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Abstract

Despite tinnitus being too common and present (prevalence: 13%, remission rate: 17.1%), it is still unclear as to why tinnitus is generated and why it persists, thus making it challenging to come up with definitive treatments. For better understanding of tinnitus mechanism, it is warranted to analyse, how the tinnitus transits from its initial onset or acute stages (duration of tinnitus less than 4 weeks) until its chronic stage (duration of tinnitus greater than 6 months). We were motivated to carry out a tinnitus study with an aim to unravel the neurobiological basis of tinnitus by studying its initial onset until its subsequent chronification. We hypothesized that the neural processes such as synchrony and gain (auditory hypersensitivity) linked to tinnitus will be maximal around the onset and reduces over time as a regression to the mean. The study involved individuals with Acute Tinnitus, who were monitored longitudinally for six months post-onset, alongside individuals with Chronic Tinnitus and a Control group matched to the Acute Tinnitus cohort. Our results culminating multiple measures (subjective and objective) of tinnitus reveal that the neural processes linked solely to the tinnitus activity were maximal around the time of onset and reduced over time which is in line with our hypothesis. We further established that measures of generalized auditory sensitivity (gain) do not significantly differ between the groups. Increased dynamic range adaptation, were observed in the Acute Tinnitus group, but not in the Chronic Tinnitus or Control groups thus indicating that tinnitus is an outcome of excess auditory hyperactivity as an invariance (mitigator) to central gain through properties of increased dynamic range adaptation that tends to persist through modes of sustained attentional networks. This would explain why tinnitus generates and persists as it seeks to modulate excessive hypersensitivity within the auditory system.

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Tables and Figures

Glossary of Abbreviations & Units:

ABR - Auditory Brainstem Response

ACC - Anterior Cingulate Cortex

ADR - Amplitude Dynamic Range

AM - Amplitude Modulation

AMPA- α-Amino-3-Hydroxy-5-Methyl-4-isoxazole Propionic Acid

ANCOVA - Analysis of Covariance

ASSR - Auditory Steady State Response

CLS - Categorical Loudness Scaling

DAN Dorsal Attention Network

dB - Decibel

dB HL – Hearing Level

dBSL – Decibel Sensation Level

dB SPL – Sound Pressure Level

DCN – Dorsal Cochlear Nucleus

DMN - Default Mode Network

DRA - Dynamic Range Adaptation

EEG – Electroencephalogram

EEG - electroencephalography

ERP – Event-Related Potential

fMRI – functional Magnetic Resonance Imaging

FFT - Fast Fourier Transform

GABA - Gamma-aminobutyric acid

HCN - Hyperpolarization-activated Nucleotide-gated

HFD - High Frequency Deviant

HPA - Hypothalamic-Pituitary-Adrenal

HQ - Hyperacusis Questionnaire

Hz - Hertz

IC – Inferior Colliculus

ICA- Independent Component Analysis

IDAEP - Intensity Dependence of the Auditory Evoked Potential

IHC – Inner Hair cells

IHS - Inventory of Hyperacusis Symptoms

kHz - Kilohertz

LAER - Late Auditory Evoked Responses

LDAEP - Loudness Dependence of the Auditory Evoked Potential

LFD - Low Frequency Deviant

M- Mean

MANCOVA- Multivariate Analysis of Covariance

MANOVA - Multivariate Analysis of Variance

MD - Median

MML - Minimum Masking Level

MMN – Mismatch Negativity

MRS- Magnetic Resonance Spectroscopy

ms – Milliseconds

μV – Microvolts

N - Number of Samples

NMDA – N-methyl-D-aspartate

NS - Novel Stimuli

OHC - Outer Hair Cells

PHG - Parahippocampal Gyrus

PTA - Pure Tone Audiometry

RM-MANOVA - Repeated Measures Multivariate Analysis of Variance

SD – Standard Deviation

SDR - Stimulus Dynamic Range

sLORETA - standardized Low Resolution Electromagnetic Tomography

SPSS - Statistical Package for Social Science

SS - Standard Stimuli

SSRIs - selective serotonergic reuptake inhibitors

TFI - Tinnitus Functional Index

THI - Tinnitus Handicap Inventory

ULL - Uncomfortable Loudness Level

Chapter 1 Introduction

1.1 Tinnitus Epidemiology

Tinnitus is characterised as a phantom auditory experience, wherein sound is perceived without any accompanying acoustic or mechanical stimuli in the cochlea. Tinnitus is a prevalent and uncomfortable otologic condition that induces many physiological and psychological illnesses, adversely affecting quality of life (Han et al., 2009). The prevalence of tinnitus is estimated to be around 14.4% globally and, in the UK, it is estimated that 1 in 7 individuals are affected from the condition totalling around 7 million individuals (Jarach et al., 2022). Despite its high prevalence, the underlying mechanisms that lead to the onset and persistence of tinnitus remains poorly understood. It is still unclear why tinnitus emerges in some individuals and not others, and why, in many cases, it continues over time—even in the absence of any identifiable external sound or effective treatment. This lack of clarity poses significant challenges in developing targeted and lasting therapeutic approaches (Chung & Lee, 2016). Recent studies have further highlighted that prevalence of tinnitus does not differ by sex but differs by age with a higher prevalence in the older aged individuals (23.7%) when compared to middle age (13.7%) and young age (9.7%) (Jarach et al., 2024). Another recent study further indicated that out of the 26.7% of adults that experience tinnitus, 20% of them report it to be bothersome (Perez-Carpena et al., 2024). Tinnitus also exceeds other medical conditions like diabetes (8.5% prevalence) and cardiovascular disorders (7.69% prevalence) and despite this high prevalence, there is currently no definitive treatment for tinnitus warranting the need for enhanced research in this area (McFerran et al., 2019; Glovaci et al., 2019).

1.2 Tinnitus characteristics

Tinnitus is typically classified into two categories: objective and subjective. Objective tinnitus refers to a rare type of tinnitus that can be heard by both the affected individuals and examiner as a sound originating from the structures surrounding the ear canal, while subjective tinnitus is perceptible solely to the patient and is typically regarded as lacking an acoustic cause that is generated peripherally or centrally (Han et al., 2009). Subjective tinnitus can be categorised as pulsatile tinnitus and non-pulsatile tinnitus, with non-pulsatile

tinnitus further divided into continuous tinnitus and intermittent tinnitus (Chung & Lee, 2016; Sonmez et al., 2007). For the purposes of this study, we will concentrate exclusively on continuous non-pulsatile tinnitus, which is crucial to understanding the mechanisms of tinnitus and the absence of effective treatments.

Based on the level of distress, tinnitus can also be classified as bothersome and non-bothersome tinnitus where intensity of tinnitus if present below 20 dBSL is classed non bothersome and tinnitus intensity above 20 dBSL is classified bothersome (Makar et al., 2014; Seimetz et al., 2016; Esmaili & Renton, 2018). Tinnitus can also be present with or without hyperacusis (abnormal sensitivity to everyday sounds) (Hofmeier et al., 2021). Tinnitus is usually characterised by one or more predominant pitches typically exceeding 3 kHz, often manifesting as either tonal or narrow band noise (Han et al., 2009). Tinnitus individuals may face symptoms beyond the acoustic characteristics that mostly include symptoms linked to distress such as stress, anxiety, insomnia, and difficulties during situations like speech perception in noisy backgrounds (Han et al., 2009). These symptoms, however, tend to be maximum during the period following onset and reduces over time due to habituation of the tinnitus for a majority of individuals (Wallhäusser-Franke et al., 2017). However, when tinnitus persists over an extended period, it can become increasingly distressing and, in some cases, debilitating, significantly impacting an individual's quality of life (Han et al., 2009).

1.3 Tinnitus Generation and Persistence Mechanisms/Models

The mechanism of tinnitus, including its origin, propagation, and changes, can be explained by the prevalent models proposed by various authors. The models can be succinctly categorised as peripheral models and central models of tinnitus with all current popular models pertaining to central tinnitus. The peripheral models encompass those associated with tinnitus linked to the cochlea and auditory nerve, whereas the central models pertain to the pathways implicated in tinnitus beyond the auditory nerve (Baguley, 2002).

1.3.1 Peripheral Models for Tinnitus

Models at the cochlear level have been linked to the onset of tinnitus. Jastreboff (1990) introduced the discordant damage theory of the cochlea where they stated that during cochlea damage post hearing loss, there is an imbalance in dysfunction between the Outer Hair Cells (OHCs) and Inner Hair Cells (IHCs) where damage to these hair cells especially the OHCs

would result in reduced input levels (as OHCs are essential for cochlea amplification) that would further lead to hyperactivity of the auditory neurons. Further models (such as the biochemical model) theorizes that cochlear damage would lead to an abnormal release of neurotransmitters like glutamate that would further result in aberrant phenomena such as glutamate excitotoxicity, increased oxidative stress, calcium dysregulation, and inhibitory dysfunction that would facilitate hyperactivity in the peripheral auditory system leading towards the persistence of tinnitus (Sahley & Nodar, 2001). (Møsller, 1984) further emphasised the potential existence of cross talk among the neurones of the VIII cranial nerve, which may lead to the synchronisation effect resulting in tinnitus. The medial efferent system's role in the peripheral model is significant; following damage to hair cells, the ascending input is reduced, which further reduces the efferent system's functionality, particularly its inhibitory capacity to mitigate noise, consequently leading to heightened activity and the onset of tinnitus (Veuillet et al., 1991). Over the past decade, discussions regarding cochlear synaptopathy/hidden hearing loss, and tinnitus have emerged. Although ribbon synapse loss is not detectable through audiometric testing, it may lead to heightened neural gain due to diminished input to the auditory system, potentially exacerbating tinnitus activity and potentially leading to hyperacusis (Liberman et al., 2016). They have further emphasized that cochlear synaptopathy is primarily associated with damage to high-threshold auditory nerve fibers, a specific subset of Type I spiral ganglion neurons. These fibers are less sensitive to soft sounds and typically respond to higher-intensity stimuli. Although they do not contribute significantly to hearing in quiet environments, they play a critical role in hearing in noisy conditions, sound level discrimination, and dynamic range encoding.

Overall, the peripheral models highlight that damage to the hair cells would lead to increased spontaneous firing in the auditory nerve or beyond, potentially leading to the perception of tinnitus. Supporting the mentioned peripheral models, studies highlight that the sectioning of the VIII cranial nerve can, in certain cases, help reduce or improve the percept the tinnitus (Barrs & Brackmann, 1984).

1.3.2 Central Models for Tinnitus

Increased neural activity

Eggermont (1984) highlighted that the increased spontaneous activity at the level of auditory nerve is highly unlikely to cause tinnitus, as mostly damage to the hair cells is more likely to cause reduced activity at the auditory nerve fibres rather than increased activity. The increased activity is likely to be more at the level of dorsal cochlear nucleus as recorded by animal studies which is more likely to cause the percept of tinnitus rather than at the previous level (level below the cochlear nucleus such as auditory nerve and cochlea) (Baguley, 2002). Increased synchrony and bursting have been positively correlated with the behavioural presence of tinnitus at the level of dorsal cochlear nucleus in animal studies with one study reporting increased spontaneous firing rate at the fusiform cells of the dorsal cochlear nucleus in the animal with tinnitus (Wu et al., 2016). Additional observations indicate heightened spontaneous activity in the dorsal cochlear nucleus following hair cell loss, suggesting that this nucleus may play a role in the initiation of tinnitus (Kaltenbach, 2007; Kaltenbach & McCaslin, 1996). Other theories highlight the possibility of the generation and persistence of tinnitus to be around the inferior colliculus and medial geniculate body with increased spontaneous activity seen at both these regions (Berger & Coomber, 2015). Noreña & Farley (2013) primarily focused on the mechanisms related to tinnitus generation linked to the central nervous system. Their model challenge theories that posit the cochlea as the primary origin of tinnitus, citing evidence such as the persistence of tinnitus following auditory nerve section, which suggests that peripheral mechanisms alone cannot fully account for the condition (Bell et al., 2016). They propose that sensory deprivation due to hearing loss causes changes in the central auditory system that are linked to tinnitus and the hearing loss may or may not be audiometrically detectable. The article further highlights that the changes seen in the central auditory system is increased spontaneous firing and neural synchrony that initiates the onset of tinnitus. With respect to the persistence of tinnitus, the authors emphasize that while changes in the auditory system may generate tinnitus, it is activity beyond the auditory network that facilitates its persistence. They highlight the importance of *functional coupling (interaction or synchronization between regions)*, where changes in this coupling, particularly in terms of *temporal coherence* (how synchronized neural activity is across different areas), can affect how neural activity linked to tinnitus is transmitted and processed. The authors further emphasize the role of cortical reorganization in the persistence of tinnitus along with abnormal functional connectivity with various networks. These neural correlates over time rely less on the peripheral input further resulting in centralization (Noreña & Farley, 2013).

This has been further voiced by Chung & Lee (2016) where they state that damage to outer hair cells in the cochlea, often due to noise exposure or aging, can disrupt normal sound processing and increase spontaneous firing rates in the auditory nerve and central auditory pathways. This can further lead to abnormalities in the areas like the dorsal cochlear nucleus, inferior colliculus, and auditory cortex where there is abnormal neural synchrony that contributes to the perception of tinnitus. The increased spontaneous firing activity highlighted by the authors mentioned above that contributes to the onset of tinnitus may also further result in maladaptive changes across the auditory pathway from the level of cochlear nucleus until the auditory cortex that facilitates the persistence of tinnitus (Shore et al., 2016). Other mechanisms relating to the persistence of tinnitus as highlighted by Haider et al. (2018) involve the interplay between the peripheral auditory structures, central auditory structures, and even the non-auditory regions where the initial trigger maybe a peripheral auditory event like hearing loss, but the persistence is attributed to the changes in the central auditory system. These changes include increased spontaneous neural activity, altered tonotopic maps, and increased neural synchrony. The current model highlights that the increased spontaneous activity resulting in tinnitus is present beyond the auditory nerve. A significant proportion of the evidence for altered neural activity in tinnitus arises from animal models, which presents inherent limitations in cross-species extrapolation and may not fully capture the complexity of human tinnitus perception.

Central gain theory

The central gain model synthesizes multiple theories to account for the elevated neural activity that follows peripheral auditory damage. It suggests that compensatory gain enhancement is shaped by both temporal and spectral aspects of auditory input and unfolds in three primary stages: a reduction in inhibitory synaptic input, a homeostatic increase in excitatory drive to maintain stable average firing rates, and modifications in the intrinsic excitability of neurons. These neuroplastic changes are not confined to the auditory pathway alone; rather, they extend to broader neural circuits, including limbic structures involved in emotion and arousal (Auerbach et al., 2014). A review by Gu et al. (2010) stated that elevated activation in the primary auditory cortex is specifically related to tinnitus, while elevated activation across multiple auditory regions, including the auditory midbrain, thalamus, and primary auditory cortex, is associated with hyperacusis. This suggests a more localized

cortical effect for tinnitus and a more widespread effect across the auditory pathway for hyperacusis. The authors hypothesize that the tinnitus-related activation in the cortex may be due to increased attention drawn to the auditory domain which amplifies evoked responses and elevates spontaneous activity at rest. The central gain theory is an integration of previous literature related to increased neural activity following hearing loss that contributes to tinnitus. Moreira et al. (2024) explored the contribution of central gain enhancement to tinnitus by examining auditory brainstem responses (ABRs) in young adults with chronic tinnitus compared to matched controls. By analysing the amplitudes and interwave ratios of ABR components, the study found significantly elevated wave III/I and V/I ratios in the tinnitus group. Their findings indicate increased neural activity within brainstem auditory pathways, likely reflecting compensatory central gain mechanisms in response to diminished cochlear input. The results support the central gain hypothesis of tinnitus and suggest that ABR metrics may serve as objective biomarkers for evaluating tinnitus severity and monitoring treatment outcomes. Further Knipper et al. (2013) in their review suggested that tinnitus and hyperacusis, which are both frequently associated with hearing loss, arise from different brain responses to varying degrees of deafferentation. While tinnitus may result from the brain's inability to adapt to reduced peripheral input, hyperacusis may stem from an excessive increase in response gain in subcortical brain regions. These differing responses are potentially linked to the extent of deafferentation, with larger extents triggering tinnitus and lesser extents linked to hyperacusis. The authors also further hight the influence of factors like stress at the time of acoustic trauma suggesting a role in cochlear vulnerability that results in subsequent development of tinnitus/hyperacusis.

Central Noise/ Central Variance theory

Zeng (2013) and Zeng (2020) explore the roles of mechanisms like central gain, central noise, and central variance that distinguishes both tinnitus and hyperacusis. An active loudness model by Zeng (2013) presents a unified model to explain tinnitus and hyperacusis through altered central gain mechanisms, particularly in response to peripheral auditory deficits. In this framework, tinnitus arises from an increase in central neural noise. Following hearing loss, the reduced peripheral input prompts the central auditory system to compensate by amplifying spontaneous neural activity, which is then perceived as phantom sound. This model suggests that tinnitus results from an additive increase in central noise—a form of

central gain enhancement in the absence of external sound stimuli. In contrast, hyperacusis is explained as a multiplicative increase in central gain, where the central auditory system, in response to reduced input, amplifies not only spontaneous activity but also external auditory stimuli. This enhanced gain leads to an exaggerated perception of sound, making normal auditory inputs feel uncomfortably loud. Thus, while both tinnitus and hyperacusis arise from central gain modifications, they differ in their perceptual consequences: tinnitus is the result of heightened internal neural noise, whereas hyperacusis is characterized by an over-amplification of external stimuli. Building upon this, Zeng (2020) introduced the central variance as another key factor for overall dynamic range stability. This work proposes that central variance, influenced by both internal noise and driven activity, can limit the effectiveness of central gain compensation for hearing loss. High central variance can exacerbate both tinnitus and hyperacusis, particularly when central gain is increased. The interplay of these three central mechanisms—gain, noise, and variance—provides a more nuanced understanding of the complex auditory phenomena of tinnitus and hyperacusis. However, these terminologies are more theoretical, and the exact details are not specified due to lack of empirical data. Figure 1.1 illustrates an active loudness growth model based on central gain and central noise for tinnitus and hyperacusis.

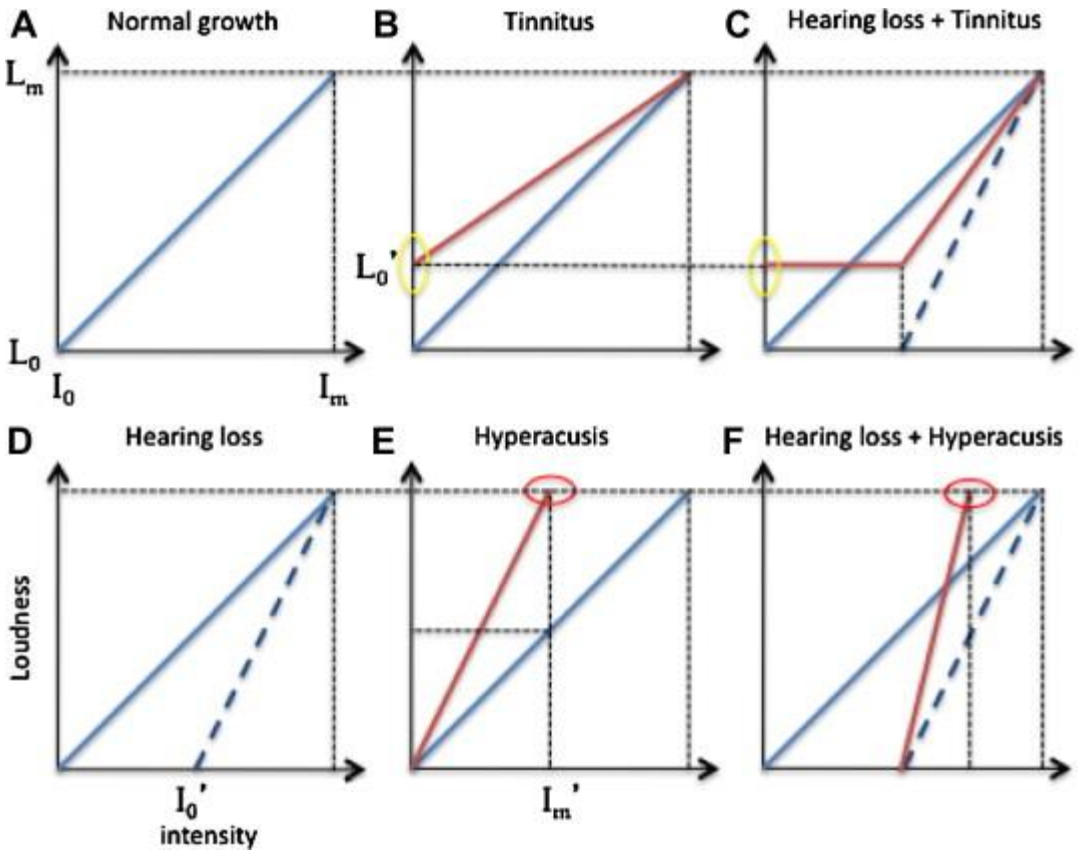

Figure 1.1. Active loudness growth model for tinnitus and hyperacusis based on central gain and central noise

The loudness increase function for normal hearing is represented by the solid diagonal line in panel A, whereas the dashed line in panel D illustrates the function for hearing loss, with I_0' indicating the higher threshold. The loudness function is altered by tinnitus (shown by a shallower line intersecting the y-axis in panel B) and associated hearing loss (seen in panel C). The loudness function is altered by hyperacusis (shown by a steeper line intersecting the dotted upper horizontal line in panel E, where I_m' represents the diminished sound level achieving maximal loudness) and associated hearing loss (panel F). The content in this figure has been reproduced from Zeng (2013).

