N100 responses were the clearest in the large difference paradigm (possibly due to the lower P50 amplitude compared to small difference). In response to raw low deviant stimuli, N100 seemed attenuated in all paradigms. All three groups showed a negativity around 200-400 ms post standard stimuli. In the single frequency paradigm, in lieu of the completely attenuated N100 responses, P50s were prolonged.

### *5.3.2 Possible underlying mechanisms*

Previous research points towards habituation likely playing a large part in the formation of the waveforms in the current findings. In repetition suppression experiments, N100 amplitude has been previously found to rapidly reduce even in response to the second stimulus in the train and continue to attenuate in the following four stimuli; P50 amplitude also decreased after the first stimulus in this paradigm, but not between the second stimulus and later stimuli [241]. Further, the N100 data were entirely consistent with other unpublished data within our group, showing that long single-frequency paradigms completely attenuate the N100 response. It is instead replaced with a positive response that extends the P50. We explain this, based on published literature, as the effect of extreme repetition positivity.

Interestingly, P50 responses to standard stimuli were least attenuated in the small frequency difference group but the N100 was least attenuated in the large difference group. It could be expected that the small difference paradigm responses may be more sensitive to changes in stimulation due to increased contrast gain [232]. This may be true for P50 responses, except the low deviants, as on average the pre-attentive reaction to both standard and deviant stimuli

was stronger than in the other two paradigms. In the low deviants, the suggestion that suppression is inversely proportional to the intensity of a second tone especially within responsive frequency fields may somewhat relate to the reduced P50 and N100 in the small difference group [238]. A detailed study on different combinations of stimuli patterns and their ability to create reproducible fundamental differences in topologically constrained small network responses concluded that networks create stable responses after repeated stimulation through various plasticity effects [242]. If such differences can be created in small networks, it could be postulated that such differences could carry into larger scale networks. However, studies performed on a small scale could show the effects of two similar frequencies in the paradigm in unexpected ways compared to whole brain studies. For example, different results were found for intracranial vs EEG in terms of N100 latencies [241]. Nonetheless, in a previous EEG study, researchers presented 115 trains of unique frequency stimuli, with one condition being wide range of frequency, and another being narrow range of frequency. They found that N100 amplitude was dependent on the frequencies presented during an experiment, with neurons adjusting to the range of stimulation history dynamically, potentially through lateral inhibition [234]. This seems similar to the dynamic range adaptation in intensity paradigms. Overall, it is possible that the differences between the paradigms that we saw are fairly fundamental to the auditory system. The single frequency and large difference paradigms may show similar responses because the field affected by changes in gain does not reach the lower frequency. This could explain the discrepant findings between the original IMA study and the first two EEG studies in this thesis.

Lower N100 suppression has been related to over-inclusion of background sounds, meaning that participants with more N100 (in the current study, large difference group) were more aware of background sounds, which the previous researchers attributed to passive attention switching towards novel sounds [243]. Within their study, the authors found that a stronger P50 suppression was related to a stronger MMN response for intensity deviants (though to 1 kHz stimuli). This seems true in the current study as well, for example, the raw deviant P50 amplitude was inversely related to MMN amplitudes, e.g. small difference group had weaker P50 but stronger MMN amplitudes in response to DDs, and the other way round for UDs. The association between P50 and MMN amplitudes was also present in the second EEG study of the current thesis, where T+H+ had a particularly decreased P50 amplitude in response to a high deviant and had a particularly strong MMN for UD, while T+H- had a similar pattern in response to low deviant and DD. An interesting hypothesis was suggested, in which the P50 suppression was related to filtering out of irrelevant stimuli and thus allowing the brain to

more efficiently detect changes in the stimulus, leading to a larger MMN [243]. Accordingly, in a round-about way, it is possible that the small difference paradigm is still creating more sensitive responses even to DDs.

While habituation has been a guiding theory, some authors argue that the inter-stimulus interval length effect on N100 is more in line with the refractoriness hypothesis than habituation [244]. The refractoriness hypothesis suggests that the N100 decrease may be due to the recovery period of particular neural generators, and not due to learning of the repeating stimuli. The two accounts differ in their predictions for the waveform in response to the stimulus after the deviant tone: the refractoriness hypothesis assumes no response recovery after a deviant tone, no exponential attenuation of N100 and no N100 amplitude change after and ISI longer than the recovery period [245]. On the other hand, the habitation hypothesis suggests continuous N100 decrease and dishabituation once a deviant tone or even tonal language, such as spoken Mandarin, are played [246]. In the current data, after particularly repetitive stimuli (single frequency group), the N100 was completely gone. Additionally, the authors state that refractoriness refers only to the immediate past prior to the current stimulus. Therefore, the different results based on the overall paradigm context would not quite fit this hypothesis. Notably, the current data cannot disprove either hypothesis as while there was no N100 in the standard response to the single frequency paradigm, N100 was not present in response to the deviants either, and the ISI used in this study was much shorter than the recovery period [247]. However, it may be interesting to pay closer attention to the behaviour of the waveform throughout the length of the experiment and particularly at and just after deviant stimuli.

### *5.3.3 Future directions*

Importantly, the observations discussed herein apply to the particular set of experimental parameters tested. There are, however, other paradigm specifics that could affect the shape of the overall waveform (particularly N100 and P200 ERPs), such as length of inter-stimulus intervals (ISI) and attention [248, 249]. For example, temporal attention can alter cortical gain, and gain in neurons responsible for predictive error signals [250]. This could potentially be due to optimisation of predictions about the sensory input, as one MEG study showed a significant difference between expected vs unexpected tones in attended condition, but not unattended condition, when the expectation was based on a prior cue that specified the instructions for every experimental block [249]. It would be useful to see how they generalise

to other experiment designs such as attended and ignored stimulus conditions, different stimulus durations, ISIs, non-isochronous, paradigms with frequency deviants, etc.

### *5.3.4 Conclusions*

Overall, the wider context of the different frequency ranges in the experimental paradigms dramatically influenced both P50 and intensity mismatch responses in a reciprocal way. Unchanging or widely separated frequencies resulted in upward intensity deviant MMNs being larger than for downward, and narrowly separated frequencies produced the opposite pattern. This effect applies even when the frequency changes are remote (e.g. tens of seconds to minutes away) from the intensity responses they influence. It is likely that similar influences occur (logically, and from group's unpublished data) when frequency changes are recent or ongoing from the intensity responses they influence.

Based on the second EEG study of this thesis, hyperacusis (with or without tinnitus) was characterised by increased upward and reduced downward intensity MMNs, the pattern related to hyperacusis might be best revealed using narrowly different frequencies, to give the maximal contrast between the intensity conditions. Conversely, tinnitus without hyperacusis was characterised by increased downward intensity deviants, and therefore may be best revealed using single-frequency or widely-spaced frequency differences.

In summary, tinnitus and hyperacusis show distinct signatures in intensity deviant MMN response profiles, as do control responses depending on the range of frequencies used in the paradigm, and future studies should consider optimising their paradigm for the condition under study based on these observations.

# Chapter 6 Intensity mismatch asymmetry in tinnitus – in which direction should participants pay attention?

## 6.0 Introduction

So far in this PhD project, we have been using subtitled movies as a passive attention task as a way to control for confounding differences in attention direction among the participants during EEG recordings. It is a possibility that the task performed while a participant is presented with the auditory stimuli may change the ERP amplitudes, or even the pattern of ERP responses across different conditions [251]. It is important to understand the effects of attention for a number of reasons, including helping to establish what role attention played in our previous findings, and whether attention is a candidate explanation for why tinnitus and hyperacusis were associated with certain differences in evoked responses profile (e.g. in Chapter 4 of this thesis). Additionally, it would be useful to establish the optimal attentional state for studying differences due to tinnitus and/or hyperacusis. Finally, the effects attention has on intensity deviant responses is an unknown factor in basic sensory neuroscience. It would be useful to understand how attention would affect responses to intensity deviants compared to each other (upward vs downwards), and compared to other sensory dimensions such as frequency.