Stochastic Resonance Model

The concept of stochastic resonance refers to nonlinear process in which the typically weak input information, such as a faint signal, can be amplified or enhanced through the presence of noise (Gammaitoni et al., 1998). Stochastic resonance, in the context of tinnitus, theorizes that a similar mechanism of an increased internal noise within the auditory system where

following hearing loss, there is an enhancement in the perception of weak signals but also the perception of phantom sounds (Krauss et al., 2016). The onset of hearing loss results in not only a reduction of input to the auditory system but also a reduction in the signal to noise ratio. The system compensates for both these alterations by increasing the internal noise and this phenomena of increasing internal noise is known as the adaptive stochastic resonance (Schilling et al., 2023). Krauss et al. (2016) further describes the increase or upregulation of this internal noise as a compensatory mechanism to the reduced input due to hearing loss that is controlled by stochastic resonance. The model further suggests that this increased noise, while benefiting for the detection of weak external sounds due reduced input, at a certain level it can also be perceived as a phantom percept (tinnitus). The stochastic resonance model explains the onset of tinnitus as a potential effect of the brain's ability to compensate for hearing loss by increasing internal noise (Krauss et al., 2016; Schilling et al., 2023). As evidence to this model, Tziridis et al. (2022) explored the potential of spectrally matched near-threshold noise to attenuate subjective tinnitus loudness through the mechanism of stochastic resonance. Twenty-four patients with tonal tinnitus and mild hearing loss were exposed to various spectrally filtered noises for 40-second intervals. The results revealed a significant reduction in tinnitus loudness in 21 of the 24 patients, with six individuals experiencing complete tinnitus suppression during stimulation. The most effective noise was a bandpass-filtered signal, centered approximately half an octave below the tinnitus pitch. These findings suggest that individualized near-threshold noise stimulation may offer a promising therapeutic approach for tinnitus management, with potential integration into hearing aids equipped with noise generators.

Thalamocortical Dysrhythmia

The thalamocortical loop comprises both the thalamus and cortex and is essential in carrying out functions related to relaying sensory information, attention, cognition, and the development of neuronal oscillations, including alpha, beta, and gamma oscillations. Damage to this loop will lead to aberrant neural firing that potentially results in hyperpolarization (Sherman, 2005; Buzsáki & Watson, 2012). Tinnitus is often associated with sensory deprivation resulting from peripheral hearing loss, which reduces the afferent input to central auditory structures. In response to this diminished input, homeostatic mechanisms within the auditory pathway may increase neural excitability to compensate as mentioned in the central

gain model. However, this compensatory mechanism can lead to hyperpolarization of thalamic relay neurons, which paradoxically facilitates pathological burst firing. Such aberrant firing patterns are thought to interfere with normal thalamocortical communication, leading to a disruption in the thalamocortical loop. As described in the model, this disruption manifests as a shift in thalamic oscillatory activity—specifically, an increase in low-frequency theta-band oscillations and a corresponding decrease in alpha-band activity (De Ridder et al., 2015). The heightened oscillations in the theta band may trigger high-frequency oscillations associated with tinnitus, leading to widespread synchronisation across several brain regions. This thalamocortical circuit may additionally contribute to the persistent awareness of tinnitus as part of a theorised global workspace model network that is discussed further below (De Ridder et al., 2015). Animal studies have been carried out supporting this model. A study by Vianney-Rodrigues et al. (2019) investigated the neural mechanisms underlying tinnitus using a rat model. They administered sodium salicylate (SS) to induce tinnitus and recorded local field potentials from the medial geniculate body (MGB) and primary auditory cortex (A1). The results showed that SS decreased theta, alpha, and beta oscillations and coherence in these bands, but increased gamma oscillations and coherence within and between the MGB and A1. Additionally, SS enhanced cross-frequency coupling between theta oscillations in the MGB and gamma oscillations in A1. These findings suggest that SS disrupts thalamocortical communication, leading to excessive cortical gamma activity, which is implicated in tinnitus. The study supports elements of both the thalamocortical dysrhythmia (TD) and synchronization by loss of inhibition (SLIM) models of tinnitus.

Dysfunctional noise cancelling system.

Tinnitus, as per Rauschecker et al. (2010), results from a complex interaction between the auditory and limbic systems. According to them, the tinnitus signal arises from a peripheral auditory injury and subsequent alterations in the central auditory system, whereas the limbic system, is essential in modulating the perception of this signal. The nucleus accumbens (part of the striatum) plays an essential role in efficiently modulating the tinnitus signal by inhibiting its transmission to the conscious percept and this is the mechanisms that healthy individuals with hearing loss and without tinnitus are inclined to possess (Rauschecker et al., 2010). When this nucleus accumbens is impaired by distress such as stress, mood disorders,

or other neuropsychological disorders, the filtering ability to inhibit the tinnitus fails facilitating the tinnitus signal to route towards the auditory cortex and be consciously perceived. Persistent perception may result in brain reorganisation, hence producing chronic tinnitus (Rauschecker et al., 2010). The dysfunctional noise cancellation model, as described in Song et al. (2015) , can be understood through two types of "gating" mechanisms that determine the perception of tinnitus:

Thalamic Gating: This first gate controls whether the tinnitus signal reaches the auditory cortex, determining whether tinnitus is experienced at all. This gate is in the auditory thalamic region, which performs the crucial function of relay station for auditory information and auditory processing. A dysfunction in this area such as reduced inhibition or increased gain would result in the aberrant neural activity that cause tinnitus to propagate towards the cortex (Brinkmann et al., 2021; Song et al., 2015). Brinkmann et al. (2021) further discusses the role of thalamocortical dysrhythmia in tinnitus, which could contribute to this dysfunctional gating. This study investigated the thalamocortical functional connectivity in chronic tinnitus patients using resting-state functional magnetic resonance imaging (fMRI). The researchers compared 31 chronic tinnitus patients with 33 healthy controls, employing a seed-based whole-brain correlation method to analyze thalamic connectivity. The results revealed decreased functional connectivity between the thalamus and several brain regions, including the middle temporal gyrus, orbitofrontal cortex, and precentral gyrus. Additionally, tinnitus distress and duration were negatively correlated with thalamic connectivity in specific regions. The study by Zhang et al. (2015) concluded that disrupted thalamocortical connectivity is associated with the neuropathological features of tinnitus, suggesting that these disturbances play a significant role in the condition's manifestation. Furthermore, the study highlighted that the reduced functional connectivity between the thalamus and auditory cortical areas may reflect disrupted thalamic gating, which is thought to regulate the flow of sensory information to the cortex. This impaired gating could contribute to the perception and severity of tinnitus.

Cortical Gating: The second gate determines whether the tinnitus signal, propagates to the auditory cortex, and enters a conscious awareness at a particular moment. The cortical gate involves the role of higher-order cortical areas, such as the limbic system, which plays an

essential function in regulation of emotional processing and attention Song et al. (2015). Rauschecker et al. (2010) further explores the interactions between the limbic and auditory regions in relation to tinnitus further suggesting that the limbic system can modulate the salience and increased awareness of the tinnitus percept. The rostral anterior cingulate cortex (rACC) as discussed by Song et al. (2015), plays a key role as a "noise cancelling mechanism" in this cortical gating that either amplifies or suppresses the tinnitus.

Therefore, the dysfunctional noise cancellation model can be conceptualized as a two-stage process: the thalamic gate determines the presence of tinnitus, while the cortical gate determines its moment-to-moment awareness. Dysfunction in either gate can lead to the persistent and bothersome perception of tinnitus. They study by Song et al. (2015) investigated 80 tinnitus patients using resting-state electroencephalography (EEG) to correlate tinnitus awareness percentage with cortical oscillatory activity and functional connectivity, focusing on the rostral anterior cingulate cortex (ACC) and its connectivity with the primary auditory cortex (A1). Significant negative correlations were found between tinnitus awareness percentage and the activity of the rostral ACC in various frequency bands (delta, theta, beta 1). Additionally, connectivity between the left A1 and rostral ACC, as well as the left A1 and subgenual ACC, showed negative correlations with tinnitus awareness. These findings suggest that the rostral ACC plays a crucial role in the noise cancellation system for tinnitus, and dysfunction in this area may lead to increased tinnitus perception.

Attention

With respect to the role of attention in the generation and persistence of tinnitus, Haider et al. (2018) attributed that the involvement of non-auditory regions, such as the limbic and autonomic nervous systems plays an essential role in the contributions towards attentional and emotional aspects of tinnitus that facilitates its persistence. Initially, the brain begins adapting to the initial trigger that generates tinnitus (continuous percept) creating a persistence percept of this ongoing continuous tinnitus. The adaptation mechanism involves the integration of complex neural networks linked to tinnitus and neurophysiological variations that further enable result tinnitus being multifaceted and a challenging condition to treat. (Roberts et al., 2013) argued that with respect to tinnitus, there is discrepancy that exists between the brain's predictions of auditory input (abnormal neural activity) and the reduced

auditory input transmitted to the brain by an impaired cochlea. In a typical auditory perception, such discrepancies facilitate different auditory attentional mechanisms and are reconciled as the brain constructs a more precise central picture of the auditory environment. In tinnitus, the discrepancy continues due to be an abnormal neuronal activity in the cortical areas impacted by hearing loss, which is not supported by sensory information from the impaired auditory system (Roberts et al., 2013). Auditory attention may persist and promote, via basal forebrain or other neuromodulatory pathways, types of neural plasticity that reinforce abnormal neural alterations associated with tinnitus persistence. De Ridder et al. (2015) discussed the global workspace model in the context of tinnitus. They propose that tinnitus isn't solely auditory phenomenon that is hyperactive but also involves a global workspace that is hyperactive. The global workspace model based on conscious cognitive processing was originally proposed by Baars (1993), suggests that multiple specialized brain modules process information unconsciously and in parallel. These modules then compete for access to a "global workspace," which allows for the broadcasting of information throughout the brain. The "winning" module gains access to this workspace, enabling its information to become consciously perceived. This model explains how diverse information sources are integrated into a unified, coherent conscious experience. De Ridder et al. (2015) adapted this model to tinnitus, suggesting that the tinnitus percept emerges from the competition between these modules and the subsequent access to the global workspace. They hypothesize that in tinnitus, the workspace linked to tinnitus might be hyperactive, contributing to the persistent and intrusive nature of the tinnitus percept. Some authors have also attributed the abnormality in the attentional networks like Default Mode Network (DMN) to contribute to the persistence of tinnitus. Schmidt et al. (2017) highlighted that DMN – Precuneus decoupling might be a potential biomarker for long term tinnitus and further highlighted that this abnormality of DMN or even the Dorsal Attention Network (DAN) is no different to the ones who have developed recent onset tinnitus when compared to controls, but the changes related to tinnitus are only visible to long term tinnitus, implying a specific role in tinnitus persistence as opposed to tinnitus onset. This was further highlighted by Hu et al. (2021) where in an fMRI study, there were major alterations in the DMN, attention system, and limbic system that may contribute to the persistence of tinnitus. Further, De Ridder et al. (2022) introduced the triple network model wherein individuals with acute tinnitus, the default mode network and the central executive network exhibit an anti-correlation. When

tinnitus becomes chronic, the anticorrelation between the two networks vanishes, and the triple network, which includes the salience network, leads to distress, embodiment, and functional impairment due to tinnitus. These connectivity patterns could serve as invariant markers of long-term tinnitus, highlighting differences between subgroups and potentially aiding in the development of diagnostic tools and interventions. The model by Sedley et al. (2016) further substantiate the attentional models by asserting that the increased prediction error resulting from deafferentation may be mitigated by the presence of tinnitus. According to them, the tinnitus is hyperactive in the initial stages and the percept must reduce over time (habituation) during its start to recalibrate perception; however, in chronic tinnitus, attention towards the condition intensifies with time, exacerbating its persistence and leading to the failure of perceptual recalibration. Predictive coding mechanism linked to tinnitus will be discussed below.

Psychological distress

Stress as a part of the onset mechanism to tinnitus

Another notion about the onset of Tinnitus is the potential influence of excessive distress that may induce Tinnitus. Mazurek et al. (2012) reviewed the interplay between stress and tinnitus, exploring both clinical observations and experimental findings. The authors highlight a linear relationship between the presence of tinnitus and the magnitude and duration of stress, especially in occupational settings. Other studies too have highlighted the role of chronic stress in tinnitus as individuals experiencing chronic stress tend to have disruptions in the hypothalamic-pituitary-adrenal axis, which is a key system that is involved in the stress response (Mazurek et al., 2015). The disruption of hypothalamic-pituitary-adrenal axis has also been observed in individuals with tinnitus, where they tend to have elevated cortisol levels (marker of stress), that further results in the perception of an intensive debilitating tinnitus (Hébert et al., 2004). While the exact mechanisms by which stress contributes to the mechanism of tinnitus remain an area of ongoing research, the current evidence point to the fact that stress plays a significant role in both the onset and exacerbation of tinnitus (Mazurek et al., 2012). Brüggemann et al. (2016) explores the multi-dimensional nature of tinnitus distress, highlighting that there is an interplay of various factors beyond the auditory nature of tinnitus. While their study primarily focused on the impact of these factors, they also acknowledge the potential role of pre-existing psychological distress in the

experience of tinnitus perception. The authors postulate a diathesis-stress model, suggesting that individuals with pre-existing psychological distress may experience they symptoms of tinnitus to be more severe. The model also posits that the tinnitus acts as a stressor, interacting with the individual's vulnerability to distress, thereby increasing the negative impact of tinnitus. Furthermore, the study also highlights those pre-existing disorders of distress, such as depression and anxiety, may precede the onset of tinnitus in some cases and may even facilitate its generation (Brüggemann et al., 2016). Further Patil et al. (2023), highlighted that the generation of tinnitus may also be linked to chronic stress and thereby leading to a dysregulation in the hypothalamic-pituitary-adrenal axis and persistent activation of the sympathetic nervous system. The combination of these factors with increased occupational noise exposure significantly increases the likelihood of developing tinnitus. The authors further highlighted that the tinnitus itself can induce conditions and symptoms like stress, anxiety, depression, difficulty concentrating, and even insomnia, creating a vicious cycle. These emotional and psychological consequences can, in turn, exacerbate the percept of tinnitus making it more debilitating and difficult to manage. The authors also point to the fact that the presence of dysfunctional beliefs, negative thoughts, and hyperarousal in both chronic tinnitus and primary insomnia, suggesting a shared mechanisms that contributes to the persistence of both these conditions.

Stress as a mediator to the reaction of tinnitus

The distress related to tinnitus can vary depending on the extent of tinnitus. The neurophysiological model proposed by Jastreboff (1990) was the first to attribute that distress can be associated with tinnitus, particularly the negative reinforcements of fear and anxiety, mediated by the limbic and autonomic systems. The model focusses on the ongoing tinnitus and its related distress. Jastreboff's neurophysiological model of tinnitus asserts that the tinnitus distress arises not only from the auditory system but also from the limbic and autonomic nervous systems. These systems mediate the adverse emotional and physiological responses to tinnitus (Jastreboff, 1990). The model indicates that although most individuals with normal hearing perceive tinnitus-like sounds in silent settings, it becomes distressing only when associated with negative emotional reinforcement such as fear or anxiety (Baguley, 2002). This negative reinforcement stimulates the limbic and autonomic systems, intensifying the perception of tinnitus and establishing a feedback loop that sustains the

discomfort Jastreboff et al. (1994). The neurophysiological model has effectively been used in tinnitus retraining therapy, that reduces the emotional burden of tinnitus and enhancing its characteristics (Jastreboff & Jastreboff, 2001). In a study by Hackenberg et al. (2023) 8539 participants were surveyed about tinnitus and its link to depression, anxiety, and somatic symptom disorders using questionnaires. Tinnitus prevalence was 28.0%, with higher rates of depression (7.9%), anxiety (5.4%), and somatic symptom disorders (40.4%) among those with tinnitus. Logistic regression showed increased odds of these conditions in tinnitus sufferers, with odds ratios of 2.033 for depression, 1.841 for anxiety, and 2.057 for somatic symptom disorders. The study highlights the strong association between tinnitus and psychological distress, emphasizing the need for mental health support for these patients.

Role of stress in the persistence of tinnitus

Despite stress and anxiety being widely accepted as mediators of tinnitus distress few authors also attribute distress such as stress, anxiety, and depression to even play a role in the persistence of tinnitus. Mazurek et al. (2012) linked the perception of tinnitus to interactions between auditory and stress systems within the brain. The stress as such has strong influences on the auditory perception mechanism via the pathway through the limbic system as it modulates the overall auditory processing through interactions with HPA axis that further impacts the auditory signal perception due to the influence of the thalamic reticular nucleus These neural pathways beyond the auditory regions when combined with factors like stress, induces a behavioural change that contributes to the onset and persistence of tinnitus. Haider et al. (2018) further attributes that the persistence of tinnitus within the context of involvement of the frontal-striatal circuits highlight that the circuits are implicated in both tinnitus and chronic pain in terms of its generation and persistence. It is still unclear as to the mechanism of tinnitus in terms of interaction with distress but the role of extra-auditory regions in chronic tinnitus suggests a presence of a complex interplay between auditory perception, emotional processing, and the ongoing nature of the phantom sound.

Memory

The memory is linked to the hippocampus and amygdala and has also been strongly attributed to the generation and persistence of tinnitus. Tavanai & Mohammadkhani (2018) explored the link between tinnitus and cognitive functions, particularly attention and memory. They

attribute that the presence of tinnitus can negatively impact cognitive performances and vice versa, the cognitive deficits can aggravate the generation and persistence of tinnitus. The authors also suggest a dysfunction in the sensory attentional gating mechanism related to sgACC/nucleus accumbens, coupled with the parahippocampal area that may contribute to the generation of tinnitus. Furthermore, they propose a role of memory mechanisms that involve the hippocampus and amygdala, in facilitating the persistence of Chronic Tinnitus. Other regions closely linked to cognition such as the prefrontal cortex and limbic system are also implicated in tinnitus mechanisms due to their involvement of top-down cognitive control and attention regulation in tinnitus. Sedley (2016) hypothesized that the persistence of tinnitus may be strongly linked to the development of robust memory traces that is associated with the phantom sound being perceived. The authors further imply that the continuous activation of the neural pathways linked to the percept of tinnitus facilitates the formation of enduring representations of memory. These memory traces could potentially become perpetuating and independent of its initial causative factors, thereby facilitating the continuous persistence of tinnitus. A recent model by Berger et al. (2024) explored the mechanisms of tinnitus persistence with respect to the role of the hippocampus and parahippocampal gyrus. The authors highlight that while these structures are often explored in the context of the limbic system and emotional responses linked to tinnitus, there is an alternative perspective beyond emotional regulations and tinnitus. They primary function of the hippocampus and parahippocampal gyrus in the persistence of tinnitus is related to the established role in memory processing. The central hypothesis present is that the tinnitus persists as an auditory object that is held in the memory maintained by the activity within the hippocampus and parahippocampal Gyrus (PHG). The authors further suggest that the hippocampus/parahippocampal gyrus may facilitate the persistence of the tinnitus percept through the communication with the auditory cortex. The article also explores the potential role of tinnitus to manifest as a learned stimulus which is continuously a replayed memory of the sensory experience that facilitates its persistence. In a study by De Ridder et al. (2006), researchers investigated the involvement of the amygdala-hippocampal complex in tinnitus perception by selectively injecting amobarbital into the anterior choroidal artery of six male patients with chronic tinnitus. The injections temporarily suppressed the function of the amygdala and hippocampus, allowing the team to assess changes in tinnitus perception. Results showed that contralateral injections led to a significant suppression of tinnitus (60-

70%) in patients with unilateral tinnitus, while ipsilateral injections resulted in a maximum suppression of 30%. No suppression was observed in patients with bilateral tinnitus. The findings suggest that the contralateral amygdalohippocampal complex plays a crucial role in the perception of pure tone tinnitus, highlighting the potential for targeted treatments in managing this condition.

Tinnitus and Neurochemistry

Researchers have indicated that the mechanism contributing to the generation and persistence of tinnitus are strongly associated with various alterations in neurochemical processes. While the mechanism of tinnitus is linked to changes in the peripheral and central system, it is primarily the variations in neurochemistry that mediate the changes in the peripheral and central systems and these neurochemical variations predominantly involve neurotransmitters and neuromodulators which will be examined in this section (Sun et al., 2007).

Glutamate

Glutamate is an essential excitatory neurotransmitter in both the peripheral and central nervous systems. Within the inner ear, the hair cells produce glutamate, which is absorbed by the dendrites of afferent spiral ganglion neurones via two distinct types of neurotransmitter receptors: non-NMDA and NMDA receptors (Niedzielski & Wenthold, 1995; Sunami et al., 1998). These two receptors function as a dual receptor system. Non-NMDA receptors encompass the kainic acid subtype and the AMPA subtype, perhaps facilitating the rapid response of spiral ganglion neurones. NMDA receptors are probably implicated in a gradual impact (Niedzielski & Wenthold, 1995; Sunami et al., 1998). With respect to tinnitus, one proposed neurochemical mechanism is glutamate excitotoxicity, wherein excessive glutamate may be released as a result of cochlear damage, potentially leading to further neuronal injury. The excitotoxic effects of glutamate have been observed in the cochlea, particularly following noise exposure or cellular ischaemia (Kuba, 2007; Sheets, 2017). This neuronal damage, especially to auditory nerve fibres, may contribute to aberrant neural activity that initiates the perception of tinnitus. Furthermore, studies using animal models of tinnitus have demonstrated alterations in the expression and function of glutamate receptors within the auditory cortex (Wu et al., 2018). These changes may disrupt the balance between excitation and inhibition in the auditory system, thereby contributing to the perception of phantom sounds. In addition, the potential therapeutic role of glutamate receptor antagonists has been

explored, with some studies suggesting that these medications may reduce the excessive neuronal activity thought to underlie tinnitus by counteracting the effects of elevated glutamate levels (Park, 2016). A treatment study by Cacace et al. (2018) investigated the efficacy of low-frequency (1-Hz) repetitive transcranial magnetic stimulation (rTMS) over the left auditory cortex as a treatment for noise-induced tinnitus using a prospective randomized single-blinded sham-controlled cross-over design. Thirty participants underwent five days of either active or sham rTMS, with pre/post measures including tinnitus loudness, Tinnitus Handicap Questionnaire (THQ) scores, and neurochemical changes via magnetic resonance spectroscopy (MRS). Results showed that active rTMS significantly reduced tinnitus loudness by 4.5 dB, improved THQ scores, and decreased glutamate concentrations in the stimulated auditory cortex. The study suggests that rTMS can modulate neurochemical and perceptual aspects of tinnitus, highlighting its potential as a therapeutic approach. The study further highlighted the presence of increased glutamate concentrations before treatment of tinnitus.