Based on Bayesian models of perception, attention may enhance the precision of sensory input (discussed in the predictive coding subtitle of the introduction to this thesis) [252]. Attentional gain modulation works through top-down modulation of sensory precision, which relies on postsynaptic gain, inhibitory interneurons and feedback mechanisms, the strength of which was positively correlated with attention [249]. Attention may be integral in the formation of optimal predictions in change detection paradigms, but also have dissociable effects from simply strengthening or weakening predictions or prediction errors [252, 253]. For example, an fMRI study used a grating orientation identification task, where a cue determined towards which side of a screen participants needed to direct their attention and respond if a visual stimulus appeared on the attended side [252]. This was done with the aim of studying the role of attention on prediction in the visual cortex. The researchers found that in the unattended condition, standard stimuli showed lower activity than deviants, however, the standards elicited larger responses than deviants when stimuli were attended and task-relevant [252, 254]. In a different study, eighteen healthy participants were tested using an auditory duration deviant oddball paradigm [251], which was presented three times, where participants were asked to either ignore auditory stimuli, passively listen, or focus on the

stimuli. Participants were overall younger than those expected to age-match with the tinnitus group in the current study. Additionally, all participants had normal hearing thresholds. The tasks in which participants focused on the stimuli or passively listened showed largest MMN amplitudes, while the smallest amplitudes were in the task where they ignored the stimuli. An important conclusion was made in a review that indicated that direction of attention was more likely to modulate the MMN responses to standard tones rather than the deviants, as MMN reflects the organisation of the prior stimuli in memory as well as the response to the deviant itself [254]. This has also been noticed in some of the previous findings in the thesis, where the main differences between conditions was the P200 response to standards but raw deviant forms did not significantly change.

Overall, previous research indicates that attention may modulate neuronal gain in healthy participants and change the amplitudes of ERPs, but any effects of attention are typically represented primarily within the modality of the changing stimuli [255], and mainly affect the responses to standard stimuli rather than deviants themselves. It has been tested how attention affects MMN more generally, but not specifically in terms to intensity paradigms. The previous chapters of this thesis have also shown that gain may respond differently in participants with tinnitus and/or hyperacusis under the same conditions, particularly at the tinnitus frequency. In the current study, the auditory system is investigated to see whether there are similarities in the way that attention affects neural prediction error correlates such as MMN, and to see how presence of tinnitus may interact with these attention-related changes. An additional important aim of this study is to understand whether the passive attention condition used in the previous EEG experiments of this thesis was adequate.

## 6.1 Materials & methods:

### *6.1.1 Participants*

Volunteers with tinnitus (N=20) were recruited from affiliated volunteer lists at Newcastle University and via Google Ads. General inclusion criteria included being over 18 years old, and able to make an informed choice about volunteering. General exclusion criteria included using ongoing sedating or nerve-acting medications, and mental health conditions severe enough to interfere with everyday life activities. To be included in the tinnitus group, participants had to have chronic tinnitus for over 6 months, with no physical source and that was not due to Meniere's disease (this was an exclusion criterion for the control group). The control participants were individually matched to participants with tinnitus based on sex, age

and an approximate match of their overall audiometric profiles. All participants completed the Hyperacusis Questionnaire.

Approval was given by the Newcastle University Research Ethics Committee, and all participants gave written informed consent according to the Declaration of Helsinki (reference number 5619/2020).

### *6.1.2 Common methods: tinnitus psychophysics and EEG*

The psychophysical assessment in which the tinnitus frequency and the intensity of sounds played during the EEG recording were determined, and the experimental design, followed the procedure in previous EEG chapters. There were three main differences in the current study. Firstly, in order to reduce the number of factors involved in the analysis, only the tinnitus frequency was included in the paradigm (similarly to the single frequency paradigm in the context study). Secondly, there were gap deviants instead of duration deviants approximately every 1 out of 10 stimuli. These were used as the control deviant condition and as targets in the auditory task. The gap deviants were equally present in every attention block type, the only difference between the tasks was that in the auditory task, participants had to respond by clicking a keyboard button. Lastly, each participant performed three tasks during their EEG recording, which were presented in randomised order. The tasks were: 1) A 10 minute visual task (x2), in which participants were asked to look at moving dots on the screen and decide whether they were moving randomly or in a specific direction. This task was adapted from code previously created for the Random Dot Kinematograms test [256], with larger gaps between each trial for similarity in task difficulty; 2) A 10 minute auditory task (x2), in which participants were asked to listen out for particularly long gaps (gap deviants within the paradigm) in the stimuli and press a button when they could hear the gap; 3) A 10 minute period (x2) in which participants were asked to watch a subtitled movie of their choice. Independent Component Analysis was used to remove ocular artefacts rather than Denoising Source Separation [109].

### *6.1.3 Statistical Analysis*

Statistical analysis was performed using MATLAB. To compare the evoked responses in participants with and without tinnitus, a three-way ANOVA was used with group, intensity, and task as factors of interest, and including interaction terms. Post-hoc analysis included Tukey Honest Significance Tests to determine any significant differences between the ERP amplitudes of the two groups in each task.

## 6.2 Results

### *6.2.1 Demographic information*

Table 6.1 shows means and standard errors (SE) of the demographic information of the two groups, their HQ scores, and THI scores in the tinnitus group. As age was not normally distributed in either group, an independent samples Mann-Whitney U test was used to show that age was not significantly different between the two group (p=0.529). However, HQ scores were significantly different between the groups (p=0.004). Therefore, based on a previous chapter, interpretation of results will have to account for presence of hyperacusis; the score in the tinnitus group is just above the updated cut-off score, but 12 of the 20 participants within the sample scored above the cut-off. A Chi-squared test was carried out to establish that there were no significant differences in sex across the two groups (Chi (1) =2.50, p=0.114). As the pure tone audiometry results were not normally distributed at the majority of frequencies/ears Mann-Whitney U tests were performed and showed that while 0.25 kHz and 0.5 kHz pure audiometry results were significantly different between the two groups, there were no significant differences between groups at 1, 2, 4, 6 or 8 kHz in right or left ears, with the smallest p-value being p=0.277. The discrepancy at the lowest measured frequencies should not be problematic for this study, as tinnitus usually appears at high frequencies and both groups had mean hearing thresholds within the normal range at 0.25 and 0.5 kHz in both ears.

| | **Age** | **Sex** | **HQ Scores** | **THI Scores** |
|---|---|---|---|---|
| **Tinnitus** | 56.20 (3.52) | 13 f, 7 m | 16.05 (1.78) | 34.20 (4.33) |
| **Control** | 57.90 (3.60) | 12 f, 8 m | 9.65 (1.34) | N/A |

***Table 6.1. Descriptive statistics of the study groups.*** *Means and standard errors are given for every group for their age, HQ scores and THI scores. The sex split is also indicated for each group.*

### *6.2.2 Time course of the stimulus response*

Grand average ERP data for channel FCz (with P9/P10 reference) across standard and deviant responses for all stimulus conditions and in each group was used to determine timeframes for quantifying P50, N100, P200 and MMN responses, based on visual inspection (Figure 6.1). To calculate the MMN difference waveform, standard responses were subtracted from their equivalent deviant conditions.

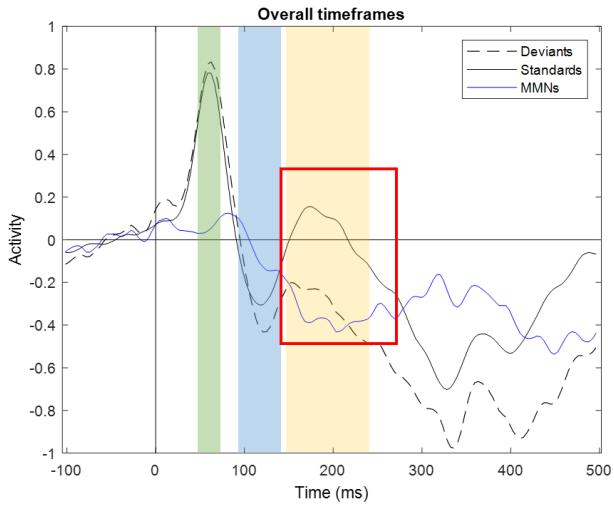



***Figure 6.1. Standard, deviant and MMN waveforms broadly combined across groups and conditions.*** *The timeframe for quantifying P50 was 50-75 ms (green), N100 was 95-140 ms (blue), P200 was 150-240 ms (orange). The MMN timeframe was 140-260 ms (red square). As the MMN and P200 overlap, P200 was only used in standards (and MMN in the difference waveform).*

### *6.2.3 Standard and pure deviant waveforms*

The average waveforms, split by task and intensity, are shown in Figure 6.2.

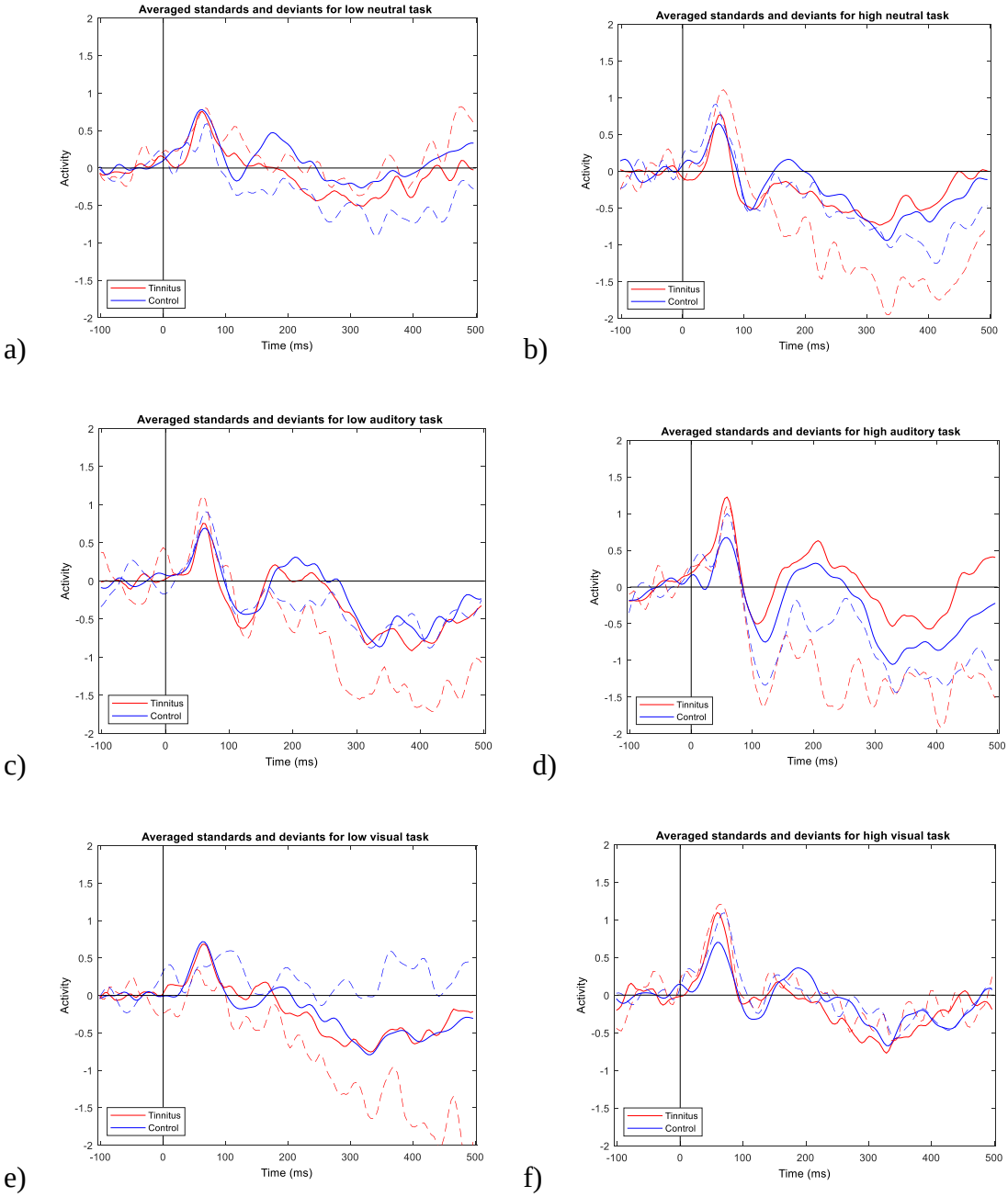



***Figure 6.2. Average waveforms to standard and deviant tones in different intensity and task conditions.*** *The tasks, from top to bottom, are neutral, auditory, and then visual. On the left, are the quieter stimulus conditions and on the right are the louder stimulus conditions. Standard responses are shown in solid lines and deviants are shown in dashed lines. The groups are colour-coded as follows: tinnitus (red), controls (blue).*

#### *6.2.3.1 P50 responses*

Figure 6.3 shows P50 responses to standard and deviant stimuli in the three tasks at two intensities. A three-way ANOVA (group, task, intensity) was carried out on P50 responses to standard tones, which found main effects of group and intensity (both $p<0.001$), but not task ($p=0.091$). There were significant interactions between group and intensity, and task and intensity (both $p<0.001$). There were also significant interactions between group and task ($p=0.005$) and group, task and intensity ($p=0.031$). From visual inspection (in keeping with the reported statistical effects), the biggest difference was that the two active tasks increased P50 to high intensity stimuli in the tinnitus group, reflecting a combination of these interaction effects. Post hoc tests showed that there were no significant differences between P50 response amplitudes to the three tasks at the low intensity. However, the neutral task had significantly smaller amplitudes than the other two tasks ($p<0.001$), driven by the tinnitus group having larger responses than the controls ($p=0.011$ for auditory and $p<0.001$ for visual).

When comparing the pure deviant responses, a three-way ANOVA (group, task, intensity) showed main effects of group, task and intensity, and a significant interaction of task and intensity (all $p<0.001$). In response to the low deviant during the auditory task, both groups had larger P50 amplitudes than during either of the other tasks. Additionally, during the visual task, both groups had particularly lower P50 amplitudes in response to low deviant compared to high deviant.

There was also a significant interaction effect between group and task ($p=0.004$). Tinnitus group had significantly higher P50 amplitudes compared to controls in response to low deviant in neutral task ($p=0.002$) and significantly lower P50 amplitudes in response to low deviant in visual task ($p=0.004$). Based on visual observation of Figure 6.2, the visual low deviant likely showed controls to have larger P50 amplitudes than the tinnitus group because there was no reduction of the amplitude due to lack of N100 in controls but not in the tinnitus group.