Gamma-aminobutyric acid

Gamma-aminobutyric acid (GABA) is an essential inhibitory neurotransmitter that is present within the mammalian central nervous system. It plays an important role in reducing excitation of neurons across the nervous system (Dhakal et al., 2012). Reduced levels of GABA activity are associated with various conditions like Generalized Anxiety Disorder and Parkinson's disease due to the inability to inhibit hyper responsive neurons (Prediger et al., 2012). Research suggests a potential link between GABA and tinnitus, with studies indicating reduced GABA levels in the auditory cortex of tinnitus patients (Sedley et al., 2015). GABAergic alterations have been studied in isolation in individuals with hearing loss too. Age-related hearing loss (presbycusis) is frequently linked to alterations in gamma-aminobutyric acidergic neurotransmission within the central auditory system. Studies utilizing Magnetic Resonance Spectroscopy (MRS) have showed that decreased GABA concentrations are present in the auditory cortex of individuals with presbycusis, potentially resulting in the impairment of speech perception in noise (Gao et al., 2015; Harris et al., 2022). Furthermore, research also suggests that GABA levels are found to be reduced in the inferior colliculus in individuals with age-related hearing loss (Caspary et al., 1995). GABA is crucial for sustaining excitation-inhibition equilibrium. Insufficient GABAergic inhibition

may underlie tinnitus, or heightened inhibition may serve as a compensatory mechanism. Alterations may manifest as modifications in gross concentration, relative tissue distribution, receptor affinity, or receptor density (Caspary & Llano, 2017). In a study on humans by Sedley et al. (2015), researchers used magnetic resonance spectroscopy to measure GABA levels in the auditory cortices of 14 tinnitus patients and 14 matched controls. They found that tinnitus patients had significantly lower GABA concentrations in the right auditory cortex compared to controls. This reduction in GABA was not correlated with hearing loss or tinnitus severity, suggesting that GABA deficiency is specifically associated with tinnitus. The findings support the hypothesis that reduced GABAergic inhibition in the auditory cortex may contribute to the pathophysiology of tinnitus, highlighting potential targets for future treatments. Caspary & Llano (2017) determined that the GABAergic modulation in the medial geniculate body may result in tinnitus, highlighting that there may be a rise or decrease in GABA levels at this site that may contribute to tinnitus. The article further indicates that a decrease in GABA may lead to reduced inhibition, thereby enhancing central gain, while an increase in tonic GABAergic inhibition may cause maladaptive oscillations in the medial geniculate body, resulting in thalamocortical dysrhythmia, which further contributes to tinnitus.

Serotonin

Serotonin has been studied in the context of tinnitus, with significant correlations identified between tinnitus and differences in serotonin levels (Fornaro & Martino, 2010a; Knipper et al., 2010). This neuromodulator plays a substantial role in sensory regulation, including the auditory system, but has a role that cannot be summarised as simply as excitation or inhibition. Animal studies have highlighted the role of serotonin across the auditory pathway. In the dorsal cochlear nucleus, serotonin (5-HT) enhances multisensory input while reducing auditory input by increasing excitability in principal cells and pathway-specific feed-forward inhibitory interneurons, thereby reconfiguring sensory representation (Tang & Trussell, 2017). Serotonin receptors in the inferior colliculus modulate auditory responses by reconfiguring auditory circuits (Hurley & Sullivan, 2012). With respect to the auditory cortex, studies on serotonin transporter (SERT) knockout mice reveal that SERT defects lead to abnormal neuronal morphology and functional organization within the auditory cortex (Pan et al., 2021). Alterations in levels of serotonin have been argued to play a role in the

generation and persistence of tinnitus. An animal study by Tang & Trussell (2015) investigated the role of serotonin (5-HT) in the dorsal cochlear nucleus (DCN) using in vitro electrophysiology and optogenetics in mouse brain slices. The researchers found that 5-HT enhances the excitability of fusiform cells by activating 5-HT2A/2C and 5-HT7 receptors, which in turn augment hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. This effect is mediated through G-protein-dependent pathways involving cAMP and Src kinase signalling. The results suggest that serotonin significantly influences the intrinsic properties of fusiform cells, potentially increasing the sensitivity of the DCN to sensory input, which may have implications for understanding auditory processing and conditions like tinnitus. Serotonin modulates glutamate and GABA, which are significant excitatory and inhibitory transmitters and of primary interest in tinnitus research serotonin levels and tinnitus distress have largely been linked, with research suggesting that those with high levels of distress may have abnormal serotonin levels. According to other research, serotonin is crucial for sensory gating, and people with tinnitus have problems with gating mechanisms because of low serotonin levels. Some also postulate the role of serotonin in driving tinnitus and responsible for its persistence. Sun et al. (2007) postulated the possible role of Serotonin in tinnitus persistence as individuals with onset of tinnitus have heightened stress and anxiety due to hypervigilance as individuals who are under stress tend to have reduced serotonergic function. On the other hand, selective serotonin reuptake inhibitors (SSRIs) can have a role in reducing tinnitus distress in the context of associated anxiety and/or depression. The generation and persistence of tinnitus may also be influenced by serotonin because hearing impairment results in a significant amount of gain at the auditory central level (Salvinelli et al., 2003). However, there has been a lack of human studies in measuring central serotonergic function. This is partly due to brain serotonin being difficult to measure (Camilleri, 2009; Audhya et al., 2012). Notably, one post-mortem study revealed a substantial reduction in serotonergic cells in the dorsal and obscurus raphe nuclei of tinnitus patients compared to controls, highlighting the potential role of the raphe serotonergic system in tinnitus (Almasabi et al., 2022). Serotonin has also been linked to hyperacusis based disorders.

Hyperacusis, the heightened sensitivity to everyday sounds, is thought to arise from central gain enhancement following reduced auditory input, mediated by homeostatic plasticity, altered inhibitory control, and descending pathway dysfunction (Brotherton et al., 2015; Pienkowski et al., 2014). Serotonin plays a complex, region-specific role in modulating

auditory sensitivity, with studies reporting both elevated and depleted levels in hyperacusis. Elevated serotonin during development, for example, may reduce GABAergic inhibition, increasing auditory cortex excitability—as seen in autism-associated hyperacusis (Sato et al., 2023). Conversely, serotonin depletion in the nucleus accumbens of rodents induces hyperacusis-like behaviour, marked by exaggerated startle responses in quiet conditions (Sato et al., 2023). These divergent effects likely reflect serotonin's action across receptor subtypes and neural circuits (Marcus & Soso, 1989). Supporting this, serotonin modulates startle responses in a dose- and site-dependent manner, with central administration suppressing and spinal administration enhancing reflexes. Serotonin reuptake inhibitors may worsen hyperacusis by elevating spinal serotonin, though low baseline serotonin in depression could also increase susceptibility (Lin et al., 2025). Together, these findings underscore the multifaceted role of serotonin in sensory gain regulation and hyperacusis. As tinnitus is linked to central gain, serotonin depletion can be linked to heightened central gain and tinnitus.

Acetylcholine

Acetylcholine is a key neurotransmitter, and neuromodulator, that has been explored in relation to tinnitus but its exact function in tinnitus along with its possible therapeutic effect has not fully been understood and needs to be explored. While some research suggests a possible relationship between altered cholinergic function and tinnitus, there isn't a definitive causal relationship that could help potentially develop effective treatments targeting this system (Sedley et al., 2015). Both animal studies and human studies have linked acetylcholine to tinnitus. The study by Zhang et al. (2019) investigated the effects of noise exposure on guinea pigs, focusing on changes in cholinergic innervation in the hippocampus. Methods included noise exposure, immunohistochemistry, tinnitus assessment, and auditory brainstem response measurements. Results showed a significant decrease in Vesicular Acetylcholine Transporter (VAChT) density in the hippocampus shortly after exposure, with partial recovery in non-tinnitus animals but persistent reduction in tinnitus animals. The findings suggest that noise-induced tinnitus is associated with long-term cholinergic remodelling in the hippocampus, highlighting its potential role in tinnitus pathophysiology. Another study by Hansen & Wei (2014) investigated the effects of aging and cholinergic neurotransmitters on the acoustic startle response in Fischer 344 rats. Methods included measuring gap-induced prepulse inhibition (gap-PPI) at different sound intensities (105, 95, and 85 dB SPL) and gap durations (1-100 ms) across three age groups (3-5, 9-12, and 15-17

months). Additionally, the effects of scopolamine and mecamylamine, cholinergic receptor antagonists, were tested. Results showed a significant "notch" in gap-PPI at 50 ms for the 105 dB SPL condition, which diminished with reduced sound intensity, indicating hyper-sensitivity to loud sounds. Scopolamine reduced the notch in young rats but had no effect on older groups, while mecamylamine showed no significant impact. The study suggests that hyperacusis-like sensitivity increases with age and that cholinergic receptors may play a role in sound processing, particularly in younger rats. (Ruan et al., 2018) in their review further proposed that the cholinergic hypofunction associated with reduced input in the auditory system may significantly contribute to the generation of tinnitus in individuals with presbycusis, especially in conjunction with cognitive impairment. The article further highlights that the reduction in acetylcholine influences a particular subtype of GABAergic inhibitory interneurons known as neuropeptide Y neurogliaform cells. Human study by Sedley et al. (2015) highlights that the cholinergic system, particularly choline concentration in the auditory cortex, is positively correlated with tinnitus severity and hearing loss. Using neuroimaging techniques, researchers measured choline levels and found a significant association with tinnitus severity. Choline is linked to neuronal membrane turnover and plasticity, which may contribute to the persistence of tinnitus. Although acetylcholine itself doesn't significantly impact the choline signal, its levels are strongly correlated with choline, suggesting it plays a role in tinnitus mechanisms. These findings indicate that targeting the cholinergic system could offer new treatment avenues for tinnitus by addressing neurochemical imbalances in the auditory cortex.

Dopamine

Recent studies suggest that the dopaminergic pathway plays a significant role in tinnitus perception, involving brain areas like the prefrontal cortex, temporal lobes, and limbic system. These areas are crucial for attention, stress, emotions, and memory, which are all influenced by dopamine (Brozoski et al., 2012). A study by Sziklai et al. (2011) investigated the effect of pramipexole, a dopamine agonist, on tinnitus in elderly patients with presbycusis through a randomized, placebo-controlled, double-blind trial. Forty patients were divided into two groups: one received pramipexole and the other a placebo, over a four-week period. The results showed a significant improvement in tinnitus annoyance and loudness in the pramipexole group compared to the placebo group, with some patients experiencing complete cessation of tinnitus. However, there were no changes in hearing thresholds or

electrocochleography results. The study concluded that pramipexole is effective in reducing tinnitus symptoms in presbycusis patients, likely due to its role in stimulating dopamine receptors, although it does not affect hearing thresholds.

1.4 Certain limitations with existing models

The primary discourse regarding the mechanism of tinnitus must focus on its onset and persistence following its initial occurrence. It pertains not only to the development of tinnitus but also to the mechanisms behind its persistence. The reason as to why the mechanism pertaining to persistence of tinnitus is essential is due to the current statistics related to tinnitus remission rate. Unlike other chronic conditions, the statistics related to tinnitus remission rate is relatively poor. The tinnitus tends to persist for most individuals when continuously present for 4 weeks and has a reduced remission rate of just 15% (Sanchez & Roberts, 2021). Another study by Vielsmeier et al. (2020) reported a full remission of just 18.4% after the onset of tinnitus when collecting patients from their initial onset of tinnitus and following them up over time at various intervals. A similar design by Wallhäusser-Franke et al. (2017) on a follow up study between Acute (tinnitus duration less than 4 weeks) and Chronic Tinnitus (tinnitus duration greater than 6 months) revealed a remission rate of approximately 11% of individuals with acute tinnitus experiencing complete remission by the end of the study period. The statistics indicate a significant likelihood that subjective continuous tinnitus will persist once it commences. It is crucial to comprehend this phenomenon, as it raises questions that if tinnitus arises from some anomaly of auditory processing, or the act of compensating for it, then why does tinnitus persist long-term, rather than the brain adapting to no longer require tinnitus? With respect to the mechanism of generation and persistence of tinnitus, so far, we have discussed mechanisms pertaining to the processing of sensory signals which include mechanism like gain, gating neuromodulation, impaired inhibition etc. But it is to note that if tinnitus were simply result of these sensory modulatory processes, we would have it in a more fluctuating/intermittent form, considering how dynamic the modulatory systems are. However, as mentioned earlier in the start of the section, tinnitus is more continuous, persistent/chronic, and unchanging with a low remission rate. This could be explained better if two sets of process related to 1) generation and 2) persistence of tinnitus are differentiated and explained better. The theoretical models mentioned above, in their differentiation of the initial generation of tinnitus from its long-

term persistence, are based on extrapolation from first-principles, other fields of neuroscience, as well as on animal studies of acute tinnitus and human chronic tinnitus studies. What is crucially needed is more empirical research focusing directly on acute tinnitus (duration of tinnitus less than 1 month), particularly in humans, as this population might provide unique insights into the neural mechanisms of tinnitus onset, perpetuation, and persistence on account of its natural history. Most animal studies involve acutely damaging the auditory system to immediately induce tinnitus and measuring the neural activity in the time period that follows (i.e. the 'acute' stage), whilst human studies almost exclusively study 'chronic' tinnitus, arbitrarily as present for over 6 months (occasionally 3 months) from its onset, which itself is often much later than the predisposing hearing damage (Sedley et al., 2019). Already, this raises the question as to whether this experimental approach in animals is representative of how most cases of human tinnitus develop. Hence humans with acute tinnitus followed up until the chronic stage of tinnitus would be an ideal population to help better understand the generation and persistence of tinnitus. A limited number of functional connectivity studies have investigated acute and chronic tinnitus using resting state EEG/fMRI measurements. Lan et al. (2021) identified a notable enhancement in power within the middle frontal gyrus and parietal cortex at the gamma band for acute tinnitus in comparison to the chronic tinnitus group. Cai et al. (2020) conducted a functional connectivity study that demonstrated an enhancement in both functional and causal connectivity between the superior temporal gyrus, amygdala, and nucleus accumbens in the acute tinnitus group relative to persons without tinnitus. Vanneste et al. (2011) posited that patients with newly developed tinnitus exhibit heightened activity in the auditory cortex, supplementary motor area, dorsal anterior cingulate cortex, and insula. The major limitation with these studies is that they study resting state brain activity alone and predominantly miss other measures like central gain which feature prominently in tinnitus models. Along with that a follow up of acute tinnitus individuals until their chronic stage would help better understand the generation and persistence of tinnitus rather than a cross-sectional comparison due to the possible heterogeneity that exists within tinnitus patients and that may act as a confound during cross sectional comparisons (Cederroth et al., 2019). The computational model of tinnitus based on prediction is a standalone model that helps distinguish between generation and persistence of Tinnitus using reference from Acute Tinnitus (Sedley et

al.,2016). However, the predictive coding model would need further evidence to substantiate the changes linked to the generation and persistence of tinnitus.

1.5 Predictive coding model.

The predictive coding model is based on the principles of Bayesian predictive inference in the brain; it posits that the brain possesses an internal model, dynamic in nature and serving as a probabilistic representation of the sensory environment, which further allows for a comparison between the internal model (probabilistic representation of the sensory environment) and incoming sensory data (Knill & Pouget, 2004). Instead of the brain passively processing the incoming sensory input, it actively generates predictions about the world based on prior experiences and internal models that is compared with the sensory input. During comparisons, if any discrepancies are present between the predicted and actual input it results in what is termed 'prediction errors', which are used to update and refine the internal models (Knill & Pouget, 2004). The continuous cycle of prediction, error detection, and internal model adjustment enables the brain to efficiently process incoming sensory information in relation to the sensory environment that would help make a better sense of this environment (Knill & Pouget, 2004). In the context of auditory information processing the brain anticipates the incoming sounds based on previously learned patterns and context. An anticipated or learned behaviour is present for an incoming sound and when an unexpected sound occurs which is different from the predicted sound pattern, a prediction error is generated that signals the brain to pay extra focus and attention to this unexpected sound and update its model. (Sedley et al., 2016). This Bayesian predictive characteristics of the brain are based on the probability theorem of Bayesian statistics or Bayes theorem and predictive coding provides a framework to understand how the brain processes incoming information by integrating prior beliefs (predicted information) and sensory evidence (likelihood) that generates prediction errors to form posterior distributions (Sedley et al., 2016). The term *Priors* represent the brain's pre-existing expectations about the surrounding environment, *likelihood* indicates the probability of observing the incoming sensory data given at a particular hypothesis (i.e. cause of that sensory input). The integration of priors and likelihood generates the *posterior distribution*, which reflects the updated belief about the current state of the environment after incorporating new sensory evidence (Sedley et

al.,2016). This inferential process is modulated by *precision*, which represents the confidence that is assigned to either the prior or the sensory data, determining which among the prior or sensory data has influence on the posterior. In predictive coding framework, hierarchical cortical structures use predictions generated from priors to interpret incoming sensory input, with mismatches between predictions and sensory data producing *prediction errors* (De Ridder et al., 2014). These errors when detected further propagate upward in the cortical hierarchy to refine the priors and further minimize uncertainty or prediction errors. Higher precision in sensory input amplifies the impact of new data on belief updating, while higher precision in priors strengthens the brain's reliance on its expectations.

Concerning tinnitus, the reduced auditory input due to hearing loss prior to its onset creates a discrepancy between the predicted signal (appropriate incoming signal) and the actual input (reduced incoming signal), leading to a prediction error. To reduce this prediction error that has been resulted from a sensory deafferentation, the brain often compensates for absent information by increasing the internal noise, which may manifest as tinnitus. The oscillatory brain activity that facilitates predictive coding is mostly the delta and theta waves which further aids in the anticipation of sound occurrence, while alpha and beta waves assist in identifying the nature of the sound. Abnormalities in these oscillatory patterns contribute to the impression of tinnitus (De Ridder et al., 2014). According to the integrative tinnitus model proposed by Sedley et al. (2016), spontaneous activity in the subcortical auditory pathway constitutes a "tinnitus precursor." This precursor is typically disregarded by the brain as imprecise evidence against the prevailing prediction of silence. Essentially, the brain filters out this neural noise as insignificant. However, certain factors can increase either the intensity or the precision of this precursor. Increased intensity could be due to factors like acute deafferentation (loss of sensory input), while increased precision could be linked to stress or attention. When the precision, or postsynaptic gain, of the precursor rises sufficiently, it surpasses the brain's filtering mechanisms and is perceived as tinnitus (Sedley et al.,2016). The transition to persistent tinnitus involves a recalibration of the brain's predictive mechanisms where the default prediction shifts from "silence" to "tinnitus." This means the brain now anticipates the tinnitus signal and incorporates it into its internal model of the auditory environment. Even if the precision of the precursor subsequently decreases to its pre-tinnitus level, the brain continues to perceive tinnitus because it is now the most plausible explanation for the ongoing neural activity. This shift in the default prediction,

coupled with attentional focus, which further enhances the precision of the precursor, contributes to the perpetuation of tinnitus. Over time, due to readjustment, the brain relies less on the internally generated tinnitus signal and recalibrates the internal model where there is less expectation of the sensory input (tinnitus) as the brain's precision-weighting mechanism adjusts and balances its sensitivity to external versus internal sensory signals. The persistence of tinnitus in chronic tinnitus individuals is facilitated by brain's ability to update its generative model to learning to predict the tinnitus and accepting it as a permanent auditory feature. Along with the model update, attentional mechanism facilitates its propagation where even if the initial trigger to the onset of tinnitus reduces, there is perpetuation of tinnitus contributing to its chronicity. The fact that tinnitus characteristics such as intensity, distress reduce over time due to recalibration of the brain's internal model and the roles of attention in its persistence frames the baseline for our hypothesis that is discussed in the upcoming section. Figure 1.2 depicts variations in prediction updates from the onset of tinnitus until its subsequent chronic stages.

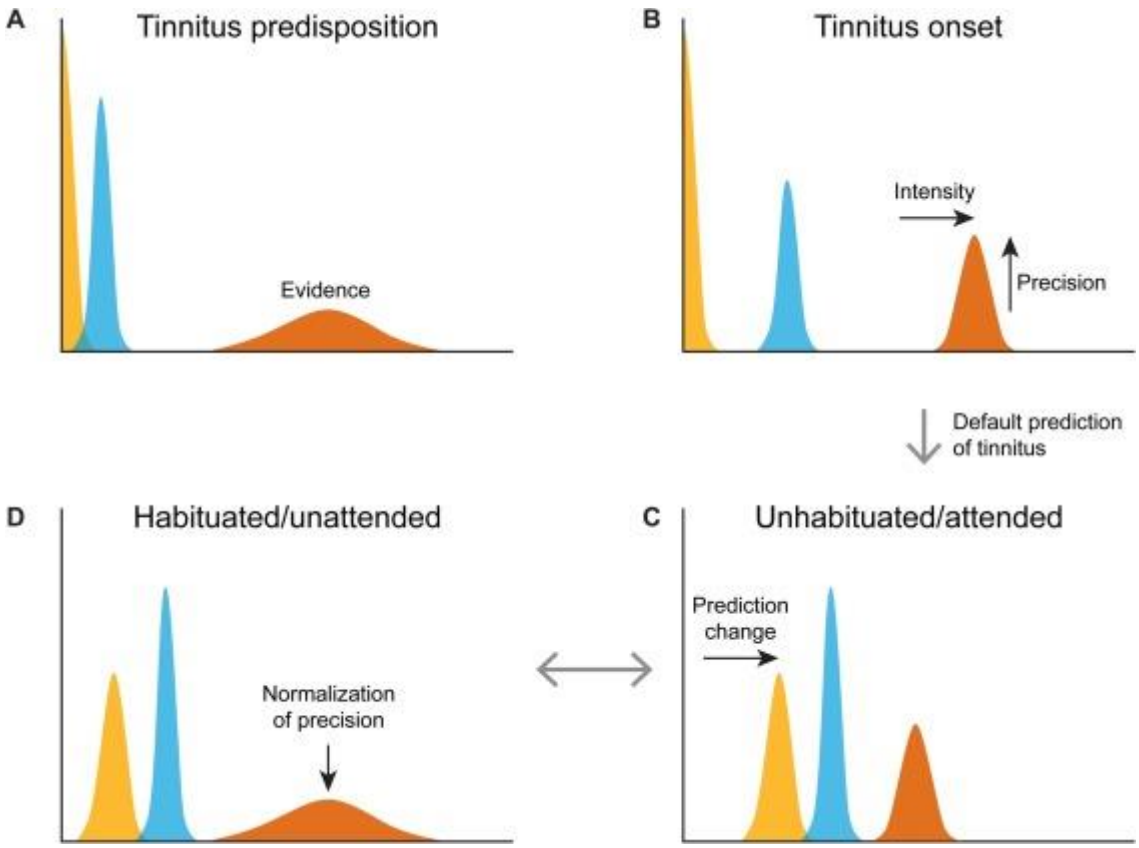

Figure 1.2: Depiction of predictive coding in tinnitus.

In each panel, the perceived intensity of tinnitus is represented by the location of the posterior distribution (blue) along the horizontal axis. (A) In cases of hearing loss, the sensory evidence for tinnitus, or its precursor (orange), lacks the precision necessary to surpass the default prediction (yellow) of silence. (B) The precursor, with enhanced precision, affects perception, resulting in an altered posterior perception of tinnitus. At this time, a window of reversibility may exist. If the default prediction is adjusted to anticipate tinnitus (often less intense than the precursor), the condition becomes chronic due to experience-dependent plasticity. Reducing the precision of the precursor to its pre-tinnitus state leads to habituation, but not the elimination of tinnitus, due to plastic alterations in previous predictions. X axis across the four graphs indicates parameter values and y axis indicates the probability density. The content in this figure has been reproduced from Hullfish et al. (2019)

1.6 PhD background and aim

It has been argued that the mechanisms of tinnitus cannot be comprehensively understood merely through chronic tinnitus research and animal studies, both of which have inherent limitations. The principal objective of tinnitus research is to understand the mechanisms underlying its onset and chronicity, as the condition is predominantly persistent in many individuals. Investigations need to be determined as to why, despite the brain's intricate mechanisms to prioritise novelty and behavioural relevance, tinnitus does not remit, especially as chronic tinnitus should have increased precision towards its prediction, and reduced prediction error related to its source, and it is no longer novel. The brain acts to reduce the increased prediction error and accepts tinnitus while doing so. Hence the activity of tinnitus remains unmitigated, as the aforementioned models do not address the reasons for its persistence in the majority of cases. Acute tinnitus studies and monitoring the transition from acute to chronic tinnitus are essential for achieving this objective. Existing literature on acute tinnitus has attempted cross-sectional comparisons between acute and chronic tinnitus; however, it lacks longitudinal comparisons within groups from acute to chronic. Individual differences in tinnitus characteristics and heterogeneity may affect cross-sectional differences, confounding the understanding of the acute to chronic transition (Cederroth et al., 2019). Existing acute tinnitus studies have mostly overlooked central gain measurements

and changes in auditory sensitivity from acute to chronic stages, despite their relevance to tinnitus research. Keeping these limitations in mind, and our motivations understanding tinnitus mechanisms better, we aim to unravel the neurobiological basis of tinnitus by studying its initial onset and subsequent chronification. This thesis builds upon the hypothesis that the neural changes underlying tinnitus, principally central gain, and neural synchrony, are maximal around the time of tinnitus onset, but later subside by way of regression to the mean (Sedley et al.,2016) as according to the sensory precision model (which posits that resetting of default predictions takes over their role in sustaining tinnitus). The reduction in tinnitus over time can be considered as a **regression to the mean,** where the brain's heightened sensitivity to the tinnitus signal gradually normalizes, and the perceived intensity of tinnitus may decrease due to less reliance of the internally generated signal. The above-mentioned sensory precision model illustrates that due to recalibration of the internal model post tinnitus which is accompanied by adaptation and habituation, the neural activity of tinnitus are maximal around the onset and reduce over time (Sedley et al., 2016). This process reflects the **brain's plasticity** and its tendency to return to a more stable state over time, especially if there is no longer a need to rely as heavily on internal signals to compensate for a lack of external auditory input. If correct, this might explain why animal studies consistently find increased neural synchrony in the central auditory pathway, whereas positive results in human tinnitus patients, compared to healthy controls, have not been replicated when controls are matched for hearing loss, i.e. these neural activity alterations are consequences of hearing damage, but a subset of these, in excess, can cause tinnitus (Sedley 2019). The study of tinnitus in humans in both the acute and, subsequently, chronic stage, provides the opportunity to address the following fundamental questions:

- What are the alterations in neural activity that are critical to initiating and driving tinnitus, and how can we set these apart from other consequences of hearing damage that are not directly relevant?

- For how long after tinnitus onset do these neural mechanisms of tinnitus remain excessively active?

- Conversely, are there neural processes related to tinnitus perpetuation that are absent in the acute stages but develop subsequently?

- Are there acute tinnitus-related changes in phenomena linked to specific neurochemical processes, which might identify neurotransmitter or neuromodulator systems as potential targets for acute or chronic tinnitus?

We intend to address these questions through our constructed hypotheses in each chapter based on our principal hypothesis that neural response such as neural synchrony and central gain tends to be maximal around the onset of tinnitus and reduce over time as a regression towards the mean.

Chapter 2 Changes in Tinnitus distress from acute to chronic stage: longitudinal observation in a community-based sample

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2.1 Introduction

Fundamental neural and perceptual mechanisms of acute tinnitus can be studied in three ways. The first is through objective means, for example, electrophysiology and neuroimaging, which can aid in a better understanding of the structural and functional changes that are causes, contributors, correlates, or consequences of tinnitus (Cai et al., 2020). The second way is through subjective perceptual-based tests such as psychophysics which can assist in quantifying the tinnitus characteristics based on the physical properties of the individual's Tinnitus, and their wider perceptual processing of auditory signals more generally (Hébert et al., 2004). Finally, one can assess tinnitus symptoms through subjective questionnaires and examinations which can give a better idea of the individual's experience of tinnitus (Zhang et al., 2023). All three modes of assessment together are essential in giving an overall understanding of tinnitus onset and its course of alteration over time.

Bearing in mind that predominantly tinnitus tends to persist over time, it is important to understand the natural history (unaltered natural course) of tinnitus symptoms. These symptoms over time may differ from individual to individual and factors like onset, duration, severity, habituation, emotional impact, effects of daily life, and treatment response may influence the course of tinnitus over time. Most studies that document the evolution of tinnitus symptoms over time are carried out in people who already have chronic (i.e. longstanding) tinnitus.

In the small number of acute tinnitus natural history studies, the participants studied have been patients seeking help in specialist clinics, rather than recruited from a community sample of tinnitus who have not had any medical or specialist consultation pertaining to their tinnitus. Patients from specialist clinics tend to receive active treatment and/or professional counselling which may interfere or confound with studying the tinnitus course, along with the placebo (or treatment) effect of any clinical intervention. A study by Wallhäusser-Franke et

al. (2017) focused on predictors for the development of chronic Tinnitus from the acute stage ("under 4 weeks") over a period of six months. The authors established that the distress and loudness of Tinnitus did not significantly change over time. Another study on Acute Tinnitus distress reported that Tinnitus-related distress improved over time, with maximum distress reported during the onset. Additionally, Zhang et al. (2023) utilized various distress measures which included tinnitus, depression, and anxiety scales to document the clinical characteristics of acute and chronic tinnitus groups. In a cross-sectional comparison between groups, the chronic tinnitus group tended to have an increased distress score on all measures, and that increased anxiety and sleep disturbances were negatively associated with the course of tinnitus suggesting that acute tinnitus individuals may be prone to increased anxiety and reduced sleep, however chronic tinnitus individuals were more prone to depression (Nyenhuis et al., 2013; Zhang et al., 2023). Examining tinnitus symptoms from its onset over a period in a community-based sample of people who have not sought professional help would reduce the potential confound of interaction with a specialist on a person's tinnitus and allow more accurate examination of the unaltered natural history of tinnitus. This is important for providing accurate information to people who have newly developed tinnitus, and to know what changes to compare against in single-arm treatment trials in acute Tinnitus. Although a few studies have documented tinnitus-related distress changes over a period of time, they have mostly used anxiety, depression, and other distress-related scales to quantify the changes, rather than tinnitus-specific symptoms. It is also important to involve validated tinnitus questionnaires to help us understand more precisely the distress and other symptoms resulting from tinnitus. By adding other questionnaires such as those assessing hyperacusis symptoms, we can get a much broader perspective on the level of overall impact. To our knowledge, no study has used hyperacusis scales on the acute tinnitus population to see the level of changes in subjective sound sensitivity over time. Hyperacusis has overlapping and distinct neural mechanisms and symptoms with tinnitus, and it would be helpful to try to disentangle these as much as possible (Cederroth et al., 2020). In our current study, we used a community-based sample of acute tinnitus and analysed their changes in distress over a period. Based on literature review and our central hypothesis (refer Chapter 1), in the current chapter we hypothesize that an initial onset tinnitus results in an increased distress due to the unfamiliarity of tinnitus, and subsequently reduces over time.

2.2 Methods

2.2.1 Participants

We examined 51 individuals with Acute Tinnitus, defined as lasting between 3 days and 6 weeks, with a mean duration of 4.06 weeks (SD 2.22 weeks) and a median duration of 4 weeks. Please refer figure 2.1 for the frequency distribution of tinnitus duration among Acute Tinnitus participants. Participants were recruited via community advertisements on Google Ads and internally from Newcastle University's research volunteer pool. Fifty-one participants with Acute Tinnitus were invited for reassessment after a minimum of six months from the onset of Tinnitus, indicating their chronic stage, and 26 of them volunteered for and completed this further testing. This is designated as the 'Post Acute' group to differentiate it from the cohort recruited during the chronic phase of Tinnitus. Out of the 25 participants who were unable to attend the follow-up sessions, they cited reasons such as lack of time to schedule an appointment or failure to respond to follow-up appointment reminders. When asked about their tinnitus status, one reported their tinnitus to be intermittent, four reported no changes in their tinnitus, one reported the tinnitus to be more progressively severe and also notified slight discomfort to participate further in a four-hour long experiment, one cited their tinnitus to completely cease after the general physician changed the dosage of their medication (Oxcarbazepine: SSRI). The rest of the participants did not respond to follow up reminders. The criteria for inclusion for Acute Tinnitus is a continuous non-pulsatile Tinnitus present for between 3 days and 6 weeks Exclusion criteria includes participants with Meniere's disease, Epilepsy, and Middle ear pain or infections.

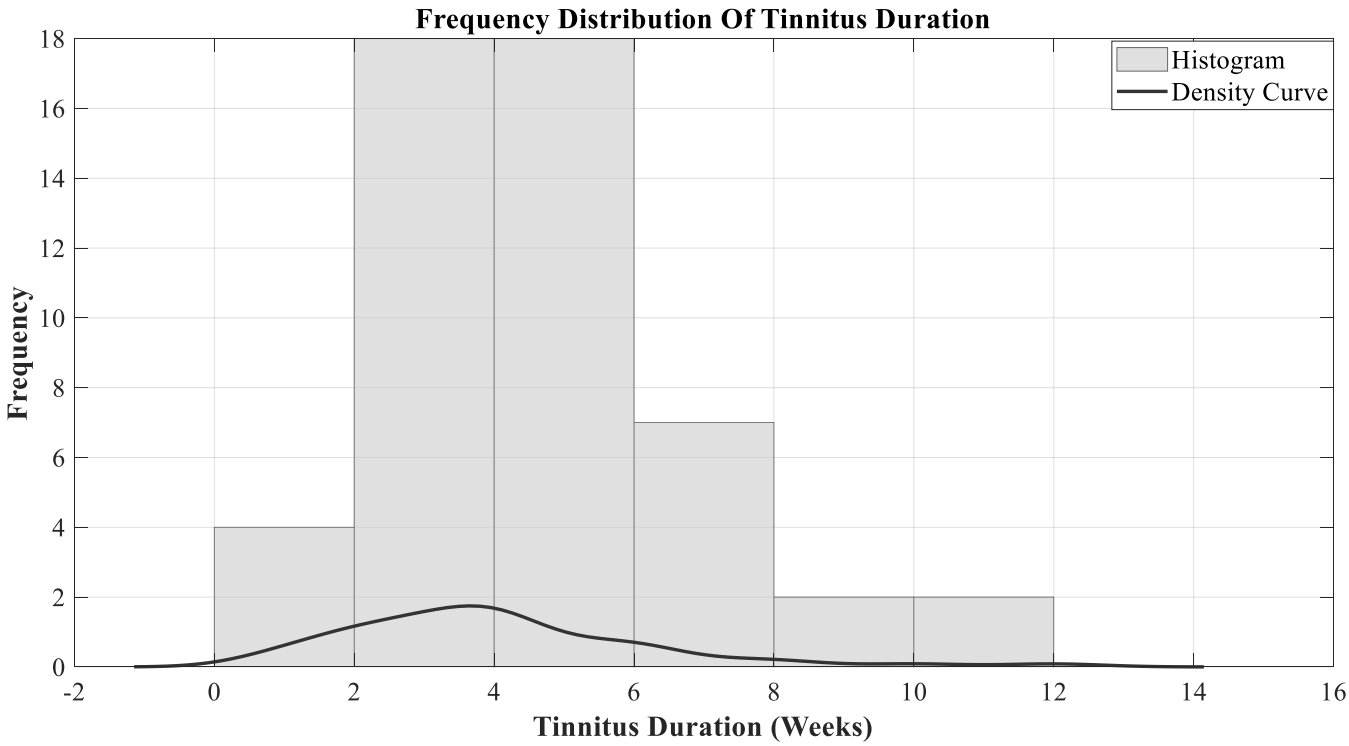

Figure 2.1: Frequency distribution of duration of tinnitus among Acute Tinnitus participants. *With a mean duration of 4.06 weeks (SD 2.22 weeks) and a median duration of 4 weeks, the frequency distribution curve is demonstrated in the above figure.*

With respect to the medications taken by acute tinnitus, of the 23 participants who disclosed their treatments, eight have been utilising either serotonin modulators via SSRIs or GABAergic medications. Please refer table 2.1 for further details on the participant's medication

SUBJECTS	**MEDICATIONS**
S1	Inhalers, contraceptive pill
S2	Thyroxine
S3	Bimatoprost
S4	Perinopril
S5	Oxcarbazepine*

S6	Duloxetine*, Statins, Aspirin, Omeprazole, Clobetasol Cream.
S7	Methotrexate, Folic Acid, Atorvastatin, Omeprazole.
S8	HRT
S9	Panadol
S10	Fluoxetine*
S11	Fexofenadine, Betahistine*
S12	Levothyroxine, Fluoxetine*
S13	Amlodipine, Lipitor, and Aspirin
S14	Sertraline*, HRT, Sumatriptan
S15	Levothyroxine, Omeprazole, Cetirizine.
S16	Asthma inhalers
S17	Amlodipine, Bisoprolol, Apixaban, Lansoprazole, Citalopram*.
S18	Allopurinol, Lisinopril.
S19	Salbutamol inhaler
S20	Colazide, Amlodipine
S21	Losartan, Statins, Aspirin
S22	Apixaban, Ramipril, Bisoprolol
S23	Mirtazapine*

Table 2.1: The table presents the classifications of medications administered to the participants with Acute Tinnitus. *Of the 51 participants, only 23 provided information on their current medications, while the remaining individuals reported not being under any*

pharmacological treatment. The asterisk (*) indicates that the subjects were taking either serotonergic or GABAergic modulator medications.

2.2.2 Audiological Assessment

The experimenter interviewed the subjects before the experiment, verifying a medical history consistent with subjective tinnitus and the absence of atypical symptoms or concerns regarding an undetermined underlying cause. Before this, participants completed a concise, individualised pre-screening questionnaire that evaluated the inclusion and exclusion criteria. Participants submitted demographic data, including age and sex, alongside details regarding their tinnitus, including type (tonal, noise-like, or other), duration, affected ear(s), and a historical account of physical and mental health, particularly any otological disease. The impact and distress associated with tinnitus, and any co-existent hyperacusis symptoms, were assessed using four standard validated questionnaires, which were completed in the order listed below:

Tinnitus Handicap Inventory (THI)

The THI is a self-assessed 25-item questionnaire developed by (Newman et al., 1996) to assess the individual's perceived tinnitus severity. It mainly assesses three domains namely functional, emotional, and catastrophic with severity ranging from very mild to catastrophic. The psychometric properties of the THI indicate a good internal validity and test-retest reliability (Newman et al., 1996).

Tinnitus Functional Index (TFI)

Another tinnitus distress-related questionnaire, the Tinnitus Functional Index (TFI) assesses the impact of tinnitus on individual's daily functioning and quality of life (Meikle et al., 2012). It assesses various aspects including emotional distress, sleep disturbances, and interference with daily activities. is a 25-item questionnaire with 10 questions per item assessing eight subscales (intrusive, sense of control, cognitive, sleep, auditory, relaxation, quality of life, and emotional). Concerning the psychometric properties of the TFI, Fackrell et al. (2016) demonstrated a high internal consistency, high reliability, and high correlation with the THI questionnaire.

Hyperacusis Questionnaire (HQ)

The Hyperacusis Questionnaire (HQ) assesses the symptoms of hypersensitivity to external sounds and related degree of distress (Khalfa et al., 2002).The psychometric properties reflect a high internal consistency and reliability (Fackrell et al., 2015).

Inventory of Hyperacusis Symptoms (IHS)

To complement the HQ to assess sound sensitivity, a 25-item IHS developed by Greenberg & Carlos (2018a) was used. The tool was developed to create a standardized assessment of symptom severity. A recent study by (Aazh et al., 2021) concluded a high internal consistency and reliability for the questionnaire to diagnose hyperacusis. In our paper, we do not aim to classify people as 'having hyperacusis', or not, based on their scores, but use these measures as continuous variables to comment on the individual's sensitivity and reaction to sounds (i.e. degree of hyperacusis symptoms).

Pure-tone audiometry

Participants underwent a Pure Tone Audiometry (PTA) test to establish hearing thresholds at octave frequencies ranging between 250 Hz up to 8 kHz. Thresholds were estimated based on the initial presented level at either 40dBHL or above depending on the participant's residual hearing (Carhart & Jerger, 1959). If perceived audible, the stimulus was continuously reduced by 15dBHL until the participant did not perceive it as audible. From the first reversal, a 5 dB up, 10 dB down staircase procedure was then used until the final threshold was established by two positive responses out of three trials (Carhart & Jerger, 1959).

2.2.3 Tinnitometry

The frequency and loudness of tinnitus were assessed using a *tinnitometry* (i.e. psychoacoustic measurement of tinnitus characteristics) approach for patients with acute or chronic tinnitus. In instances of unilateral Tinnitus, matching stimuli were delivered contralaterally, but in bilateral tinnitus, they were administered bilaterally. Participants initially matched the loudness of their tinnitus, followed by matching its frequency, commencing at a reference frequency of 6 kHz. In bilateral cases, supplementary modifications were implemented to equilibrate the two ears when tinnitus presented asymmetrically. A procedure was implemented in which the loudness and then frequency were repeatedly modified until the sound resembled the individual's tinnitus as closely as possible, at which point no further adjustments were deemed required by the participant. The

technique was conducted over three trials, and the average of these trials was determined as the final loudness and pitch of the tinnitus. The stimulus employed for this method was either a pure tone or narrow band noise, contingent upon the subjective characteristics of the tinnitus.

The Minimum Masking Level (MML) was assessed for the tinnitus ear in cases of unilateral tinnitus and for each ear separately in instances of bilateral tinnitus. To ascertain MML, a narrowband noise was introduced at the threshold level and incrementally raised in 1dB steps until subjects perceived total masking of the tinnitus by the noise.

2.2.4 Data Analysis

Questionnaires were calculated as their total score. Descriptive and inferential statistics were carried out using (SPSS) version 28.0. A Shapiro Wilk's statistical test score ($p>0.05$) was obtained for all questionnaires across the two groups, prompting use of parametric statistical tests. To assess longitudinal changes between Acute and Post-Acute Tinnitus, we performed a Repeated Measures Multivariate Analysis of Variance (RM-MANOVA) to compare groups on several dependent variables, including the THI, TFI, HQ, and IHS questionnaires. Additionally, a Bonferroni correction was applied to account for multiple comparisons.

2.3 Results

Out of the 26 participants who were followed up from their initial onset over a period of six months, 22 of them filled the online questionnaires at both time points and the remaining 4 participants had either one or more questionnaires incomplete in at least one of the two time points. Individual distress scores for all 22 participants are tabulated in table 2.2. There was an overall effect seen across distress scores for both the groups of tinnitus ($F(4,18) = 4.811$, Wilk's $A = 0.483$, $p = 0.008$)

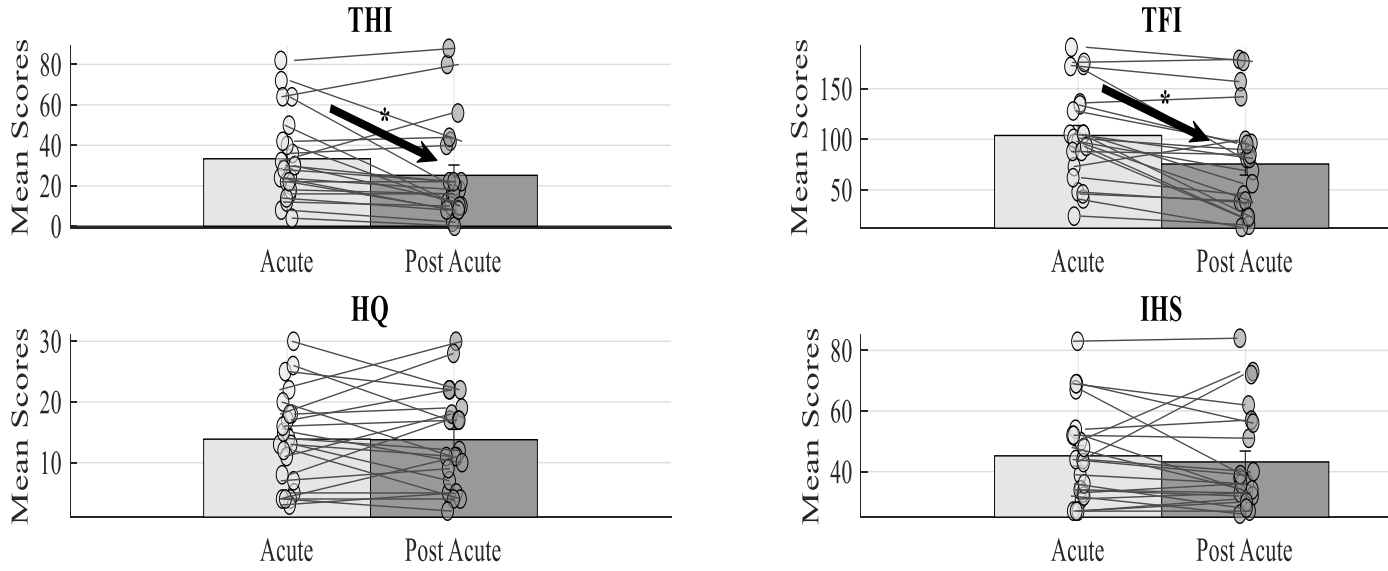

Figure 2.2: Longitudinal changes between Acute and Post Acute Tinnitus for all questionnaires. *THI indicates Tinnitus Handicap Inventory, TFI indicates Tinnitus Functional Index, HQ indicates Hyperacusis Questionnaires, and IHS indicates Inventory of Hyperacusis Symptoms, the error bars indicate the Standard Error of Mean and '*' indicates the presence of statistically significant different.*

2.3.1 Tinnitus Distress

Between the time points of Acute and Post-Acute, there was a significant difference with reduction in both distress questionnaire (THI and TFI) scores: THI ($F(1,21) = 5.37$, $p = 0.031$); TFI ($F(1,21) = 19.409$, $p < 0.001$). The Acute Tinnitus was associated with higher mean scores of 33.63 ± 21.27 (Mild Severity) and 103.77 ± 46.28 (high severity) for THI and TFI respectively than the Post Acute Tinnitus stage, which were 25.18 ± 23.86 (Mild severity) 75.55 ± 51.26 (Upper moderate severity) respectively for THI and TFI. Figure 2.2 and table 2.2 depicts the mean scores for the distress scales between the groups. Upon running the Pearson's correlation between THI and TFI, there was a strong positive correlation between THI and TFI which was statistically significant ($r = 0.898$, $N = 44$, $p<0.001$).