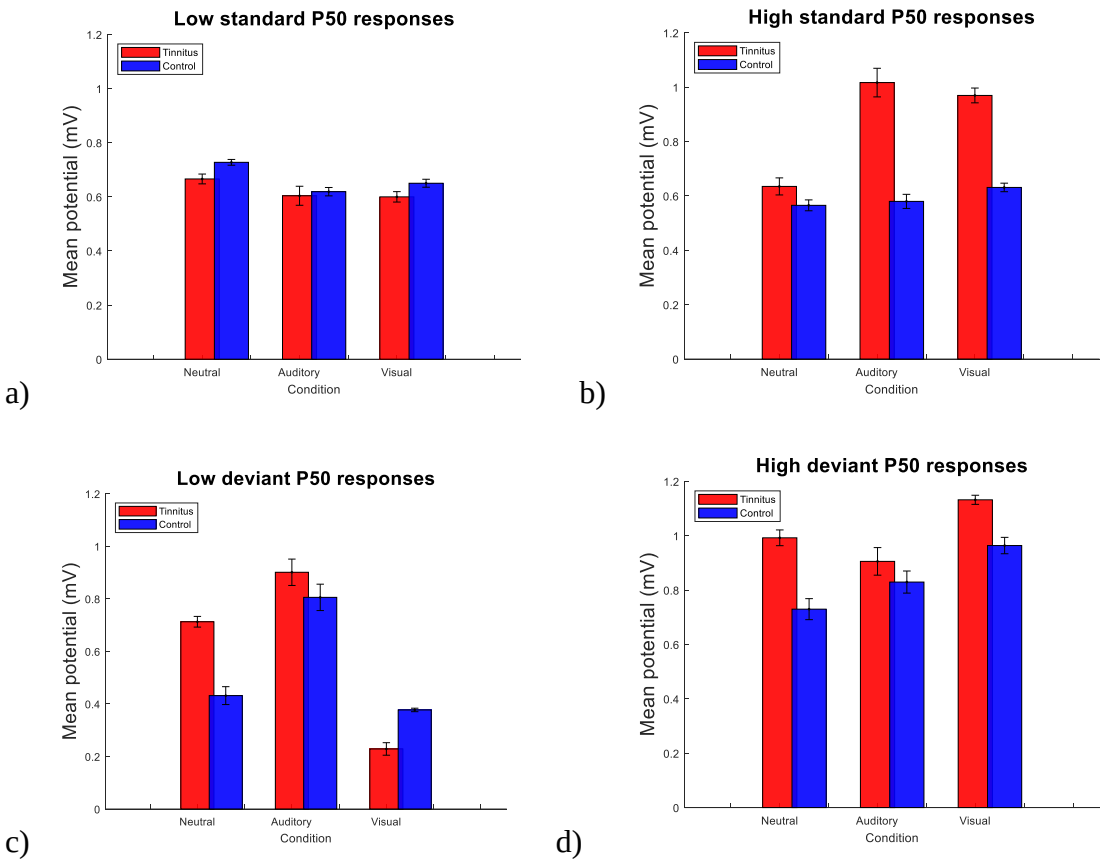



***Figure 6.3. Standard and deviant responses in the P50 timeframe.*** *Top two charts represent averaged responses to standard stimuli and bottom charts represent averaged responses to deviant stimuli. Tinnitus group is represented in red and controls are represented in blue.*

### *6.2.3.2 N100 responses*

Figure 6.4 shows the mean amplitudes of N100 responses to standard and deviant tones in the three tasks. A three-way ANOVA (group, task, intensity) showed main effects of all three factors, and interactions between task and intensity, and group, task and intensity (all $p<0.001$). There was also a significant interaction between group and task ($p=0.022$). At the low intensity standards, N100 amplitudes for the auditory task were significantly lower than for both neutral and visual task ($p<0.001$ and $p=0.006$, respectively). At high intensity standard, N100 amplitudes were significantly weaker in the visual task than neutral and auditory tasks ($p<0.001$). Post hoc tests showed significantly weaker responses in tinnitus group compared to controls within the neutral and visual tasks at low intensity standards ($p=0.003$ and $p=0.002$, respectively), and at high intensity standards within auditory and visual tasks ($p<0.001$ for both). Based on visual inspection of Figure 6.2, the tinnitus group did not have a clear N100 in response to low standards in the neutral task. The tinnitus group

had non-significantly stronger N100 amplitudes than the controls for the low standard in the auditory task and for the high standard in the neutral task.

For deviant tones, a three-way ANOVA (group, task, intensity) showed significant main effects of task and intensity, as well as significant interactions between group and task, task and intensity, and group, task and intensity (all $p<0.001$). At the low intensity deviant, the tinnitus group showed significantly weaker N100 than the controls in the neutral task ($p<0.001$) but significantly stronger N100 than controls in the visual task ($p<0.001$). For the high deviant, the auditory task elicited significantly stronger responses than the other two tasks overall ($p<0.001$), with the visual task showing the lowest amplitude for N100 ($p<0.001$ compared to the others). In the neutral task, the tinnitus group had significantly lower N100 amplitude than the controls ($p<0.001$).

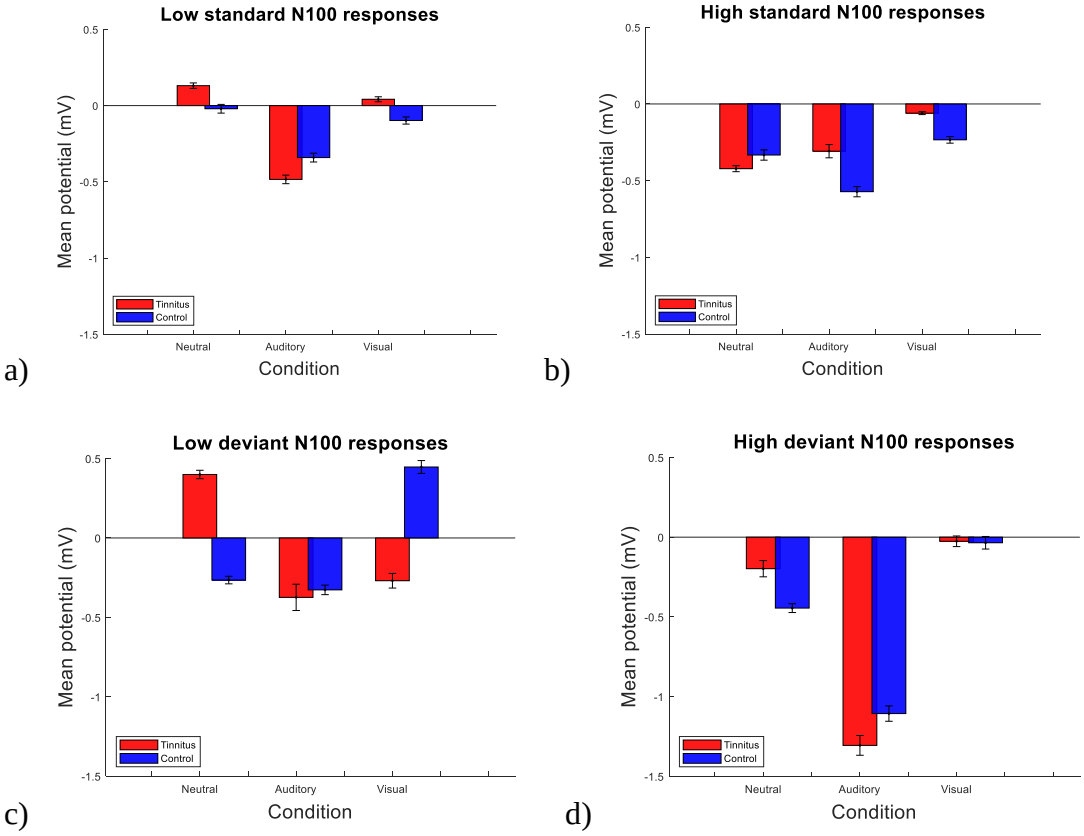
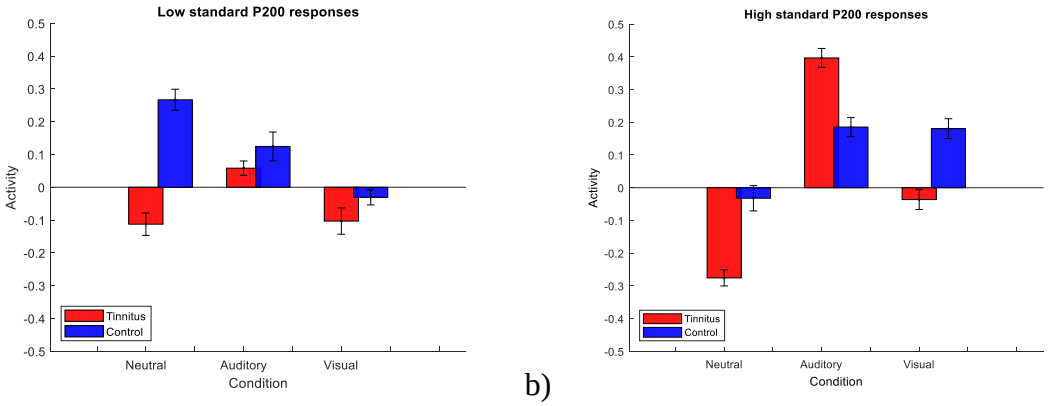



***Figure 6.4. Standard and deviant responses in the N100 timeframe.*** *The top two charts represent averaged responses to standard stimuli, bottom charts represent averaged responses to deviant stimuli. The order of the groups in all charts is: neutral task, auditory task, visual task.*

### *6.2.3.3 P200 responses*

Figure 6.5 shows the mean amplitudes of P200 responses to standard tones in the three tasks. A three-way ANOVA (group, task, intensity) showed main effects of all three factors ($p=0.036$ for group, $p<0.001$ for task and intensity), as well as an interaction between group and task, task and intensity, group task and intensity (all $p<0.001$), and group and intensity ($p=0.009$). In response to both intensities during the neutral task, the tinnitus group had significantly lower amplitudes than the controls (both $p<0.001$). For high intensity standards during the auditory task, there was a significantly larger response in the tinnitus group compared to controls ($p<0.001$), possibly due to the prior N100 amplitudes. Additionally, the controls had a significantly stronger response to high standards in the visual task ($p<0.001$). At both intensities, the tinnitus group had higher P200 responses to the auditory task than neutral or visual tasks ($p<0.001$), and lower P200 amplitude in the high intensity responses during the neutral task ($p<0.001$).




a) b)

***Figure 6.5. Standard responses in the P200 timeframe.*** *The order of the groups in all charts is: neutral task, auditory task, visual task.*

### *6.2.4 Mismatch Negativity (MMN)*

A difference waveform was calculated by subtracting standard waveforms from deviant waveforms, which was used for MMN analysis (Figure 6.6).

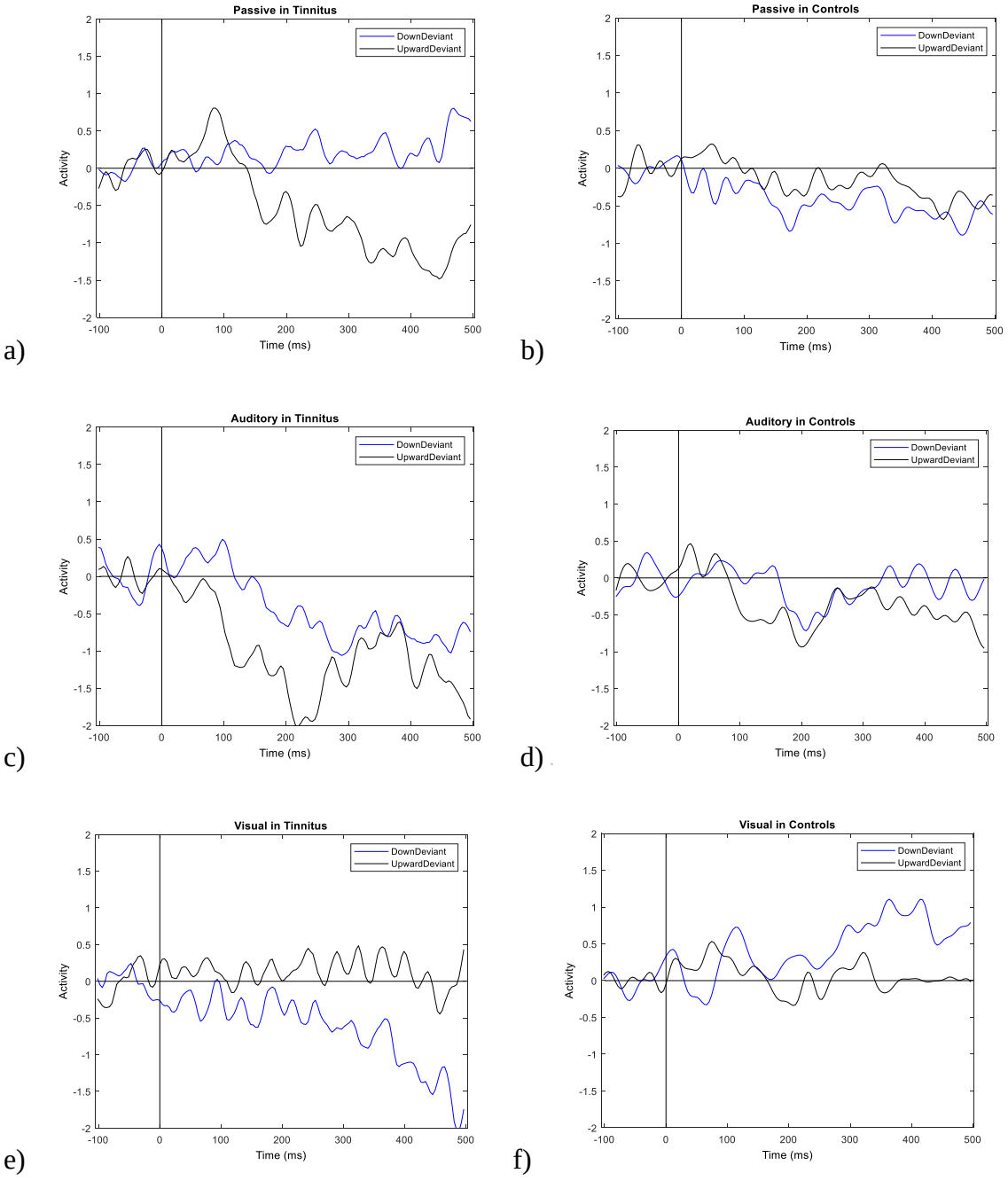
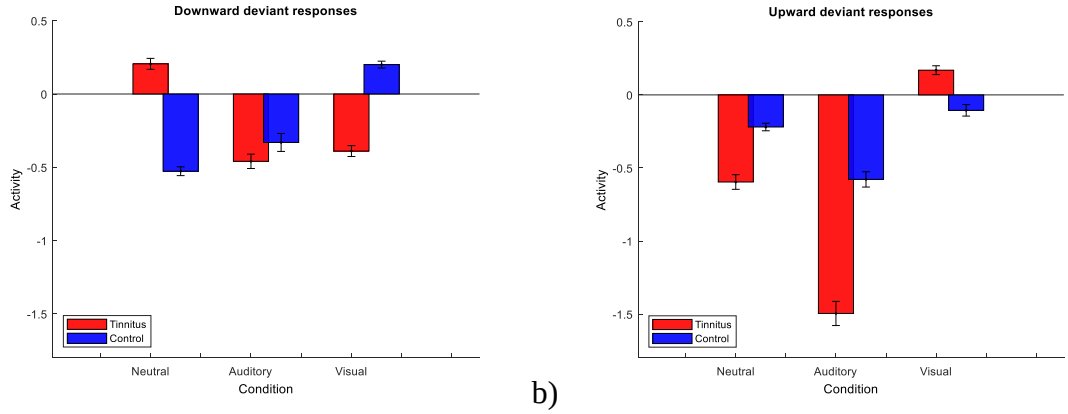



***Figure 6.6. Average difference waveforms in different intensity and task conditions.*** *The tasks, from top to bottom, are neutral, auditory, and then visual. The tinnitus group is on the left and the controls are on the right. The conditions are colour-coded as follows: DD (blue), UD (black).*

Figure 6.7 shows MMNs in the three tasks. A three-way ANOVA (group, task, intensity) showed significant main effects of all three factors, as well as significant interactions between all pairs and between all three factors (all $p<0.001$). Post hoc tests showed that at each intensity and in each task, except DD for the auditory task ($p=0.058$), there was a significant difference between tinnitus and control groups (all $p<0.001$), though the largest difference was in the auditory group in response to UDs. Tinnitus group had weaker MMNs compared to controls only for DDs but stronger MMNs for UDs in the neutral task, but the opposite pattern was seen for MMNs during the visual task.




a) b)

***Figure 6.7. MMN responses.*** *The order of the groups in all charts is: neutral task, auditory task, visual task.*

## 6.3 Discussion

### *6.3.1 Main findings*

Results of the passive task condition were consistent with previous studies. Auditory attention magnified MMN in response to upward deviants, while visual attention attenuated it. Auditory attention selectively enhanced downward deviant MMN in the tinnitus group (compared to passive attention).