2.3.2 Hyperacusis symptoms

No significant differences were observed between Acute and Post Acute Tinnitus for both HQ ($F(1,21) = 0.05$, $p = 0.944$) and IHS ($F(1,21) = 0.596$, $p = 0.449$) questionnaires. These

results are displayed in Figure 2.2 and Table 2.2. Upon running the Pearson's correlation between HQ and IHS, there was a strong positive correlation which was statistically significant ($r = 0.835$, $N = 44$, $p<0.001$).

Parameters	**Acute Tinnitus**	**Post Acute Tinnitus**	**Significance Overall**
Tinnitus Frequency (Hz)	M = 6305.11 SD = 1899.22	M = 6551.35 SD = 1402.15	t = 0.521 p = 0.607
Tinnitus Loudness (dBSL)	M = 12.61 MD = 9.83 SD = 11.88	M = 8.51 MD = 6.08 SD = 9.26	z = -2.18 p = 0.029*
THI	M = 33.36 SD = 21.27	M = 25.18, SD = 23.86	F = 5.37, p = 0.03*
TFI	M = 103.77, SD = 46.28	M = 75.54, SD = 51.26	F = 19.409 p < 0.001*
HQ	M = 13.86 SD = 7.77	M = 13.77 SD = 7.98	F = 0.005 p = 0.945
IHS	M = 45.23 SD = 15.64	M = 43.23 SD = 16.82	F = 0.596 p = 0.449

Table 2.2: Longitudinal changes between Acute and Post Acute Tinnitus.

THI indicates Tinnitus Handicap Inventory, TFI indicates Tinnitus Functional Index, HQ indicates Hyperacusis Questionnaires, and IHS indicates Inventory of Hyperacusis Symptoms, F test indicates an RM-MANOVA carried out, and z test indicates the non-parametric Wilcoxon signed rank test.

2.4 Discussion

Overall, the results indicate peak tinnitus distress and hyperacusis symptoms during the initial acute stage that tends to reduce over the initial six months. However, with respect to the Hyperacusis questionnaire, there were no significant differences between the Acute and Post-Acute Tinnitus, possibly indicating that this measure is insensitive to longitudinal changes or alternatively indicating that the subjective degree of hyperacusis did not change in our cohort. We also observed a reduction in tinnitus loudness over time between the groups further indicating the possible role of adaptation of the tinnitus signal. With respect to the lack of differences seen in both hyperacusis questionnaires, our results indicate that the degree of hyperacusis (one of the subjective measures of gain) does not vary between the onset of tinnitus and its subsequent chronic stage which is an interesting finding to report. However, it is important to note that the HQ scale may lack robust psychometric properties, potentially affecting its reliability and validity. This limitation could have influenced the way our results were reflected (Greenberg & Carlos, 2018). The findings of a reduction in tinnitus severity are in accordance with our hypothesis about changes in distress over time. Other studies have assessed changes in tinnitus distress from its new onset into chronic stages (Wallhäusser-Franke et al., 2017; Vielsmeier et al., 2020; Zhang et al., 2023; Nyenhuis et al., 2013). The study by Wallhäusser-Franke et al. (2017), involved 47 participants experiencing tinnitus for no longer than four weeks, who completed questionnaires at four intervals: inclusion (T1), 6 weeks (T2), 3 months (T3), and 6 months (T4) post-onset. Audiograms were conducted at T1, and assessments included tinnitus loudness, distress, sound sensitivity, depression, anxiety, and coping strategies. Results indicated that 11% of participants achieved complete remission by T4, while 30% of those with high depression levels at T1 experienced worsening tinnitus-related distress. Both Vielsmeier et al. (2020) and Nyenhuis et al. (2013) report a significant reduction in distress from the onset of tinnitus. With these studies in mind, our findings are not novel in showing a reduction of tinnitus distress, but are, to our

knowledge, the first demonstration that this tendency for symptom reduction applies to the wider tinnitus population, rather than just those attending specialist clinics, and does not necessarily require any clinical intervention to achieve (Vielsmeier et al., 2020). However, Wallhäusser-Franke et al. (2017) did demonstrate a highly distressed subgroup that did not improve over the initial six months, even with management in a specialist clinic. Our study highlights the need to consider each individual's case when prognosticating and deciding on the need for specialist treatment (Wallhäusser-Franke et al., 2017). Other similar findings to the current study were reported by Zhang et al. (2023) with increased anxiety and sleep disturbances during the acute stage, increased depression and high frequency hearing loss during the chronic stage thereby highlighting a positive association between depression scores and the course of tinnitus and negative association between anxiety, sleep disturbances with the course of tinnitus (Zhang et al., 2023).

With respect to the usage of both hyperacusis questionnaires in the current study, the HQ and IHS can serve a dual purpose by not only evaluating the existence of hyperacusis in tinnitus but also accounting for another aspect of distress amongst tinnitus individuals. As far as we are aware, there are no literature documenting the HQ and IHS in Acute Tinnitus, but some have been documented generally in Chronic Tinnitus. Various research has highlighted the importance of the utility of hyperacusis questionnaires on tinnitus distress. Studies have highlighted that hyperacusis questionnaire scores positively correlate with tinnitus questionnaires scores and can be utilized as an additional tool for overall tinnitus distress evaluation (Degeest et al., 2016; Cederroth et al., 2020). These reports highlight the utility of hyperacusis questionnaires for overall tinnitus distress analysis, which is why we included both the HQ and IHS. Hyperacusis questionnaires might also give an indication of the degree of central gain subjectively experienced by individuals (Bigras et al., 2024).

Based on the current study's findings, the tinnitus loudness demonstrated a decreased score over time indicating that the tinnitus neural activity/intensity of the tinnitus signal might have been maximal around onset and reduced over time as increased neural activity is directly correlated with increased tinnitus loudness. Also, increased distress increases tinnitus loudness too (Eggermont & Roberts, 2004) and if correct, this would be in line with the proposal from the sensory precision model by Sedley et al. (2016) where precision of tinnitus tends to be maximal around the time of onset, as a causative factor in tinnitus onset, but regresses to the mean over a period of time and returns to approximately pre-tinnitus levels.

In this model, increased sensory precision is only relevant to the initial onset of tinnitus, and not required for chronic tinnitus to persist. Interestingly, in our study, both tinnitus loudness and tinnitus distress reduced over the initial six months but the hyperacusis scores remained unaltered, hence making it unclear whether if the intensity of the tinnitus signal reduces, why does the central gain remain unaltered? Is central gain and tinnitus distinct and if so, what are the neural correlates of tinnitus?

Questionnaires alone cannot distinguish these possibilities, which will require complementary avenues of scientific studies involving other subjective and objective physiological responses. These will be addressed in the upcoming chapters giving us a more wholistic measurement of tinnitus variations between its onset and chronic stages. The Acute and Post Acute time periods showed significant differences in tinnitus loudness but not on the hyperacusis questionnaires which is in support to the research carried out by Fackrell et al. (2015) where they have noted that hyperacusis questionnaires such as the HQ may not offer a comprehensive measure of sound hypersensitivity, particularly in individuals with tinnitus. These tools along with their limiting psychometric properties often fail to capture the full spectrum of auditory discomfort experienced in this population, which may limit their utility in accurately assessing hyperacusis severity or its interaction with tinnitus symptoms (Greenberg & Carlos, 2018). It is possible that the patients exhibited minimal hyperacusis and reported near normal hyperacusis scores that did not significantly change, as they were already close to the normal range.

In addition to the questionnaires that investigate tinnitus distress, neurophysiological studies can help understand the brain mechanisms of distress during initial onset. A cross-sectional comparison between Acute Tinnitus, Chronic Tinnitus, and healthy Control groups by Lan et al. (2021) documented the alterations in brain activity using electroencephalography (EEG). They found out that when compared between Acute and Chronic Tinnitus, the Acute Tinnitus had an increase in oscillatory power in the middle frontal gyrus and parietal cortex in the gamma band frequency. Comparing both the tinnitus groups with Controls, Acute Tinnitus had a reduction of power in the superior frontal cortex across all frequency bands. The Chronic Tinnitus had a reduction in power in the superior frontal cortex at beta 3 and gamma bands and an increase in the inferior frontal cortex at delta band and superior temporal cortex at alpha 1 band. Vanneste et al. (2011) examined the

correlates of tinnitus duration over a timescale of years and revealed that there are several changes in cortical structure and function which include increased activity in the auditory cortex, supplementary motor area, dorsal anterior cingulate cortex, and the insula. These studies indicate that various brain regions may be more active during the onset of tinnitus when compared to its chronic stages. The high distress in tinnitus during the initial stages could also be attributed to novelty processing, with attention to novel stimuli tending to subside as they become familiar, resulting in adaptation/habituation along with distress reduction (Czornik et al., 2022). Distress may also create an increased focused attention toward the tinnitus signal during the initial stage, along with the contribution of top-down cognitive factors, which is akin to increasing the precision of the tinnitus signal. Tinnitus being novel or new during its onset makes it more noticeable (Sedley et al., 2016).

Over time with respect to the tinnitus chronicity, there is, acceptance, adaptation, and habituation to the tinnitus signal that may reverse some of these effects of being more noticeable thus reducing attention/precision on the tinnitus signal. It is presently unclear whether these factors simply determine the level of tinnitus distress over time, or whether they have a more causal role in perpetuating tinnitus, allowing it to persist chronically rather than remitting altogether in the early stages. In the sensory precision model of tinnitus, the initial onset of tinnitus typically causes increased attention to the tinnitus signal, resulting in increased precision of the tinnitus signal. This increased attention towards the tinnitus might be a causal and perpetuating factor for the tinnitus to become chronic. Persistence of the tinnitus causes an update in the precision resulting in tinnitus becoming the default sensory prediction (Sedley et al., 2016). Roberts et al. (2013) similarly highlighted that, individuals with Chronic Tinnitus in general, have increased auditory attention towards tinnitus, and that this is a causative factor in the persistence and/or maintenance of tinnitus. These authors also state that for some tinnitus individuals, attention towards tinnitus might be negligible but still its characteristics remain the same. For highly distressed tinnitus individuals, it is this increased focused attention that causes maladaptive changes. Based on the understanding that there is a maladaptive plastic change in individuals with high distress. Our study suggests increased distress during the acute stages thereby increasing the tinnitus loudness and focussed attention towards it and resulting in a maladaptive plastic change which can be a possible causative factor to tinnitus persistence.

The transition of Acute to Chronic Tinnitus has received little attention, but from other sensory perspectives such as pain, psychological predictors such as emotional distress, helplessness-hopelessness, and negative emotions have been highlighted as some of the causative predictors from the transition of acute to chronic pain (Hasenbring et al., 2014). With respect to tinnitus, Wallhäusser-Franke et al. (2017) highlighted the importance of depression and anxiety as predictive factors for the continuation of high levels of tinnitus distress from acute to chronic stages. However, tinnitus is not completely analogous to pain, as pain by definition requires a negative emotional component, whereas tinnitus can persist as either an emotionally negatively valent percept or as an emotionally neutral one. The actual tinnitus can still exist with and without a negative component unlike pain which has a negative component (Gilam et al., 2020).

Our current study recruited a community sample of acute tinnitus and is not based on clinical patients. Tinnitus populations recruited from clinical settings would likely tend to have a high amount of distress as they seek clinical care due to the inherent severity of their respective tinnitus along with the issues that they may not be able to seek clinical care in a timely manner due to longer wait times in general physician practices (Thorlby et al., 2019). In our case, we recruited a community sample who may not be similar to the clinical population, but interestingly, the reduction in distress and tinnitus loudness we observed indicates some similarity in tinnitus progression between a community sample and a clinical population. There tends to be a natural reduction in distress over a period of time even without any professional healthcare services for tinnitus. McFerran et al. (2019) iterated the positive news/information from internet or social media that can have benefits but also cautions the adverse effect of these information on tinnitus distress taking into account the quality and reliability of information. This could be a larger reason to the natural reduction of distress where individuals with Acute Tinnitus tend to have accessed self-help information which would have had a positive impact on their tinnitus.

The tinnitus does habituate eventually after its onset, whether it is an emotional habituation or a physiological habituation, but the key question is why tinnitus persists over a period of time and why there isn't a remission despite a form of habituation being present. Here we considered whether the mechanism of increased distress and increased hypersensitivity (central gain) during the onset of tinnitus plays a role in the persistence of tinnitus due to a form of maladaptive plasticity as a consequence of the distress or is solely a consequential

symptom. Further examination into neural changes from acute to chronic stages based on the increased distress during onset may provide insight to any causal relationship.

Our study had certain limitations. Firstly, the sample size of the cohort was relatively small, which reflects the challenge of recruiting people so early in their tinnitus course, and of achieving retention of a group for whom their condition is of decreasing impact. Secondly, the follow-up to one year (beyond six months) could have given more detailed information about the time course of tinnitus symptoms as a one year period would definitely give the complete picture of the time course. Thirdly, there were significant amount of drop outs during the second phase of the experiment (49.02%) which could have led to possible selection biases considering majority of them did not respond to follow up reminders. Additionally, only THI and TFI were considered as distress questionnaires, whilst other non-tinnitus questionnaires such as anxiety and depression scales could have been used. Future studies could expand the current research with more subjective and objective measures.

2.5 Conclusion

The current study aimed to assess and monitor the level of distress and sound sensitivity changes from acute to chronic tinnitus. With changes in distress and tinnitus loudness, we conclude that both sensitivity, distress and the individual's reaction to tinnitus do tend to be maximum during the acute stage, and in most cases improves spontaneously over at least the initial six months.

Chapter 3 Exploring the short and long-term changes in auditory sensitivity between acute and chronic Tinnitus measured using categorical loudness scaling

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3.1 Introduction

3.1.1 Background

It is well established from the previous two sections that tinnitus is a phantom auditory percept and one of the major theories of tinnitus is that hearing loss results in abnormal increase in central gain (refer to Chapter 1, section 1.3). The auditory system possesses sophisticated mechanisms to adapt to the loss of processing incoming external stimuli. Tinnitus is argued to be a maladaptive by-product of this hyperexcitability or homeostatic plasticity, manifesting through increased spontaneous firing when intrinsic noise in the auditory pathways is amplified (Auerbach et al., 2014). Some other theories suggest that tinnitus arises from the auditory system's inability to sufficiently enhance central gain, with tinnitus being an outcome of failure to adapt to reduced input and hyperacusis being an outcome of over adaptive increase in response to gain (Knipper et al., 2013). These models suggest that tinnitus is linked to central gain, either through excessive hyperexcitability or an inability to adjust gain augmentation. Therefore, a primary focus in understanding the mechanism of tinnitus should involve assessments of central gain.

3.1.2 Tinnitus and hyperacusis stem from similar but distinct mechanisms

Hyperacusis is a condition where every day noises are regarded as abnormally loud, even excruciatingly so in some situations (Brotherton et al., 2015). One of the mechanisms of hyperacusis is a consequence of homeostatic plasticity where the central nervous system tries to stabilize neural firing rates following a loss of input in conjunction with the dysfunction of the descending auditory pathway and variations in the balance of excitation and inhibition which is similar to tinnitus (Pienkowski et al., 2014). Alternative approaches have suggested that tinnitus and hyperacusis may arise from distinct underlying mechanisms. Hyperacusis

can result from cochlear damage without significant hearing loss, such as the loss of ribbon synapses at the basolateral regions of inner hair cells, near the afferent synaptic contacts with type I spiral ganglion neuron dendrites. In contrast, tinnitus is typically associated with cochlear damage accompanied by measurable hearing threshold elevation, except in cases involving cochlear synaptopathy, where synaptic damage may occur without overt audiometric loss (Knipper et al., 2013; Kujawa & Liberman, 2015). Knipper et al. (2013) further proposed that while tinnitus arises from the inability of the brain to enhance central gain, hyperacusis in contrast arises due to overcompensation with central gain. With respect to slope (which is defined as the rate of changes in the output with respect to the change in input) an alternative hypotheses propose that tinnitus acts as a source of central noise, which elevates baseline neural activity without altering the input/output slope, due to its additive nature, while hyperacusis on the other hand increases the slope due to its multiplicative function in contrast to the additive function of tinnitus (Zeng, 2013). Auerbach et al. (2014) further highlighted in their central gain theory that tinnitus results from spontaneous hyperactivity in the central auditory pathway in the absence of an input while hyperacusis is caused by excess central gain to the auditory input thereby increasing sound intolerance. tinnitus has found to coexist with hyperacusis as a recent study by Cederroth et al. (2020) established that individuals with tinnitus are approximately three times more likely to exhibit hyperacusis, with the likelihood further increasing in cases of severe tinnitus. In contrast, not all individuals with hyperacusis are strongly associated with tinnitus, suggesting that while hyperacusis may occur independently, a degree of sound intolerance is commonly present in tinnitus patients. This shared feature points to the involvement of abnormal auditory gain. Therefore, assessing auditory gain—or changes linked to central gain mechanisms—may be crucial for understanding the underlying neurophysiological processes of both tinnitus and hyperacusis

3.1.3 Tinnitus heterogeneity and the need for Acute Tinnitus measures

The other aspect of tinnitus is that although it involves mechanisms of central gain, it is not equivalent across people. There are inter-subject variations amongst tinnitus sufferers with subjective continuous tinnitus lasting more than six months (chronic). Some instances of tinnitus may have varying degrees of distress (Beukes et al., 2021), sound intolerance or hyperacusis (Raj-Koziak et al., 2021; Hébert & Carrier, 2007), and hearing loss (Hébert &

Carrier, 2007), and even variations in tinnitus aetiology (Hiller & Goebel, 2006). The current literature in chronic tinnitus risks possible confounding by tinnitus heterogeneity and the question must be asked as to how much these factors would have an impact on tinnitus sensitivity, and how tinnitus would behave in isolation if these factors were controlled for. Examining the natural history of tinnitus within the same individuals might be an appropriate approach to remove some of these confounding factors. This pertains to the changes in tinnitus symptoms over time, without any medical or therapeutic interventions, or other major changes in physical health, mental or social health. It elucidates the progression, transformation, and potential resolution of tinnitus over time providing insight into the mechanism of tinnitus. By examining these characteristics over time, significant heterogeneous confounds and variables would be mitigated. This revisits our initial hypothesis regarding the changes in tinnitus from its acute phase to its eventual chronicity over time, which not only taps into its natural history but also aids in comprehending the transition from acute to chronic and ensures that the study remains unaffected by potential confounding variables related to heterogeneity between individuals. In Chapter 2, we obtained results in a community-based sample where longitudinally tinnitus distress reduced over time attributing to emotional and physiological habituation. The habituation of tinnitus in individuals is believed to be present over the time course from its onset until its chronic stages (Han et al., 2009). Regardless of habituation, the tinnitus tends to persist long-term in most individuals when continuously present for 4 weeks and has a reduced remission rate of just 15% (Sanchez & Roberts, 2021). Even with habituation that tend to reduce the impact of tinnitus over time from its onset, it is believed to be bothersome when present for a significantly prolonged period of time (Han et al., 2009). These variations in tinnitus from its onset necessitate the assessment of gain-related functions in both acute and chronic tinnitus to elucidate why, despite excessive gain inducing tinnitus, complete remission does not occur over time, even with stability in tinnitus characteristics.

3.1.4 Subjective measurement of gain in tinnitus

Measurement of tinnitus has variably used either or both of subjective and objective measures. Objective measures of tinnitus include usage of neuroimaging measures such as functional Magnetic Resonance Imaging (fMRI) or electrophysiological resting state functional connectivity measures to isolate the activity of tinnitus and its involvement of

various brain regions (Elgoyhen et al., 2015; Husain & Schmidt, 2014). Additionally, evoked electrophysiological measures involve usage of brain's response to sound which include both early and late potentials with each of these sometimes-demonstrating changes in amplitude as a potential biomarker for the possible presence of tinnitus activity (Fan & Li, 2022). Subjective gain measurements involve usage of questionnaires to assess tinnitus severity and distress (Brüggemann et al., 2016) or utilizing measures of Uncomfortable Loudness Level (ULL) or Categorical Loudness Scaling (CLS) to measure subjective levels of sound tolerance in tinnitus (Hébert et al., 2013). ULLs, defined as the threshold at which a sound is perceived as excessively loud, have been identified as predictive of both the presence and severity of tinnitus (Hébert et al., 2012). CLS on the other hand measures loudness growth (in the form of loudness growth curves) where the function of physical intensity of sound and perceived loudness is measured with physical intensities ranging from just audible to uncomfortably loud (Theelen-Van Den Hoek et al., 2014). Tinnitus and hyperacusis are thought to both stem from a shared basis of abnormal central gain enhancement; hence, a subjective loudness assessment of loudness intolerance would effectively quantify central gain in both conditions (Hébert et al., 2013). Hébert et al. (2013) carried out a study on chronic tinnitus by measuring the auditory sensitivity using CLS and found that sensitivity to loud sounds was increased in individuals with tinnitus when compared to normal hearing individuals, indicating a maladaptive central gain in tinnitus. Also, they revealed a presence of parallel loudness growth curves (loudness growth curves across different degree of hearing loss are parallel) in individuals with tinnitus across different degrees of hearing loss and convergent growth curves (loudness growth curves across different degree of hearing loss converge towards the high intensity) in non-tinnitus individuals across different degrees of hearing loss further indicate consistent sensitivity for tinnitus regardless of degree of hearing loss and recruitment like pattern for non-tinnitus individuals. Figure 3.1 illustrates the different growth curves (parallel and convergent) between the tinnitus and non-tinnitus individuals across different degrees of hearing loss.