#### *6.3.1.1 P50s*

P50 responses were similar in response to low standards in both groups across the three tasks. While the responses remained similar between the groups for the high standard in the neutral task, in the visual and auditory tasks the tinnitus group had larger P50 amplitudes, while control group responses remained similar to each other and to low standard. The differences in response amplitude in the tinnitus group was probably not due to the presence of hyperacusis because, as shown in the findings in a previous chapter, T+H+ group had weaker

P50 amplitudes both near and outside of tinnitus frequency (though there is also a possibility that results in the two studies may not be comparable due to the overall context of the paradigm). Regarding the overall context, it is also possible that the tinnitus group in the current study had less overall/less quick suppression to the repeating sound when they had to either focus on the stimuli, or the stimuli were interfering with focus on a task in a different modality. Participants with tinnitus may have had more difficulty gating out the stimuli compared to the control group during the attention-demanding tasks due to less ability to ignore the irrelevant stimuli [208, 257], but not in the neutral task where they did not need to pay attention to the stimuli.

Controls had larger high deviant responses and smaller low deviant responses compared to standards, except during the auditory task where low deviant responses were similarly increased to high deviant responses. Tinnitus group showed similar patterns to the controls, but neutral condition responses appeared more like auditory condition in response to low deviant. Additionally, the tinnitus group also had larger deviant P50s to both intensity directions during the neutral task. So, while tinnitus participants were not concentrating on the stimuli or on a particular task, they were more responsive to changes in the stimuli. This was further shown by the tinnitus group having higher P50s for all tasks in response to high deviants. In the auditory task, P50 amplitudes were similar for both groups in response to both intensity deviants.

As it has been suggested that P50 amplitudes reduce for repetition of the immediately preceding tone, it is possible that the tinnitus group may have reduced inhibitory function compared to controls when attention is manipulated [220, 258]. Further, while much of previous literature showed that P50 was not dependent on attention (unlike N100), P50 amplitudes may be altered by psychological stressors, both in terms of task (e.g. mental arithmetic) and more chronic difficulties such as PTSD [259, 260]. So, perhaps either hyperacusis presence or tinnitus itself relates to higher stress responses both when paying attention or purposefully ignoring tinnitus-like stimuli.

The control group responses in the neutral task were similar to results seen in response to the small frequency difference paradigm in Chapter 5.

#### *6.3.1.2 N100s*

The tinnitus group showed a similar (almost) fully suppressed N100 in response to standard tones during the low intensity condition in the neutral task and during both intensity

conditions in the visual task. In the neutral task, suppression was only present during the low standard for tinnitus, which could occur because the higher intensity stimuli drew more attention in a stimulus-driven manner in a way that does not occur with lower or decreasing stimuli. N100 has been regarded as an attention-triggering mechanism [200]. This suppression may not have been present in the auditory task because participants were focusing on the stimuli; however, the more attention was taken away from the stimuli, the less sensitive the neurons became (less gain) and the more suppression was present [252], and this did not occur in the controls because they were not used to having to ignore an auditory stimulus while completing tasks. As possible corroboration of this explanation, a previous MEG study showed that actively ignoring a particular auditory stimulus was related to reduced M100 amplitudes compared to the passive condition [261].

In the auditory task, the tinnitus group had stronger responses to the low standard but controls had stronger responses to high standard stimuli. At the high standard (but not low), this could have occurred due to the prior P50 amplitudes, which in turn may have occurred due to hyperacusis, and subsequently affected P200 in the standards. However, N100 and P200 are not always dependent on each other, again pointing towards potentially this paradigm being more useful for sensory gating/ adaptation experiments into tinnitus and/or hyperacusis rather than MMN [202].

N100 amplitudes were present and stronger overall, particularly in response to high deviants in the auditory task compared to the other two tasks, likely due to the concentration on the stimuli. In response to low deviants, N100 was not clear for the tinnitus group during the neutral task (but it was present in controls); in high deviants, N100 was also weaker in the tinnitus group than controls. The opposite pattern occurred in response to low deviants in the visual task, where the tinnitus group showed an N100 but controls did not, and both groups had weak responses to the high deviants. It would be interesting to understand why these pattern swaps occur.

#### *6.3.1.3 P200s*

P200 was more present in controls than in the tinnitus group, except during the auditory task in response to high standards. Controls had more positive P200 responses to the neutral task in low standards, but to auditory and visual tasks in high standards. In the tinnitus group, P200 was more positive during the auditory task overall, and particularly in response to high standards. P200 has been associated with allocation of attention [202]. Previous studies have found reductions in P200 amplitudes in tinnitus participants compared to controls [262],

though other studies saw differences in latencies but not amplitudes [263]. P200 amplitude could be manipulated through task demands, as well as stimulus parameters [203]. P200 tends to be larger when attentional demands are lower [264]. This may explain why P200 was larger for the tinnitus group during the auditory task compared to the other tasks in both intensity conditions: they only needed to pay attention to the auditory stimuli, which already takes some of their attention as part of tinnitus. However, both neutral and visual tasks required some focus on other modalities and tasks. Control responses, however, are harder to explain using this theory. Differences in N100 may be obscuring the relative relationships between high and low intensities.

#### *6.3.1.4 MMNs*

In the neutral task, the tinnitus group had larger responses to the upward deviants and smaller responses to downward deviants, while controls had the opposite pattern of MMN amplitudes. This finding is similar to the original study and the T+H+ group in Chapter 4. Additionally, despite the present study having only one frequency, there was no 'extreme habituation' effect that was found in response to the single frequency paradigm in Chapter 5.

In the auditory task, controls did not show much difference from the neutral task, but there was a slight increase in response to upward deviants compared to downward deviants. This difference was seemingly mediated by differences in standard P200 responses [254]. In the tinnitus group, larger responses were seen to downward deviants compared to controls. This was not driven by P200 standard responses, and perhaps was in keeping with the result of T+H- group in Chapter 4, where increased MMN to downward deviants was the sole MMN change related to tinnitus once hyperacusis had been controlled for. The larger response to the upward deviant may have been driven by P200. Aside from P200 findings, the main results were that the tinnitus group had larger downward deviant MMN in the auditory task than the passive condition.

In the visual task, MMN responses to upward deviants were largely attenuated. In response to downward deviants, MMN was somewhat more present for the tinnitus group compared to upward deviant, while for the control group MMN was abolished even compared to upward deviant.

The oddball paradigm in a previous MMN attention study mentioned in the introduction was similar to the current study [251]. The tasks in which participants focused on the stimuli or passively listened showed the largest MMN amplitudes, while the smallest amplitudes were in

the task where they ignored the stimuli. A difference between the previous and the current studies was that previously, all paradigms elicited significant MMNs while this was not the case in the current study. It is possible that duration deviants elicited somewhat stronger responses than the intensity changes in the current study, or even that the overall context of their study included word and sentence paradigms within the same session, all of which were used to elicit MMNs, and that may have changed the waveform shapes somewhat. A potential explanatory factor in the diminished responses, particularly to the visual task, could be stress or arousal, as this task likely involved higher cognitive load due to involvement of different modalities, For example, in a previous study, cortisol level was inversely related to MMN amplitudes in response to duration deviants, and psychosocial stressors overall attenuated processing of change detection [265].

Similarly to the Chapter 4 and 5 of this thesis, there was an inverse relationship between deviant P50s and MMN in all groups in response to all conditions [243]. This was particularly present in responses to the upward deviants, where during the visual task, P50s were the highest compared to the other two tasks, and MMNs were abolished. This relationship was still present in response to downward deviants, but it was somewhat less apparent, possibly due to the overall lower amplitude in response to the quieter tones. However, it may be interesting to further investigate this relationship.

Notably, the present results (whilst all at one frequency) did not resemble the single frequency results in Chapter 5 (for example, in the pattern of IMA in controls or the tinnitus group, or in the 'extreme habituation': in the presence or absence of an N100 in the current study compared to the prolonged P50 in the previous study), and more resembled the two-frequency paradigms. The attentional demands/switching with a single frequency paradigm seemingly creates similar results to the narrowly spaced frequencies in the neutral task (and extreme repetition positivity seems to be prevented either by attention, or by the breaks or interactions with the researcher between blocks). The similarities are between previous small difference paradigm and the current neutral task paradigm are particularly present in P50s and MMNs. Therefore, perhaps attention or interfering frequencies act via a common mechanism.