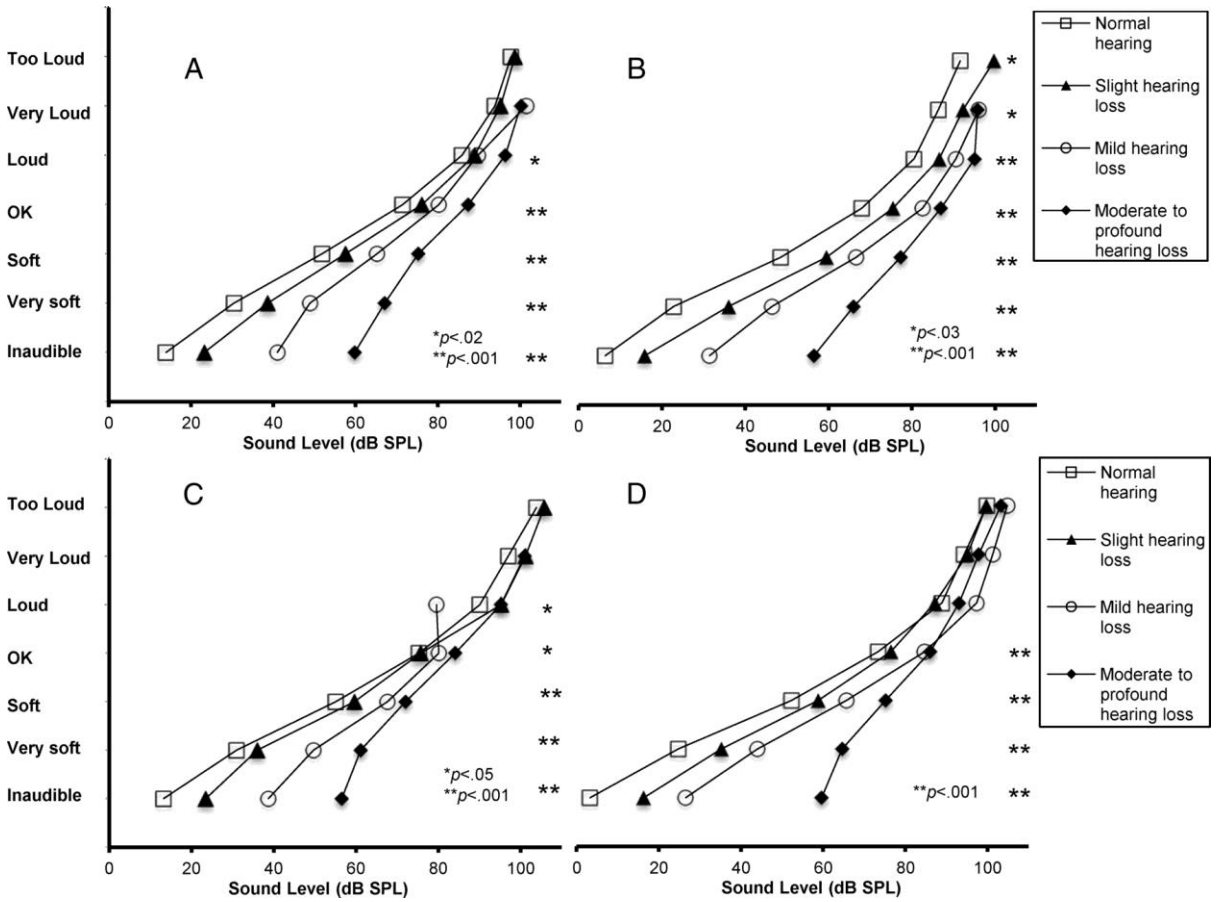


Figure 3.1: Loudness growth curves between Tinnitus (A- 1 kHz, B – 4 kHz) and Non-Tinnitus Controls (C – 1 kHz, D – 4 kHz).

The graph indicates the parallel loudness growth curves and convergent loudness growth curves for both tinnitus and control groups respectively. B (tinnitus group) has growth curves, across different degrees of hearing loss, that are parallel to each other, indicating consistent sensitivity for tinnitus individuals. Both C and D (controls) have growth curves, across different degrees of hearing loss, that converge towards the high intensities indicating recruitment like pattern. The content in this figure have been reproduced from Hébert et al. (2013)

Another study by Zeng (2013) proposed a model based on active loudness growth for both Tinnitus and hyperacusis whereby he highlighted the presence of a multiplicative increase (central gain) in the input-output function for hyperacusis thus making the slope steeper and lowering the ceiling (reduced ULL). For tinnitus on the other hand, the slope does not change, and the floor is raised, thus making the input-output function more additive (central noise) in nature. Articles related to subjective measurement of auditory sensitivity in tinnitus have highlighted the reduction of ULLs in tinnitus. Shim et al. (2021) explored ULL and Auditory Brainstem Response in Tinnitus and Non-Tinnitus and their results revealed to have reduced ULL when compared to non-tinnitus, attributing possible underlying factors beyond peripheral mechanism at the level of central auditory system. Shin et al. (2024) carried out a retrospective study on ULLs in Tinnitus attempting to correlate levels of hearing with the loudness discomfort levels and they stated that there was notable association between loudness discomfort levels and emotional factors in tinnitus rather than the auditory thresholds. According to the analyses of (Zeng, 2013) and Hébert et al. (2013) regarding alterations in auditory sensitivity associated with tinnitus, the slope of the loudness growth function is a critical metric for assessing these changes, particularly in relation to central gain, which Zeng (2013) elucidates in the context of slope function variations.

3.1.5 Aim and hypothesis

In the previous section, we have determined that the slope of subjective categorical loudness scaling (loudness levels) serves as an effective measure of central gain. The reason to consider the slope of the CLS over just the single measurement of ULL is that the differences in ULL might be attributed to change in slope or even could be due to a baseline shift of loudness levels without variating the slope (as in central noise by Zeng (2013)). The slope and individual loudness categories can aid in differentiating in central gain and central noise (Zeng, 2013). Revisiting our central hypothesis, our primary measure for tinnitus is in relation to central gain which cannot be interpreted without the function of slope and our central hypothesis is revolved around the fact that the central gain is maximal around the tinnitus onset and later reduces over time as a regression to the mean. We predict a decrease in slope values (improvement in sound tolerance) and attribute tinnitus habituation to be causing it.

3.2 Methods

3.2.1 Participants

We studied 51 individuals with Acute Tinnitus, defined as lasting between 3 days and 6 weeks, with a mean duration of 4.06 weeks (SD 2.22 weeks) and a median duration of 4 weeks. Additionally, we assessed 51 individuals with Chronic Tinnitus, defined as tinnitus persistence lasting over 6 months, with a mean duration of 8 year (SD 8 years) median duration of 5 years, and 35 Non-Tinnitus Controls age and hearing-matched to the Acute Tinnitus group. Participants were recruited via community advertisements on Google Ads and internally from Newcastle University's research volunteer pool. Fifty-one participants with Acute Tinnitus were invited for reassessment after a minimum of six months from the onset of tinnitus, indicating their chronic stage, and 26 of them volunteered for and completed this further testing. This is designated as the 'Post Acute' group to differentiate it from the cohort recruited during the chronic phase of tinnitus. The criteria for inclusion were as follows:

- Acute Tinnitus group: continuous non-pulsatile tinnitus present for between 3 days and 6 weeks.
- Chronic Tinnitus group: continuous non-pulsatile tinnitus for more than 6 months
- Age 18 or over, with ability to provide informed consent.

Exclusion criteria:

- Meniere's disease
- Epilepsy
- Middle ear pain or infections

Control participants were attempted to be matched to the acute Tinnitus group for age, sex, and hearing. Details are displayed in table 3.1.

3.2.2 Audiological Assessment

The experimenter interviewed the subjects before the experiment, verifying a medical history consistent with subjective tinnitus and the absence of atypical symptoms or worries over an undetermined underlying cause. Before this, participants completed a concise, individualised pre-screening questionnaire that evaluated the inclusion and exclusion criteria. Participants submitted demographic data, including age and sex, alongside details regarding their tinnitus,

including type (tonal, noise-like, or other), duration, affected ear(s), and a historical account of physical and mental health, particularly any otological disease. Four common, validated questionnaires—

- The Tinnitus Handicap Inventory (THI) (Newman et al., 1996).
- The Tinnitus Functional Index (TFI) (Meikle et al., 2012).
- The Hyperacusis Questionnaire (HQ) (Khalfa et al., 2002).
- The Inventory of Hyperacusis Symptoms (IHS) —were used to evaluate the impact and distress related to Tinnitus and any co-existing hyperacusis symptoms (Greenberg & Carlos, 2018).

Participants underwent a Pure Tone Audiometry (PTA) test to establish hearing thresholds at octave frequencies ranging between 250 Hz up to 8 kHz. Thresholds were estimated based on the initial presented level at either 40 dBHL or above depending on the participant's residual hearing. If perceived audible, the stimulus was continuously reduced by 15 dBHL until the participant did not perceive it as audible. From the first reversal, a 5 dB up, 10 dB down staircase procedure was then used until the final threshold was established by two positive responses out of three trials.

3.2.3 Tinnitometry

The frequency and loudness of tinnitus were assessed using a tinnitometry approach for patients with acute or Chronic Tinnitus. In instances of unilateral tinnitus, matching stimuli were delivered contralaterally, bilateral tinnitus, they were administered bilaterally. Participants initially matched the loudness of their tinnitus, followed by matching its frequency, beginning at a reference level of 6 kHz. In bilateral tinnitus cases, supplementary modifications were implemented to equilibrate the two ears when tinnitus presented asymmetrically. A procedure was implemented in which the loudness and then frequency were sequentially and iteratively modified until no further adjustments were deemed required by the participant to provide an optimal match. The technique was conducted over three trials, and the average of these trials was determined as the final intensity and frequency of the tinnitus. The stimulus employed for this method was either a pure tone or narrow band noise, contingent upon the subjective characteristics of the tinnitus.

The Minimum Masking Level (MML) was assessed for the tinnitus ear in cases of Unilateral Tinnitus and for each ear separately in instances of bilateral tinnitus. To ascertain MML, a

narrowband noise was introduced at the threshold level and increased in 1 dB steps until subjects perceived total masking of the tinnitus by the noise. To ensure experimental consistency, the high frequencies presented to Control participants were calibrated to mirror the individualized tinnitus frequency parameters used for each corresponding acute tinnitus subject. The mean frequency presentation across Control participants was ensured it matched both Acute and Chronic Tinnitus.

3.2.4 Categorical Loudness Scaling (CLS)

Following the tinnitometry procedure, a loudness scaling procedure was carried out where the participants rated the loudness of repeated stimuli in ascending intensity according to the following scale: (1) just audible, (2) very soft, (3) soft, (4) medium, (5) loud, (6) very loud, and (7) uncomfortably loud, which was adapted from (Hébert et al., 2013).
The stimulus was a one-third octave noise tailored centred on either a control frequency (1 kHz) or the matched tinnitus frequency (derived from the assessed tinnitus frequency via tinnitometry), presented in ascending sequence from lower to higher levels, with individuals aligning their respective levels to the associated categories sequentially. This process was repeated sequentially across ears and frequencies. The process was performed only once, and in an ascending intensity manner, unlike the randomized intensity presentation order of Hébert et al. (2013). Given that the additional experimental procedures—outlined in Chapters 4, 5, and 6—accumulated to a total duration of 3.5 hours, the study design prioritized minimizing participant burden. To reduce the cumulative auditory exposure and potential fatigue associated with prolonged testing sessions, only a single trial per ear and frequency was administered. This presentation aligns with established clinical audiological protocols for determining Uncomfortable Loudness Levels (ULLs), which typically employ an ascending method of limits—such as the Hughson-Westlake procedure—to identify the threshold at which sound becomes uncomfortably loud. This approach has been adapted for use in Categorical Loudness Scaling (CLS) procedures to ensure consistency and reliability in assessing loudness perception (‘Recommended Procedure Determination of uncomfortable loudness levels’, 2022a). Stimuli were generated and presented using the Matrix Laboratory (Matlab) version R2019a, using the Psychtoolbox toolbox (Pelli, 1997).

3.2.5 Data analysis

Data were averaged across ears in cases of bilateral tinnitus and in case of unilateral tinnitus, only the measures for the tinnitus ear were considered. For Controls, they were averaged across ears for all participants. The slopes (expressed loudness category per dB increase) across five categories (soft, medium, loud, very loud, uncomfortably loud) with categories as independent and loudness levels as the dependent variables, were computed using linear regression for each frequency for each participant, and the resultant slope values were compared among groups. Subsequent statistical analyses were conducted using SPSS version 28. The slopes were compared between groups of Acute Tinnitus, Chronic Tinnitus, and Controls using individual One-Way Analysis of Variance (ANOVA) for 1 kHz and Tinnitus frequency, as both exert unique effects on the dependent variable (CLS slope) which might have otherwise been obscured. Analysing them independently enabled us to concentrate on their individual consequences without interaction effects, along with a targeted hypothesis specifically for each frequency. If a significant effect was found, a Tukey's post hoc test was implemented. Separate analyses were conducted for each category across the groups utilising One-way Multivariate ANOVA, treating each loudness category as a dependent variable across both frequencies, with Tukey's post hoc test applied if a significant main effect was identified. A paired t-test was conducted between the Acute and Post Acute Tinnitus groups to determine the significance of any longitudinal changes in the overall slope, and individual loudness categories, for each frequency as this was the primary outcome measure being a longitudinal design.

3.3 Results

This section is divided into five sections: 1) demographics and hearing between the groups; 2) differences in Tinnitus distress between the groups; 3) differences in levels of hyperacusis; 4) CLS slope across and within groups; 5) differences across loudness categories.

3.3.1 Demographics and Hearing

Participant groups were attempted to be matched for age, sex, and hearing. There were no significant differences across the groups for age ($F = 1.60$, $p = 0.204$) but significant effects found in sex ($X^2 = 7.046$, $p = 0.029$), with Chronic Tinnitus having more males than both Acute Tinnitus ($X^2 = 5.784$, $p = 0.016$) and Controls ($X^2 = 4.443$, $p = 0.035$). With respect to

hearing, using the 1 kHz, 4 kHz, and 8 kHz hearing thresholds, an attempt was made to pair the groups. There were no significant differences across the groups for 1 kHz ($F(2,132) = 2.344$, $p = 0.1$), however differences were present at 4 kHz ($F(2,132) = 13.04$, $p < 0.001$) with differences present between Acute ($M = 23.97$ $SD = 15.57$) and Chronic Tinnitus ($M = 36.57$, $SD = 18.36$) ($p = 0.012$), differences between Chronic Tinnitus and Controls ($M = 19.37$, $SD = 14.96$) ($p < 0.001$), and no differences between Acute Tinnitus and Controls (0.378). Similar results were obtained for 8 kHz ($F(2,132) = 13.84$, $p < 0.001$) with differences between Acute Tinnitus ($M = 34.31$, $SD = 19.72$) and Chronic Tinnitus ($M = 50.56$, $SD = 20.99$) ($p = 0.01$), Chronic Tinnitus and Controls ($M = 27.43$, $SD = 23.08$) ($p < 0.001$), and none between Acute Tinnitus and Controls ($p = 0.28$). In all instances of significant differences, Chronic Tinnitus had higher hearing thresholds than both Acute Tinnitus and Control groups. Despite efforts to match participants for hearing ability, the chronic tinnitus group in our study exhibited unmatched high-frequency hearing loss. This suggests that prolonged tinnitus distress may be associated with progressive deterioration in the high-frequency regions of the auditory spectrum. For further details please refer to table 3.1.

Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Age (Years)	N = 51 M = 54.86 SD = 12.78	N = 49 M = 57.9 SD = 10.56	N = 35 M = 53.29 SD = 13.65	F = 1.6 p = 0.204	NS
Sex(M/F)	20/31	31/18	14/21	$X^2 = 7.046$ p = 0.029*	Acute – Chronic: p = 0.016* Chronic – Control: p = 0.035* Acute – Control: p = 0.942

Tinnitus Ear (B/L – U/L)	27/24	29/20	N/A	$X^2 = 0.392$ p = 0.52	NS
Hearing (1 kHz) dBHL	M = 12.31 SD = 8.20	M = 15.34 SD = 15.10	M = 10.75 SD = 11.48	F = 0.801 p = 0.453	NS
Hearing (4 kHz) dBHL	M = 23.97 SD = 15.57	M = 36.57, SD = 18.36	M = 19.37, SD = 14.96	F = 13.05 p < 0.001*	Acute – Chronic: p = 0.012* Chronic – Control: p < 0.001* Acute – Control: p = 0.378
Hearing (8 kHz) dBHL	M = 34.31, SD = 19.72	M = 50.56, SD = 20.99	M = 27.43, SD = 23.08	F = 13.84 p < 0.001*	Acute – Chronic: p = 0.01* Chronic – Control: p < 0.001* Acute – Control: p = 0.28

Table 3.1: The participants demographics for each group are shown in Table 3.1.

*N denotes number of participants, M denotes mean, SD denotes Standard Deviation, MD denotes Median, F statistic indicates a One Way ANOVA has been carried out, H statistic with X^2 indicates a non-parametric Kruskal Wallis test, X^2 indicates a chisquare goodness of fit test, NS denotes No Significant difference. * indicates a statistical significant difference at p < 0.05. Hearing thresholds were calculated by averaging the left and right ear values in*

cases of bilateral Tinnitus and the ear of Tinnitus in case of unilateral Tinnitus across 3 frequencies.

3.3.2 Symptom Scores for Tinnitus Distress

Cross-sectional comparisons between the Acute and Chronic Tinnitus groups for THI and TFI yielded no significant differences (THI: t (90) = -1.12, p = 0.263, TFI: t (90) = -1.634, p = 0.105). A paired comparison between the Acute and Post-Acute Tinnitus groups revealed a significant reduction over time in THI score (t(21) = -2.317, p = 0.03), TFI score (t(21) = -4.4, p < 0.001) with Acute Tinnitus having higher THI (M = 33.36, SD = 21.27) and TFI (M = 103.77, SD = 46.28) scores when compared to Post-Acute Tinnitus (THI - M = 25.18, SD = 23.86, TFI – M = 75.54, SD = 51.26).

3.3.3 Symptom Scores for Hyperacusis Questionnaires

Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Tinnitus Frequency (Hz)	N = 51 M = 5994.90 SD = 2293.09	N = 49 M = 6761.72 SD = 2231.77	N = 35 M = 5909.39 SD = 2132.54	F = 2.043 p = 0.134	NS
Tinnitus Loudness (dBSL)	M = 14.58 MD = 12.5 SD = 15.1	M = 8.57 MD = 6.5 SD = 11.70	N/A	z = 1.924 p = 0.054	NA
THI	M = 34.69 SD = 21.4	M = 39.86 SD = 22.58	N/A	t = -1.12, p = 0.263,	NA
TFI	M = 104.22	M = 121.98	N/A	t = -1.634, p = 0.105	NA

	SD = 46.56	SD = 57.56			
HQ	M =13.43 SD = 7.19	M =16.02 SD = 10.69	M = 8.41 SD = 5.62	F = 7.662, p < 0.001*	Acute Tinnitus– Control; p = 0.02*
					Chronic Tinnitus – Control; p = 0.002*
IHS	M = 43.8 SD = 13.73	M = 48.52 SD = 17.5	M =32.48 SD = 7.67	F = 12.03 p < 0.001*	Acute Tinnitus– Control; p = 0.01*
					Chronic Tinnitus – Control; p < 0.001*

Table 3.2: Participant's tinnitus characteristics for each group

*N denotes number of samples, M denotes mean, SD denotes Standard Deviation, MD denotes Median, F statistic indicates a One Way ANOVA has been carried out, t statistic indicates and independent t test done, z statistic indicates a non-parametric Mann-Whitney u test has been carried out, N/A- Not Applicable, NS denotes No Significance. * indicates presence of a statistically significant difference at p < 0.05. THI - Tinnitus Handicap Inventory, TFI – Tinnitus Functional Index, HQ – Hyperacusis Questionnaire, IHS – Inventory of Hyperacusis Symptoms*

Significant main effects of group were observed in cross-group comparisons between acute, chronic, and non-Tinnitus controls $F(2,120) = 7.662$, $p < 0.001$). Tukey's post-hoc test revealed significant differences between Controls and Acute Tinnitus ($p = 0.02$) and chronic

Tinnitus (p = 0.002). Controls had lower HQ scores (M = 8.41, SD = 5.62) compared to both Acute Tinnitus (M = 13.43, SD = 7.19) and Chronic Tinnitus (M = 16.02, SD = 10.69). Consistent findings were obtained for IHS, $F(2,120) = 12.03$, $p < 0.001$, and as shown by Tukey's post hoc test, controls had lower IHS scores (M = 32.48, SD = 7.67) in comparison to Acute Tinnitus (M = 43.8, SD = 13.73, p = 0.01) and Chronic Tinnitus (M = 48.52, SD = 17.5, $p < 0.001$). For both the questionnaires, there were no significant differences between Acute Tinnitus and Chronic Tinnitus (HQ; p = 0.348, IHS; p = 0.298) Upon the paired longitudinal comparison, no change over time was observed for scores on either HQ ($t(21) = -0.071$, p = 0.943) and IHS ($t(21) = -0.771$, p = 0.448). The results of the symptom questionnaires and tinnitometry for each of the three groups are shown in table 2.1 (chapter 2) and table 3.2.

3.3.4 CLS Slope

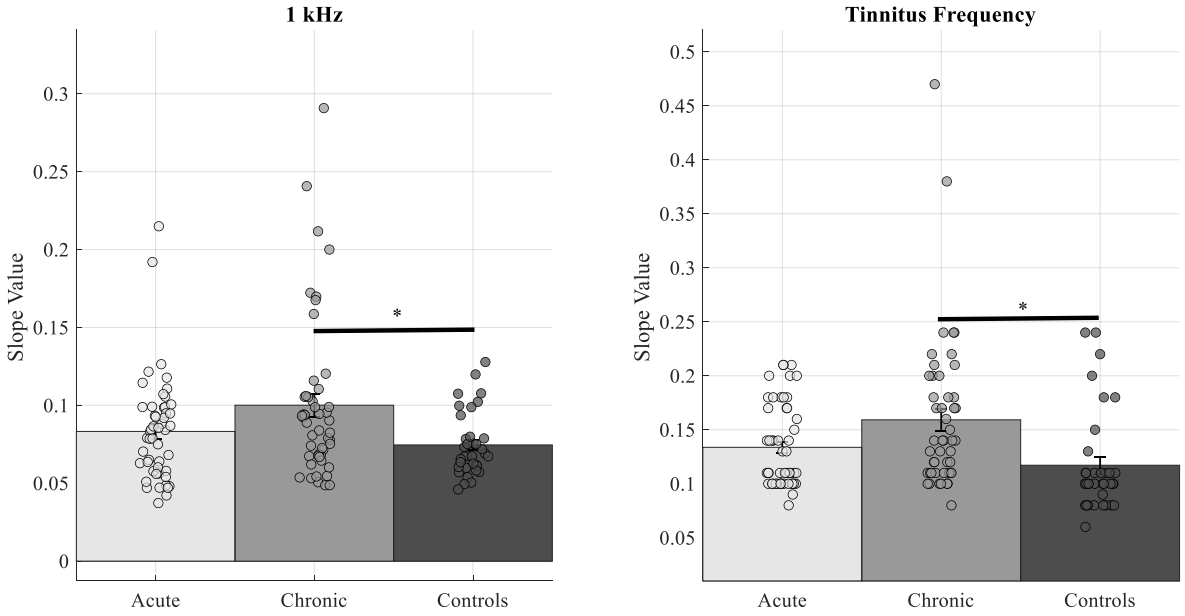


Figure 3.2: The slope differences between Acute Tinnitus, Chronic Tinnitus, and Controls. *The graph illustrates the slope for 1 kHz and tinnitus frequency and the error bars indicate the Standard Error of the Mean (SEM) and '*' indicates the presence of statistically significant different. The slope is defined as loudness category per dB increase. The slope is defined as loudness category per dB increase*

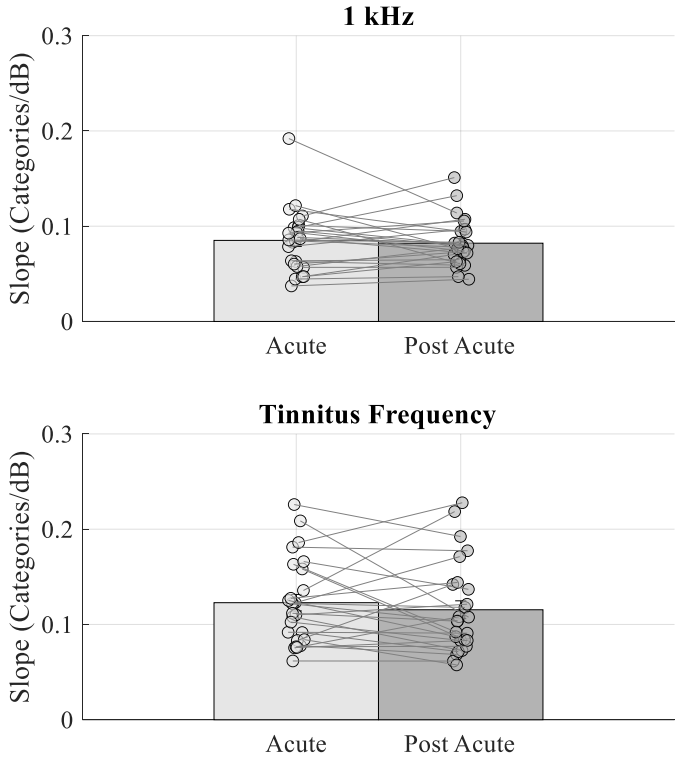


Figure 3.3: The slope differences between Acute and Post Acute Tinnitus. *The graph illustrates the slope for 1 kHz and tinnitus frequency and the error bars indicate the Standard Error of the Mean (SEM). The slope is defined as loudness category per dB increase*

The slopes (loudness category per dB) among the categories were examined across the groups of Acute Tinnitus, Chronic Tinnitus, and Non-Tinnitus Controls. The slope of the loudness growth function was determined for each participant by applying linear regression across categorical loudness scaling (CLS) data. This analysis quantified the rate of change in perceived loudness increase in stimulus intensity, thereby characterizing individual loudness growth patterns. Results indicate a significant effect among groups at 1 kHz ($F(2,132) = 4.811$, $p = 0.010$) with no significant difference between Acute Tinnitus and Chronic Tinnitus ($p = 0.081$), no significance between Acute Tinnitus and Controls ($p = 0.568$), and a significant difference between Chronic Tinnitus ($M = 0.18$, $SD = 0.1$) and Controls ($M = 0.1$, $SD = 0.04$) ($p = 0.01$) upon the Tukey's post hoc test. There was no significant correlation between sex and slope ($r = -0.003$, $p = 0.977$) implying sex was not a confound for the group differences in slope values, despite the group difference in sex distribution.

Due to the potential impact of the significant group differences in high-frequency hearing (4 kHz and 8 kHz), it was necessary to account for a potential confound of hearing loss on the measured slope. To establish the possible presence of a potential, confound of the differences in hearing upon slope, we conducted a correlation analysis between hearing thresholds at 8 kHz and loudness levels across categories (slope), which revealed a significant positive correlation ($r = 0.515$, $p < 0.001$). However, there was no significant correlation between sex and slope ($r = 0.068$, $p = 0.436$) implying sex was not a confound for the obtained slope values for tinnitus frequency.

A One-way Analysis of Covariance (ANCOVA) was conducted at tinnitus frequency with hearing thresholds at 8 kHz as a covariate. Similar results were yielded at tinnitus frequency with an overall significant effect across the groups ($F(2,132) = 4.58$, $p=0.012$) with a non-significant trendbetween Acute Tinnitus and Chronic Tinnitus ($p = 0.071$), no significant difference between Acute Tinnitus and Controls ($p = 1$), and presence of significant difference between Chronic Tinnitus and Controls ($p = 0.014$). Chronic Tinnitus ($M = 0.16$, $SE = 0.01$) had a higher mean slope compared to Acute Tinnitus ($M = 0.13$, $SE = 0.009$), and Controls ($M = 0.12$, $SE = 0.01$). *To Note: Mean values mentioned for tinnitus frequency are estimates after controlling for hearing at 8 kHz.*

Longitudinal analysis between Acute and Post Acute Tinnitus yielded no statistically significant difference between the groups, based a one tailed paired t statistic test for either 1 kHz ($t(25) = 0.569$, $p = 0.287$) or tinnitus frequency ($t(25) = 0.922$, $p = 0.183$). Figures 3.2 and 3.3 illustrate the values across groups for both cross sectional comparisons and longitudinal comparisons.

3.3.5 CLS Categories

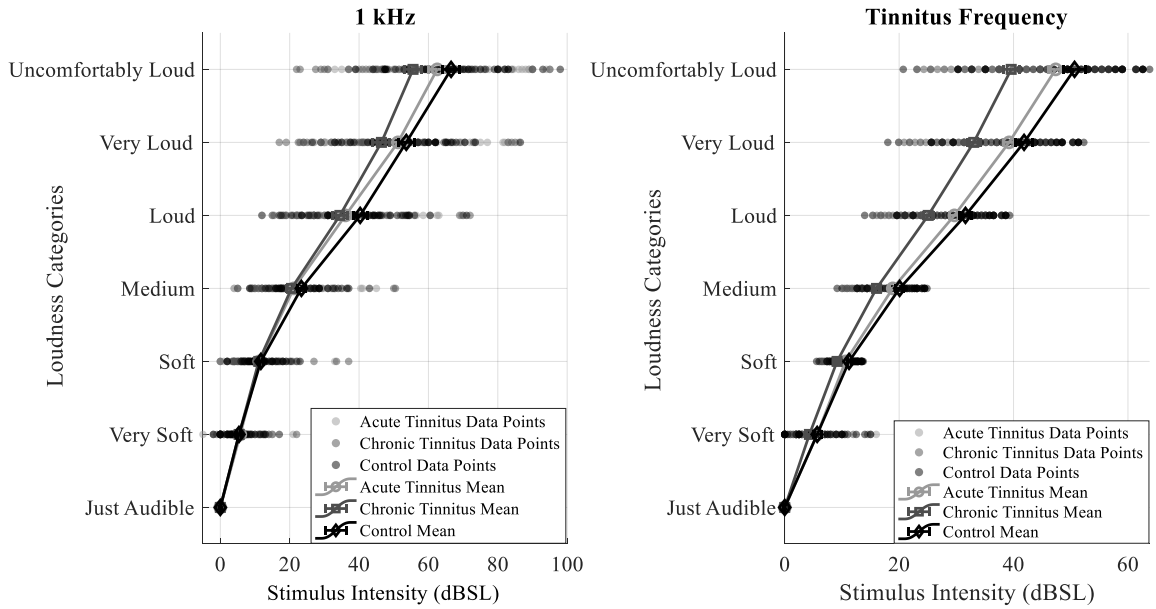


Figure 3.4: Loudness category differences between Acute, Chronic, and Non-Tinnitus Controls. *The graph illustrates the average category values among the groups for 1 kHz and the residuals after adjusting for hearing at 8 kHz at the Tinnitus frequency.*

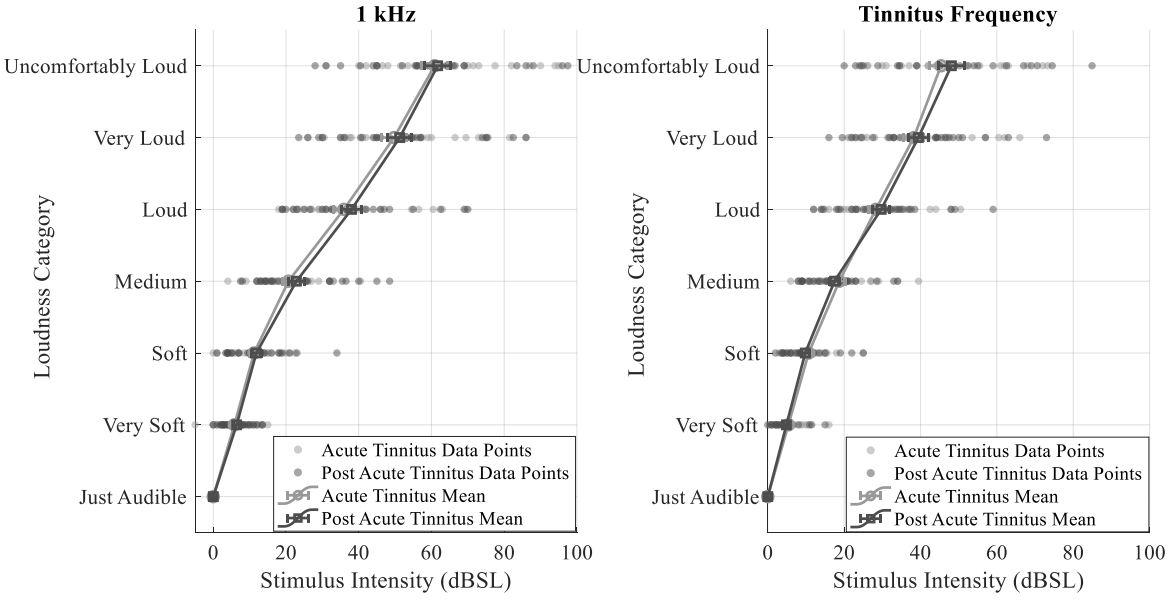


Figure 3.5: Loudness category differences between Acute and Post Acute Tinnitus. *The graph illustrates the average category values among the groups for 1 kHz and frequency.*

Loudness categories ('very soft' category to ULL) were compared cross-sectionally among groups of Acute Tinnitus, Chronic Tinnitus, and Controls using a One-way MANOVA, as well as longitudinally within the groups between Acute and Post-Acute Tinnitus using an RM ANOVA. The loudness categories were examined across the groups of Acute Tinnitus, Chronic Tinnitus, and Non-Tinnitus Controls. For 1 kHz, there was an overall main effect of group on the loudness categories ($F(12,254) = 1.899$, $p = 0.035$, Wilk's $A = 0.842$, $\eta_p^2 = 0.082$). The very soft category did not exhibit any significant effect (group difference) ($F(2,132) = 0.100$, $p = 0.905$, $\eta_p^2 = 0.002$), similarly no effect was found for soft category ($F(2,132) = 0.095$, $p = 0.910$, $\eta_p^2 = 0.001$), medium category ($F(2,132) = 1.109$, $p = 0.333$, $\eta_p^2 = 0.017$), loud category ($F(2,132) = 1.874$, $p = 0.158$, $\eta_p^2 = 0.028$), and very loud category ($F(2,132) = 2.15$, $p = 0.12$, $\eta_p^2 = 0.032$).
Significant differences were found at the uncomfortably loud category ($F(2,132) = 4.696$, $p = 0.011$, $\eta_p^2 = 0.066$), between Chronic Tinnitus and Controls ($p = 0.011$), no significant differences between Acute Tinnitus and Chronic Tinnitus ($p = 0.119$), or between Acute Tinnitus and Controls ($p = 0.835$). Chronic Tinnitus had reduced loudness for the ULL category ($M = 55.56$, $SD = 17.5$) when compared to both Acute Tinnitus ($M = 62.52$, $SD = 17.7$) and Controls ($M = 66.53$, $SD = 14.08$). The above-mentioned results were obtained after correcting for multiple comparisons using Bonferroni multiple comparison analysis. Figure 3.4 illustrates the changes in individual loudness levels for both 1kHz and tinnitus frequency across groups.

Longitudinal analysis between Acute and Post Acute Tinnitus also revealed no significant difference between the groups for loudness categories upon RM ANOVA ($F(6, 20) = 0.266$, $p = 0.946$). Figure 3.5 illustrates the changes in individual loudness levels for both 1kHz and tinnitus frequency between groups of Acute and Post Acute Tinnitus.

Due to the substantial impact of high-frequency hearing (4 kHz and 8 kHz), it was necessary to account for a potential confound of hearing loss on the measured loudness levels across categories. We conducted a correlation analysis between hearing thresholds at 8 kHz and loudness levels across categories, which revealed a significant negative correlation at the soft category ($r = -0.336$, $p < 0.001$), medium category ($r = -0.445$, $p < 0.001$), loud category ($r = -0.489$, $p < 0.001$), very loud category ($r = -0.544$, $p < 0.001$), and uncomfortably loud ($r = -0.592$). Due to the strong impact of hearing at 4 and 8 kHz on tinnitus frequency, we opted to

adjust for hearing in the supplementary analysis, thereby doing a Multivariate Analysis of Covariance (MANCOVA) with hearing at 8 kHz as a covariate.

A nearly significant difference in loudness categories was observed among the groups after adjusting for hearing ($F(12,254) = 1.599$, $p = 0.092$, Wilk's $A = 0.863$, $\eta_p^2 = 0.071$). There were no significant effect at the very soft category ($F(2,132) = 0.329$, $p = 0.720$, $\eta_p^2 = 0.005$), soft category ($F(2,132) = 0.1.175$, $p = 0.312$, $\eta_p^2 = 0.018$), medium category ($F(2,132) = 2.343$, $p = 0.100$, $\eta_p^2 = 0.035$). However, a significant effect was present for the loud category ($F(2,132) = 3.627$, $p = 0.029$, $\eta_p^2 = 0.052$) with differences between Chronic Tinnitus and Controls ($p = 0.032$) with Controls having increased intensity for loud category ($M = 34.1$, $SE = 2$) when compared to Chronic Tinnitus ($M = 27.78$, $SE = 1.7$). No significant difference between Acute Tinnitus and Chronic Tinnitus ($p = 1$) and between Acute Tinnitus and Controls ($p = 0.111$). For the very loud category, there was an overall effect between the groups ($F(2,132) = 3.627$, $p = 0.028$, $\eta_p^2 = 0.053$) with significant differences present between Chronic Tinnitus and Controls ($p = 0.024$), Controls ($M = 42.8$, $SE = 2.33$) had increased intensity for very loud category when compared Chronic Tinnitus ($M = 34.14$, $SE = 2.02$).

No significant difference between Acute Tinnitus and Chronic Tinnitus ($p = 0.703$) and between Acute Tinnitus and Controls ($p = 0.223$). Similar results were obtained for the uncomfortably loud category with a presence of overall effect ($F(2,132) = 4.996$, $p = 0.008$), with the presence of significant difference between Chronic Tinnitus and Controls ($p = 0.006$). Controls have increased intensity for the ULL category ($M = 51.95$, $SE = 2.57$) when compared to Chronic Tinnitus ($M = 40.77$, $SE = 2.23$). No significant difference between Acute Tinnitus and Chronic Tinnitus ($p = 0.428$) and between Acute Tinnitus and Controls ($p = 0.134$).

Longitudinal analysis between Acute and Post Acute Tinnitus also revealed no significant effect between the groups for loudness categories upon RM ANOVA ($F(6, 20) = 1.425$, $p = 0.254$). *To Note: Mean values mentioned for Tinnitus frequency are estimates after controlling for hearing at 8 kHz.*

3.4 Discussion

Our results reveal two primary highlights: first, the longitudinal changes between Acute and Post Acute Tinnitus showed no significant differences; even in a one-tailed test, only a non-

significant downward trend in both slope and loudness categories was observed; second, the cross-sectional comparisons indicate that Chronic Tinnitus exhibits an increase in slope and loudness categories relative to both Acute Tinnitus and Controls, even after controlling for hearing. This section elaborates the relevance of these two contrasting results.

3.4.1 Acute to Post Acute Longitudinal Changes

No alterations in slope or loudness categories were observed at the Post Acute stage compared to the Acute stage, highlighting the relatively consistent sound sensitivity over time, and not in keeping with our hypothesis of increased central gain around the onset of tinnitus and a subsequent regression to the mean with respect to reactivity to stimuli in either of the stimuli. It is noteworthy that both of our Tinnitus-related distress questionnaires (THI and TFI) exhibited a substantial reduction with time, although the hyperacusis questionnaires did not, indicating no changes in sound tolerance or auditory sensitivity or gain between the groups (Khalfa et al., 2002; Gu et al., 2010). While there is a lack of specific research on the alterations in auditory sensitivity from Acute to Chronic Tinnitus, our findings regarding the alleviation of distress over time were corroborated by Vielsmeier et al. (2020), who emphasised that although the characteristics of tinnitus remained unchanged, there was a moderate decrease in Tinnitus-related distress over time. Other findings related changes in tinnitus distress has been highlighted in chapter 2. The consistency across all these studies is that distress diminished, although the characteristics of tinnitus remained unchanged, which aligns with our findings where distress decreased, but traits associated with hypersensitivity did not. It can be further claimed that distress might in principle also create an increased focused attention toward the Tinnitus signal during the initial stage, along with the contribution of top-down cognitive factors, which is akin to increasing the precision of the Tinnitus signal. This increased attention to the Tinnitus signal during the onset, if it occurs, does not necessarily correspond to increased attention to external stimuli, as no changes in slope were seen despite reduction in distress

Our original hypothesis was based on the sensory precision model by Sedley et al. (2016) where increased central gain in the auditory system is implicated in the development and persistence of tinnitus. The model further emphasizes that this gain increase can exaggerate prediction errors resulting from reduced auditory input, initially generating, or further reinforcing the tinnitus percept. The amplified neural activity, coupled with increased

precision weighting of prediction errors increased around the onset of tinnitus and regressed to the mean over time. However, such increased central gain was not observed in this study in association with Acute Tinnitus or its evolution over time, at least manifest in subjective loudness tolerance. That said, gain can take many forms, at many levels, and this observation in isolation does not mean gain is not relevant in Tinnitus in some other form.

3.4.2 Changes between Acute, Chronic and Controls

It is noteworthy that no significant cross-sectional differences were observed between Acute Tinnitus and Controls. We previously posited that the onset of tinnitus is associated with an excess gain, a notion supported by existing data that indicates a compensatory augmentation of central auditory activity in response to diminished input, which may lead to both tinnitus and hyperacusis (Auerbach et al., 2014). However, in this instance, tighter matching of Controls with the Acute Tinnitus groups concerning age, hearing, and sex may have contributed to the present negative findings, as opposed to positive findings in previous less well-matched studies. We did not employ conventional methods of matching hearing through frequency averages; instead, we specifically matched at 4 kHz and 8 kHz in isolation, given the pitch of the tinnitus resided within this range. This aligns with Sedley et al. (2019) assertion that, despite substantial data supporting the concept of increased gain, the validation of this idea is often obstructed by the lack of well hearing-matched controls. Both Acute Tinnitus and Control groups were matched for hearing, indicating that the gain in each group appears to be comparably equivalent, resulting in no differences in their slope functions. As highlighted in the previous section regarding the independence of gain and tinnitus, this section too raises the question of whether tinnitus is directly associated with gain. Limited research emphasises the independence of central gain from tinnitus, as demonstrated by Zeng (2013) and Zeng (2020), who argued that central gain elicits hyperacusis which is a multiplicative increase in the output for an incoming input, whereas tinnitus results from central noise that constitutes an additive change to the baseline neuronal activity level. In support of this distinction, we saw no significant alteration in loudness growth slope specifically associated with acute tinnitus; concurrently, there were no differences between groups among loudness categories, indicating that not only can a shift in central gain be seen but also other parameters like central noise where no baseline shift in the loudness categories was seen for the tinnitus which contrasts with the central noise model. The question arises as

to whether the onset of tinnitus specifically modulates, or is modulated by, central gain, considering how constant gain is over time since the onset and how it is not significantly different from the tinnitus and non-tinnitus group. The current study thus highlights that central gain in the form of auditory sensitivity does neither explain Tinnitus mechanism linked to gain nor does explain the onset and persistence of tinnitus.