### *6.3.2 Paradigm recommendations*

Overall, auditory attention increased P50 responses to downward deviants, facilitated N100 formation to low intensity standards, facilitated P200 formation overall, increased upward deviant MMNs as an effect of standard P200 rather than deviant waveform, and increased N100 and MMN responses to upward deviants. In tinnitus, auditory attention exaggerated

P200 formation, exaggerated MMN enhancement to upward intensity deviants, and enhanced MMN to downward intensity deviants. It was shown that when paying attention to auditory stimuli, participants with tinnitus (though more likely this was due to hyperacusis) were particularly more responsive than controls to a change in the stimulus in the upward intensity direction. This finding was similar to the T+H+ group in Chapter 4. It may be useful to investigate whether there would be stronger difference between the groups in response to the downward deviant condition in a tinnitus sample without hyperacusis.

Using the auditory attention paradigm may be advantageous in MMN studies on tinnitus/hyperacusis because the observed differences would be particularly large. Similarly, this task would be useful in investigating sensory gating or habituation processes due to its effect on P50, N100 and P200 amplitudes. However, to avoid both boredom/fatigue and extreme repetition positivity, the length of the experiment would need to be shortened, or some breaks would need to be implemented. The extreme repetition positivity affects the neutral task as well, but only in the single-frequency paradigm as seen in Chapter 5, so the frequencies involved may also need to be taken into consideration to achieve optimal outcomes.

Passive listening (in the neutral task) gave overall similar response profiles, though often much weaker, to auditory attention, meaning that one can generally compare the results of attended and passive studies meaningfully. Therefore, results of the previous IMA studies within this thesis as well as the original study, in which passive attention task was utilised, were unlikely to be due to attentional differences in tinnitus and/or hyperacusis, because attention to auditory stimuli in the present study exaggerated differences between tinnitus and control groups rather than causing or abolishing them. The neutral task elicited most similar standards across the two groups at P50 and N100 ERPs, while the auditory condition showed the strongest responses on average. Therefore, responses to neutral task would probably be the most representative for later components such as P200 or MMN, however they will not be as strong as in the auditory attention task. For particularly long paradigms, or those that cannot include breaks, this may be the more optimal paradigm, as it would show similar, albeit less striking results compared to the auditory paradigm and would not be as potentially taxing for participants.

Visual attention largely had the opposite effects to auditory attention: abolishing N100, especially to high intensity standards and deviants, and MMN. However, in some limited instances in tinnitus subjects it had similar effects to auditory attention (increasing P50 to high

standard stimuli, and increasing N100 and MMN to downward deviants), and these changes may be more indicative of state of arousal/stress/readiness/task-engagement than sensory attention per-se. As such, this paradigm may be used to study the distress-related symptoms or responsiveness to stimuli in tinnitus or possibly hyperacusis.

### *6.3.3 Conclusions*

Overall, the results of this study could occur due to alterations of central gain, such as enhancement with directed attention and inhibition with an interfering task [252]. Within the Free Energy formulation, attentional gain modulation works through top-down modulation of sensory precision, which is controlled by post-synaptic gain [249]. Attention acts by increasing post-synaptic gain/precision, to the point where they can be considered interchangeable. There might have been some differences in adaptation mechanisms between groups, including altered dynamic range adaptation and sensory gating in the tinnitus participants, which was affected both by task and intensity conditions. Dynamic range adaptation of neural firing controls the excitability of the neurons to a desired rate, and therefore improving efficiency of neural coding of intensity (both increasing and suppressing activity in response to mean sound level in the environment) [30]. A reduced dynamic range leads to increased sensitivity to sound intensity, which has been found in people with tinnitus/hyperacusis [266]. While participants could adjust the loudness of the sounds they listened to, they may have continued to be more responsive to the presence of the sounds, as indicated through less sensory gating. In that case, the comparisons made by the error units in participants with tinnitus would not follow the patterns of controls. The results seen in this study could be due to the different weighting participants with tinnitus assign to prediction errors (based on previous chapters within this thesis), as well as different functioning of sensory gain and inhibitory interneurons. As such, researchers should consider attentional modulation in the design of future studies, depending on the primary focus of experiments.

# Chapter 7. Overall discussion

## 7.1 Sound therapy conclusion

A study was carried out to assess the effectiveness of a novel covariance-cancelling sound therapy. These sounds were designed to reduce synchronous activity between neurons that are responsible for frequencies around the tinnitus frequency of an individual. Daily listening of around 60 minutes for 6 weeks lead to a reduction in perceived tinnitus loudness in the active sound group but not the sham sound group. This effect in the active group continued even three weeks after the participants stopped listening to the sounds. The tinnitus assessments were done online through a process of approximating the nearest tinnitus frequency. This required no direct input from the researchers. There were no face-to-face visits that usually take place as part of sound therapy for tinnitus.

While the findings were very promising, there are many potential next steps that can be taken in order to optimise the sounds themselves as well as the delivery system, in terms of optimising and automating a platform from which tinnitus patients can access these sounds. Some of the next steps may include estimating the timeframe in which the sounds were effective, e.g., does it work immediately after listening even on the first day, is it the cumulative effect of listening over a few days/weeks, is there a point at which participant stops getting benefits from listening, how long would be the optimal amount of listening/should it be done in one block or several small blocks throughout the day? Furthermore, stimulus properties could be further adjusted for optimal benefit, and the parameters used so far are just a starting point. The modulation can then be applied to existing music that participants enjoy. Finally, we would like to build an accessible platform for sound to be delivered to any tinnitus sufferer once the modulation is optimised.

## 7.2 Summary of EEG findings

This project started with an experiment in which an attempt to replicate the original IMA findings using a similar paradigm, and optimise the paradigm for identifying individuals with tinnitus and/or hyperacusis. However, as the one significant difference in design from the original study, I included 1 kHz as the second frequency used [95, 179]. The results did not follow the expected pattern in controls and did not show the strength of findings in the original study. Based on these unexpected findings, I changed direction from the original plan, and sought to focus on explaining these different results patterns, and in the process to better understand what the paradigm was revealing about brain responses to intensity changes.

As well as understanding caveats and potential confounds introduced by differences in the paradigm, I was also keen to focus on the other major confounds frequently overlooked in tinnitus research, namely hyperacusis and attention.

My next EEG study investigated differences in intensity deviant responses between parametrically controlled combinations of the presence (T+) or absence (T-) of tinnitus and/or hyperacusis (H+/H-) (determined by the Hyperacusis Questionnaire scores). In response to repetitive stimuli (standard), the presence of tinnitus reversed the effects of hyperacusis on P50 amplitudes, but by the timeframe of P200, the effect of hyperacusis was seen in both H+ (both with and without tinnitus) groups in terms of a much larger P200 amplitude compared to either of the H- (with or without tinnitus) group. The main finding within the tinnitus frequency was that the T+H- group had increased MMN amplitude to downward deviants, but both H+ groups showed increased MMN responses only to upward deviants. The controls had similar responses to the results in Chapter 2 of this thesis, where the same frequencies were used in the paradigm, but had the opposite pattern to the control group responses in the original study. This led to a hypothesis that the discrepant results were due to differences in the experimental paradigm: specifically, whether the two frequencies used were closely or widely spaced. Therefore, the next step was to understand the MMN patterns to intensity deviants in a healthy group of participants with no tinnitus and no hyperacusis in paradigms where either similar frequencies or distant frequencies were present in different blocks.

I used 6 kHz as an average tinnitus-like frequency, which was a similar frequency to average tinnitus in all three previous IMA studies. A striking difference in patterns was seen between the small frequency difference group compared to both single frequency and larger difference groups. The small frequency difference group showed a similar pattern to the control group in the original study (larger MMN to downward intensity deviants), corroborating the thought that two similar frequencies being present create some sort of interference that reverses the amplitudes of MMNs to downward versus upward deviants. Conversely, the single frequency and widely spaced frequencies yielded similar results to my two previous EEG studies, with larger responses to upward than downward intensity deviants. These findings reconciled the results from all previous studies, showing both the reproducibility of the findings, and also the implications of different choices of paradigm depending on the type of intensity deviant response abnormality one is trying to highlight. However, there still remained the question of possible attentional influences on MMN amplitudes in the two groups, including whether attention might mediate the differences between tinnitus, hyperacusis and control group

responses, and/or between the results of the different frequency context versions of the paradigm.

Therefore, in Chapter 6 of this project I studied effects of passive listening compared to auditory attention and to visual attention on the EEG responses. The findings from the passive listening were consistent with the previous study, but also, I found that the auditory attention task enhanced the responses in the tinnitus group, but without qualitatively changing the profile of MMN responses to intensity deviants. This was a helpful finding because MMN literature in tinnitus has used both auditory attention tasks and passive attention tasks in the past, and the results of these studies can be meaningfully compared. Earlier evoked responses differed more in their patterns depending on attention, and the more nuanced implications for studies focusing on these are discussed in the respective chapter.

### *7.2.1 Main interpretations*

Hyperacusis with versus without tinnitus differs more in its early responses (P50) to stable stimulation but show more similar responses to intensity changes. It has been shown that stimulus-specific adaptation is affected in autism, migraine and other conditions that are often comorbid with hyperacusis, possibly due to excitation-inhibition imbalance [267]. Adaptation is the process of decreased neural responses across repetitive stimulation, whilst habituation is more closely related to learning processes [268]. This heightened responsiveness early on to auditory stimuli may explain the particularly high amplitudes/overall presence of P200 in the hyperacusis group that was seen in Chapter 4. In hyperacusis without tinnitus, it is possible that lack of adaptation is a more fundamental underlying mechanism than the central gain that is thought to underlie tinnitus-related hyperacusis; this might cause heightened responses to the early components such as P50 and N100. Despite the differences in early components, similar heightened MMN amplitudes in response to upward deviants were seen in the T+H+ group compared to T-H+ group, perhaps highlighting aspects of the neural correlates of hyperacusis that are common across different aetiologies. Similar patterns to the T+H+ group were seen in tinnitus groups in other chapters where the tinnitus groups contained mostly participants who also had hyperacusis. Therefore, it is possible that tinnitus presence in hyperacusis either counteracts these early differences in adaptation, or hyperacusis with versus without tinnitus have separate underlying mechanisms. In the latter case, tinnitus with hyperacusis may show less altered adaptation, but the amplified MMN response still happens due to increased central gain. A similar heightened response was also seen in controls during the auditory attention task in Chapter 6. Therefore, it is possible that we saw hyperacusis-

mediated pervasive auditory stimulus–driven attention enhancement even in the passive tasks. This, however, was not present in tinnitus without hyperacusis.

The main finding seen in participants with tinnitus but no hyperacusis was the stronger MMN amplitude in response to downward deviants. T+H- group reflected the closest thing to a 'pure tinnitus' measure obtained. This was not due to attentional influences as I did not see a similar outcome in Chapter 6. However, a similar result was seen in controls in Chapter 5 during the small frequency difference paradigm, pointing towards a specific interference effect (possibly reflecting contrast gain theory mechanisms); in controls, the interference was due to alternating between two nearby stimulus frequencies, whereas in tinnitus similar interference could have occurred due to alternation between (or simultaneous perception of) the tinnitus itself and the presented tones. In either instance, I posit that high levels of adaptation or habituation to a single frequency selectively attenuate downward intensity deviants and facilitate upward ones, whilst the interference between close frequencies limits this adaptation/habituation. It remains to be determined whether the altered pattern of results is an epiphenomenon of tinnitus, or whether it might hold insights into key underlying tinnitus mechanisms.

Finally, a potential reason why the larger response to downward deviants was not seen in the T+H+ group is that the effects of tinnitus are counteracted by the stronger effects of hyperacusis, similarly to what happens to effects of hyperacusis earlier on in the response waveform.

In the present project, participants were able to personally set the stimulus intensities subjectively based on individual preference/tolerance. The potential benefits of this approach (compared to a fixed sound presentation level or sensation level) is that it perhaps adjusts for differences in individual sound level tolerance, and therefore it would be expected that the results would be less influenced by differences in central gain. As such, it is interesting that I still saw differences related to hyperacusis, even when the subjects were likely to have lowered their sound presentation levels to compensate for their hyperacusis. However, in instances where there were smaller responses in hyperacusis this could be one potential explanation. In future studies including hyperacusis, it may be useful to establish a subjective 'discomfort level' of the stimuli and perhaps aim to keep these levels similar across participants, while keeping note of average dB SL levels in each group.

A potential limitation of the current project is that all EEG studies were not longitudinal. It may help to understand how the differences in sound processing develop over time in

tinnitus/or hyperacusis from the onset, as this could shine light on the development and divergence of the alterations in different mechanisms related to each condition/combination of the two conditions.

### *7.2.2 Future directions*

An interesting future direction would be to test the extent to which intensity deviant response changes associated with tinnitus generalise across frequencies remote from tinnitus. For example, participants with tinnitus could be tested using 1 kHz frequency only versus tinnitus edge frequency only, to see whether their usual pattern would be diminished with 1 kHz if they were no longer hearing a tinnitus-like sound that unintentionally created the "small frequency difference" situation. Some slight changes would need to be made to the paradigm to avoid any extreme habituation effects, though based on findings from Chapter 5 and the above hypotheses, this effect should not occur.

Further studies and/or analyses are needed to better characterise mechanistic differences in intensity deviant responses between hyperacusis only and hyperacusis with tinnitus, as well as tinnitus only groups, both through source localisation methods and possibly through time-resolved decoding methods that would allow to disentangle time courses of neural processes, such as Multivariate Pattern Analysis [269]. The latter might also allow optimised classification of tinnitus and/or hyperacusis status of individuals, which would be particularly beneficial as a biomarker for animal studies.

It may also be interesting to study change detection of more naturalistic sounds or with certain cues, as this would shine light on the more everyday life processing of sound and whether this is affected in people with tinnitus. In hyperacusis, of course, sound processing is clearly affected in everyday life, but it may be helpful to understand effects of e.g. in the expectation of the uncomfortable sounds versus not expecting any discomfort in the near future. For example, in healthy participants, piano sounds with clear harmonicity produced larger MMNs than inharmonic sounds in response to multiple deviant types [270, 271]. Visual cues and auditory emotional cues (happy versus sad music) can also alter responses; visual cues could alter responses even to standard tones when a misleading cue had been presented beforehand [272, 273]. Studying differences in cue-based auditory predictions, and their violations, could add a whole additional layer of understanding to our knowledge about differences in sensory processing associated with tinnitus and hyperacusis.

#### *7.2.2.1 Different generators of ERPs*

N100, MMN and Repetition Positivity (RP) have all been found to have different generators [251]. For example, it has been shown that MMN elicited by a supra-temporal component (mastoids) was significantly altered by attentional focus [251]. Some research has suggested that the supra-temporal MMN occurs earlier and sends a signal to the frontal generator, and process abstract pitch-relation features of the stimuli [274, 275]. Additionally, different RP generators may process stimuli based on multiple timescales, meaning that adaptation also occurs on multiple timescales [276]. While only a roving paradigm was able to elicit RP, the mastoids showed RP in both types of paradigm in an auditory deviant EEG experiment, while MMN was seen in both conditions at either generator site [228]. The authors suggested that short term stimulus history was represented by the auditory cortex generator (completely restarting its generation after a deviant stimulus), while the frontal generator activity was based on more longer stimulus history [228]. As hyperacusis may be related to reduced adaptation, we may see differences in short-term as well as long-term RP resets compared to tinnitus only and control groups, while it may take participants with tinnitus only longer to reach a certain RP amplitude due to the altered habitation mechanism.

### *7.2.3 Conclusion*

Based on the EEG studies included in the present project, paradigm choice is essential in exposing the differences between both tinnitus and hyperacusis compared to controls, especially in light of their different and partially opposite response profiles. Both auditory attention and passive attention elicit similar patterns in auditory change responses, but active auditory attention may yield stronger findings, particularly in MMN responses. These observations may be important in finding an individual-level diagnostic tool for tinnitus (and possibly hyperacusis), and may eventually serve as a bridge between human and animal biomarkers of the two conditions. Not only is it important to study patient groups, but it is also necessary to understand how any particular paradigm may affect the responses in the control group, in order to make any meaningful conclusions.

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