The differences between Chronic Tinnitus and Controls, characterised by increased sensitivity in chronic Tinnitus, are corroborated by ,Hébert et al. (2013) where they observed analogous results of heightened auditory sensitivity in Tinnitus relative to controls, suggesting a maladaptive central gain in Tinnitus. Hébert et al. (2013) further substantiated this claim by demonstrating that uncomfortable loudness levels in individuals with tinnitus were diminished in comparison to control subjects. The current results for Chronic Tinnitus saw noticeable shift across loudness category levels when compared to Controls indicating a baseline or additive shift in sensitivity across categories further validating the central noise model. Despite the current sample of controls not being matched for high-frequency hearing, the results were nonetheless replicated after statistically adjusting for the hearing variable. Notably, there was a non-significant trend of increase in slope function in Chronic Tinnitus compared to Acute Tinnitus, which contrasts with the longitudinal data seen (of a non-significant trend towards decreasing slope over time). This raises the question of why Chronic Tinnitus would exhibit distinct behaviour from Post-Acute Tinnitus, despite both groups being Chronic. There can be multiple factors contributing to the current findings in Chronic Tinnitus. Firstly, our recruitment relied on advertisements from a community-based sample, and the Acute Tinnitus group likely represents the broader tinnitus population more accurately, as individuals experiencing recent-onset tinnitus often seek information and support online during the initial stages of their condition. This behaviour aligns with observed patterns of health information-seeking, where individuals encountering new health issues turn to the Internet for guidance and understanding (Fackrell et al., 2012). Conversely, among the Chronic Tinnitus cohort, we believed that the individuals experiencing ongoing Tinnitus-related distress are more inclined to search online in its chronic stages, and therefore to find out about and participate in our study. This would ultimately lead to a selection bias that favours recruitment to the Chronic Tinnitus group of people prone to long-term Tinnitus distress. Conversely, whilst beginning at a matched level of Tinnitus distress to the Chronic

Tinnitus group, the Acute Tinnitus group had significantly reduced Tinnitus distress over the initial months.

Another potential reason might be the inter-subject variability among individuals with chronic subjective continuous tinnitus lasting over six months as discussed previously in relation to Tinnitus heterogeneity. The disparity between Chronic Tinnitus and Controls is evident, indicating a potentially considerable elevation of hyperacusis in individuals with Chronic Tinnitus relative to Controls. Cederroth et al. (2020) further substantiated this by stating that the occurrence of Hyperacusis in individuals with severe tinnitus can be as high as 80%. This is further supported by the research by Hébert et al. (2013) where they highlight that tinnitus groups displayed increased hyperacusis due to which alterations in slope (gain) was seen with respect to the tinnitus and Control groups. Shim et al. (2021) further highlighted the possible presence of certain of degree of hyperacusis in unilateral Tinnitus individuals where both Tinnitus and Non-Tinnitus ears tend to show reduced ULL and increased sensitivity when compared to Controls. We could attribute from these findings which also supports our cross-sectional analysis of Chronic Tinnitus and Controls that long term bothersome tinnitus can be strongly linked to higher degree of hyperacusis.

Although habituation and adaptation typically diminish the effects of tinnitus, it is considered bothersome when experienced for an extended duration (Han et al., 2009). This has been reported by literature where they indicate that long term presence of tinnitus may mostly result in the increase of mental health factors associated with tinnitus. Zhang et al. (2023) highlighted the presence of depression during the chronic stages of Tinnitus even though anxiety and sleep disturbances tend to be reduced. Simões et al. (2021) reported tinnitus characteristics and distress to not change over time. One of the predictors of tinnitus discomfort as reported by Scott et al. (1990) was the duration of tinnitus as they imply that the greater the duration the louder the tinnitus and more the discomfort of tinnitus. Our cohort of Chronic Tinnitus subjects might represent the severity group as mentioned above. They may or may not represent long term distress as tinnitus distress over time may only apply to a subset of people with naturally non-habiting tinnitus and potentially the subset of the current study maybe a group of Chronic Tinnitus individuals with recruitment bias that tend to show increased distress. We also saw that distress did not differ between Acute and Chronic Tinnitus cross sectionally, however, there were changes in the auditory sensitivity

for loudness categories potentially indicating that long term tinnitus distress may not be captured by the typical use of questionnaires. Revisiting our prior hypothesis with these two measures, the question arises whether there is a relationship between gain and tinnitus at all as; 1) Acute Tinnitus did not differ from controls, 2) Acute and Post Acute Tinnitus did not differ in either loudness categories or CLS slope, and 3) Chronic Tinnitus might have differed with Controls but there were indications of greater hyperacusis and distress. So, in all indications we could infer that once factors of recruitment and hyperacusis are isolated, tinnitus in isolation does not have an association with measures of central gain (loudness sensitivity).

3.4.3 Tinnitus may not be entirely explained by central gain

Our current assertion is that tinnitus *per se* is independent of central gain and does not relate to auditory sensitivity if hearing and hyperacusis are controlled. But it is also to indicate that there may be subgroups of individuals with tinnitus that maybe strongly associated with increased central gain as seen in our Chronic Tinnitus subgroup where they did not differ in hyperacusis with Acute Tinnitus cross sectionally but did have reduced sound tolerance when compared to them which warrants further exploration into the sensitivity of quantifying long term Tinnitus distress. Similar explanations were given by Knipper et al. (2013) where they stated that the onset of Tinnitus might be due to the systems failure to increase central gain after deafferentation post hearing loss. The stochastic resonance model of tinnitus suggests that weak signal due to hearing loss can be partially compensated by adding an appropriate level of noise. This added noise can enhance the signal detection by making the otherwise subthreshold signal cross the threshold for perception, thus, becoming audible as tinnitus (Schilling et al., 2021). The lack of significant difference between the groups may indicate that if tinnitus merely represents an augmentation of lower-level noise within the system, it need not affect total gain and can remain constant without modification. In conclusion, central gain may not explain tinnitus entirely and the variations in central gain measures in certain sub-groups of tinnitus may be influenced by level of hyperacusis present and long-term distress.

3.4.4 Limitations and future directions

A few limitations are to be highlighted, firstly, our Chronic Tinnitus group may exhibit selection bias. Secondly, we reviewed only four questionnaires, including those for tinnitus

and hyperacusis as measures of distress; a more comprehensive evaluation is necessary, combining additional questions for stress, anxiety, and depression to establish a holistic psychological profile of tinnitus and there is a necessity to establish predictors of non-habituation or a less favourable time course of tinnitus, to longitudinally study groups with contrasting clinical courses. Thirdly, the follow-up of Acute Tinnitus was conducted at just two time points, and consistent results may have been obtained with additional evaluation and follow-up intervals. Fourthly, the methodology employed for Categorical Loudness Scaling involved a straightforward assessment of loudness in ascending order during a single trial; in the future, identical predictive or non-predictive methods may be integrated for several trials. A definitive correlation with evoked potentials would have substantiated the present finding which we presented in the upcoming chapters. We conclude that tinnitus does not exhibit alterations in gain right from its onset and that the changes obtained cross sectionally between Acute and Chronic Tinnitus, or between Chronic Tinnitus and Controls, may result from selection bias for the Chronic Tinnitus group which warrants further dedicated research attention.

Chapter 4 Alterations in steady-state synchronisation between Acute and Chronic Tinnitus suggests reduction in both tinnitus and central gain.

4.1 Introduction

4.1.1 Chapter 3 summary and background

In Chapter 3, we focused solely on alterations in subjective sound sensitivity, by categorical loudness scaling, and determined that there were no changes between Acute and Post-Acute Tinnitus. We observed cross-sectional variations in slope between Chronic Tinnitus and Controls, with Chronic Tinnitus exhibiting abnormally elevated slope values, indicative of heightened sensitivity, potentially influenced by hyperacusis, as evidenced by the significant differences in hyperacusis scores between the groups. Building upon our initial hypothesis of reduction in neural responses such as neural synchrony and central gain subsequent to the onset of tinnitus (after a transient elevation), as a regression towards the mean, we propose to also conduct objective physiological assessments of central gain. While the slope values in categorical loudness scaling may suggest alterations related to central gain, they could be affected by other factors, including hyperacusis. They may also not capture key processes important in tinnitus, thus not demonstrating differences once the confounding factor of hyperacusis has been controlled for. Objective metrics of gain might provide us with additional insights beyond our subjective sound sensitivity results.

4.1.2 Auditory steady state response as a potential measure for tinnitus activity

One of the objective markers of gain is the use of Auditory Steady State Response (ASSR) (Stapells et al., 1988). Amplitude Modulation (AM) results in neurons exhibiting a *mixed coding* response at the level of primary auditory cortex, which encompasses both phase locking to the modulation frequency, and an elevated firing rate in response to the unmodulated tone (Yin et al., 2011). The cortical source of ASSR at an AM of 40Hz is found to be at the level of primary auditory cortex with ASSR amplitudes increasing with stimulus intensity but reducing with increasing stimulus frequency (Ross et al., 2003; Wienbruch et al., 2006). Auditory steady-state responses (ASSRs) are also related to middle latency responses, which arise during the transition from early to late potentials, and even tapping into the inhibitory function (Yasoda-Mohan et al., 2024).

The 40 Hz ASSR has been used in tinnitus studies as a potential measure of neuronal sensitivity in tinnitus. Most studies attribute increased ASSR in tinnitus patients when compared to non-tinnitus controls. A study conducted by Sadeghijam et al. (2023) examined the steady-state response across three groups: individuals with low distress tinnitus, individuals with high distress tinnitus, and non-tinnitus controls. Their findings indicated an elevation in ASSR for the low distress groups relative to the controls, whereas a decrease in ASSR was observed in the high distress group compared to the controls. Studies by Schlee (2006) and (Schlee et al., 2008) observed ASSR amplitude to be increased in tinnitus patients, and the response amplitude of the tinnitus frequency correlated with tinnitus loudness and distress, even after controlling for hearing loss. A recent study found out that there were enhanced 40 Hz and 80 Hz ASSR in tinnitus patients when compared to controls, attributing these differences to potential sensory impairments in higher-order auditory regions in individuals with idiopathic tinnitus. It is to be noted that these studies carry out measurements at a single intensity and infer changes in gain based on response amplitude (Ghasemahmad et al., 2024).

Tinnitus has been found to originate most commonly because of hearing damage, which can be either an audiometrically detectable hearing loss or an undetectable kind of ‘hidden’ hearing loss such as cochlear synaptopathy (Kujawa & Liberman, 2015). A study by Paul et al. (2017) found that hidden hearing loss, characterized by damage to low-SR auditory nerve fibers, may be a key factor in causing tinnitus. Despite normal audiograms, individuals with tinnitus showed impaired temporal coding and worse amplitude modulation detection thresholds. This suggests that hidden synaptic losses affecting both low-SR and high-SR fibers could explain the presence of tinnitus, highlighting the need to consider hidden hearing loss in understanding its mechanisms. These low-SR fibers are crucial for processing sounds in noisy environments. The damage to these fibers, which is not detectable through standard audiograms, leads to impaired temporal coding of sounds. This impairment may result in the brain generating the perception of sound (tinnitus) in the absence of external stimuli. Hidden synaptic losses affecting both low-SR and high-SR fibers are thought to contribute to the development of tinnitus. In principle, for optimal functioning of the auditory system, each hierarchical level should have an input/output function that maintains a stable and optimum range of output values despite potentially large changes in the range of inputs. But, due to

peripheral auditory insults, input to the central auditory system is reduced, thus altering the equilibrium of the input/output function. In order to maintain the equilibrium of the input/output function, the brain typically changes the slope function by increasing the rate of excitatory to inhibitory firing to achieve homeostasis (Auerbach et al., 2014; Sedley, 2019). This alteration of slope in the input/output function is gain and helps achieve homeostatic plasticity (Schaette & Kempter, 2006). The slope typically dictates the degree of excess input/output function, which cannot be fully deduced from a single data point (intensity) measurement and requires at least 2 data points (intensities). Therefore, it is necessary to do an intensity dependence analysis (across different intensities) of the steady-state response, which will provide the slope function to facilitate the determination of central gain related to the ASSR. Another advantage of examining the intensity dependence of the ASSR is that it allows us to leverage each participant's dynamic range, providing insight into how neural response amplitude changes in relation to perceived loudness growth. The intensity dependence has mostly been employed in auditory evoked potentials (N1-P2) to assess serotonergic activity (Carrillo-de-la-Peña et al., 2006). They highlight that IDAEP is often used to assess serotonergic neurotransmission and can indicate central nervous system sensitivity. In general, a stronger intensity dependence suggests weaker inhibitory mechanisms and heightened sensitivity to sensory stimuli, which is relevant in conditions like fibromyalgia and migraine. Chapter 5 discusses the intensity dependence of auditory evoked potentials in detail, whereas in this chapter we are employing an intensity dependence measure of the ASSR to determine the slope that may permit central gain measurement. The ASSR also varies with respect to attention modulations which has been highly attributed in tinnitus (Roberts et al., 2013)

From the above-mentioned literature, ASSR demonstrates adequate sensitivity to measure neural mechanisms of tinnitus and hence would be an ideal objective tool to explore the primary hypothesis that posits that central gain diminishes as a regression towards the mean from the Acute to the Chronic stage, leading us to predict a decline in ASSR slope and amplitude over time.

4.2 Methods

4.2.1 Participants

We studied 55 participants with Acute Tinnitus (which we defined as duration 3 days to 6 weeks, with one outlier having a duration of 12 weeks), 57 participants with Chronic Tinnitus (defined as duration more than 6 months), and 39 hearing-matched (to the acute tinnitus group) non-tinnitus controls. Participants were recruited through community advertising on Google Ads, and internally within Newcastle University's research volunteer pool. The 55 Acute Tinnitus participants were invited for reassessment after a minimum of 6 months from tinnitus onset (which we took to indicate their chronic stage), and 26 of them volunteered for and completed this further testing. We refer to this as the 'Post Acute' group, to distinguish it from the group recruited during the chronic stage of tinnitus. Please refer chapter 3 section 3.2.1 for inclusion and exclusion criteria.

Control participants were matched to the acute tinnitus group for age, sex, hearing, and stimulus frequency and presentation ear. Details are displayed in table 4.1.

4.2.2 Audiological Assessment

Refer chapter 3, section 3.2.2.

4.2.3 Tinnitometry

Refer chapter 3, section 3.2.3.

4.2.4 ASSR Stimuli

The ASSR stimuli comprised 15 stimuli per frequency (5 stimuli for low, medium and high intensities respectively) within a total experiment duration of 15 minutes. These stimuli were Amplitude Modulated sounds with a modulation frequency of 40Hz and a modulation depth of 100% at 1 kHz and the participant's matched tinnitus frequency, each lasting 30 seconds, with an interstimulus interval of 2 seconds. In the Control group, tinnitus frequencies were not self-reported but were assigned based on age-, sex-, and hearing-matched counterparts from the Acute Tinnitus group. This approach allowed for consistent frequency-based comparisons across groups

For every frequency, three different intensities were presented. Stimuli were presented in one block, with intensities and frequencies fully randomised within that block. To account for

hearing loss, loudness recruitment and hyperacusis, whilst keeping the stimuli clearly audible yet comfortable, stimulus intensities were presented in accordance with each participant's dynamic range, i.e. the range between their hearing threshold and uncomfortable loudness level, which were established for the specific experimental stimuli at both 1 kHz and the tinnitus frequency. Hearing thresholds were established using an ascending-descending run of 3 dB steps until at least 50% positive responses were obtained (i.e. 2 correct responses out of 4 trials) (refer Chapter 3, section 3.2.2). The uncomfortable loudness level was established by presenting an ascending run of 3 dB step size from the threshold until the participant just perceived the tone to be uncomfortably loud or showed signs of discomfort ('Recommended Procedure Determination of uncomfortable loudness levels', 2022b). Dynamic range was defined as uncomfortable loudness level (ULL) minus hearing threshold. Stimulus intensities were determined as follows:

a. Low Intensity: hearing threshold plus 60% of Dynamic Range
b. High Intensity: ULL – 10 dB
c. Mid Intensity: Mean of Low and High Intensity

For those with unilateral tinnitus, stimuli were only presented in the tinnitus ear; for those with bilateral tinnitus, they were presented in both ears. Participants with Acute Tinnitus were matched individually with controls of the same age, sex, and hearing. Control participants were presented with stimuli that matched the frequency and presentation ear(s) of their matched tinnitus participant but were individualised in intensity to the control participant based on the same dynamic range procedure. Table 4.1 shows the ear and stimulus presentations for each group.

4.2.5 ASSR Recording

EEG was recorded using a 64-channel Active Two system (Biosemi) in a soundproof room. Electrode offset was kept at the manufacturer's recommended limits of ±10mv with a sampling rate of 256Hz.

The experiment was a passive task, during which the participants watched a silent subtitled movie of their choosing. All stimuli were generated and presented using Matlab version R2019a, using the Psychtoolbox toolbox (Brainard, 1997; Pelli, 1997; Kleiner, Brainard, Pelli 2007).

4.2.6 ASSR Pre Processing

Data were processed in MATLAB, version R2019a, using the EEGlab toolbox (Delorme A & Makeig S, 2004) and customised code. Data were re-referenced to the P9/P10 (Linked mastoids) montage. The data were further segmented into 30 seconds epoch around each trigger which was followed by a baseline correction. Following the segmentation of the EEG data into 50% overlapping 1s Hanning-windowed sub-epochs, a frequency analysis was conducted utilising a Fast Fourier Transform (FFT) to transfer the time-domain data into the frequency domain, facilitating the identification and extraction of spectrum peaks, notably at 40Hz.

4.2.7 Data Analysis

After pre-processing, we used customised code to perform automatic peak detection at 40Hz and signal to noise ratio calculation to determine the ASSR amplitude and signal to noise ratio. We did this using data from FCz to measure the peak amplitude for ASSR at 40Hz for both 1 kHz and tinnitus frequency. Similar analysis was done for noise floor estimation, where the FFT amplitudes were averaged across the neighbouring frequencies between 38 and 39, and between 41 and 42, Hz of modulation rate. The presence of a meaningful ASSR was ascertained via visual evaluation of the response at 40Hz and objectively through F-statistics (Korczak et al., 2012), which analysed the variance of peak amplitude and noise floor values; a meaningful response was judged to be present only if the ASSR response was significantly larger than the noise floor values based on a value of $p < 0.05$. Each subject had amplitude and noise floor calculated for 3 intensities across 2 frequencies of 1 kHz and tinnitus frequency. From these, the ASSR slope function at each frequency was calculated as the quotient of the Amplitude Dynamic Range (ADR: relative difference of the high and low ASSR amplitude) and Stimulus Dynamic Range (SDR: relative difference of the high and low stimulus intensity).

$$\frac{\text{High intensity response amplitude} - \text{Low intensity response amplitude}}{\text{High intensity stimulus intensity} - \text{Low intensity stimulus intensity}}$$

Units of the ASSR slope are thus uV/dB. Note that the mid-intensity stimuli were not used for analysis but were judged important to include in the paradigm for their influence on the

overall statistics of presented stimuli, and adaptation occurring in the auditory system. Figure 4.1 depicts a sample ASSR response along with its noise floor.

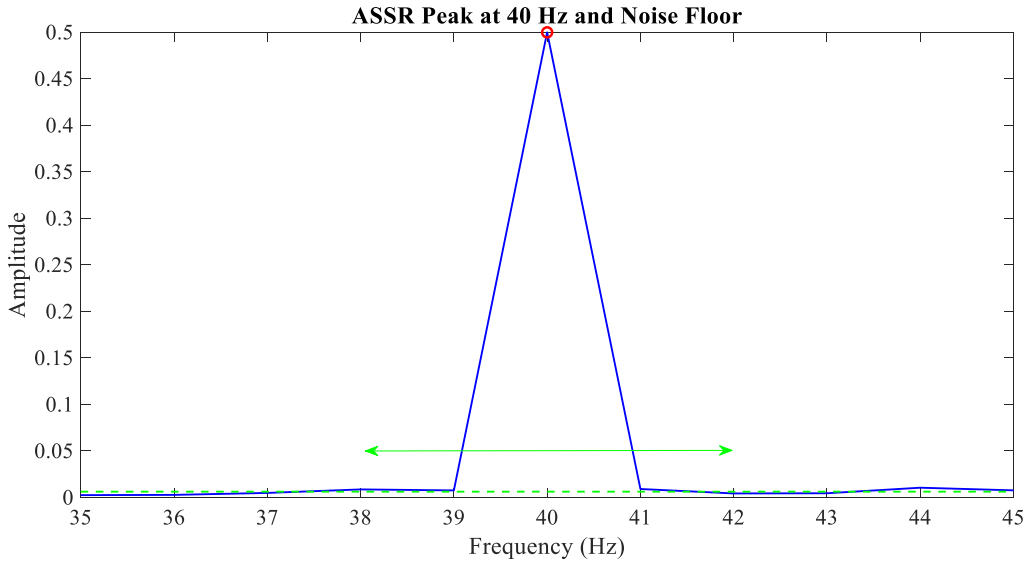


Figure 4.1: Example of an ASSR response at 40Hz along with its noise floor. *The blue peaked line denotes the peak ASSR at 40Hz, while the green dotted lines indicate the noise floor across various modulation frequencies. The red circle denotes the peak amplitude of ASSR at 40 Hz, while the green double arrow represent the noise floor between 38 Hz and 42 Hz that is considered for statistical analysis. The F statistic will lie between the amplitude values indicated by the red circle and those of the noise floor (green dotted lines).*

Statistical analysis was performed using the Statistical Package for Social Science (SPSS). Based on the Shapiro-Wilk test of normality, the data were normally distributed across the three groups for 1 kHz, and hence parametric statistics were used. However, the data were not normally distributed for tinnitus frequency, and hence a non-parametric statistic was used for these. The statistical analysis was done in two stages.

In the initial analysis, the ASSR amplitude values obtained at different intensities and frequencies were compared between groups both cross-sectionally and longitudinally. Individual MANOVA (Multivariate Analysis of Variance) was performed at a frequency of 1 kHz and the tinnitus frequency with group as independent factors and intensities as dependent due to fact that the number of subjects included in the analysis differed between the two

frequencies due to variations in signal-to-noise ratio (SNR) across frequencies. If a significant effect was found, a Tukey's post hoc test was implemented.

As a second analysis, the ASSR slope values for each frequency were compared between the groups. Since there existed sample size inequality between the stimulus frequency groups (table 4.1), statistical analysis was conducted independently for each frequency instead of a factorial analysis with group and frequency. Significant differences across the groups for 1 kHz were determined using a one-way ANOVA (Analysis of Variance), and for the tinnitus frequency groups, the Non-parametric Kruskal Wallis test was used. To compare the groups between the Acute phase and the follow-up Post Acute phase for both frequencies, a paired t test was employed. Figure 4.2 is an illustration that presents a sample waveform from a single subject.

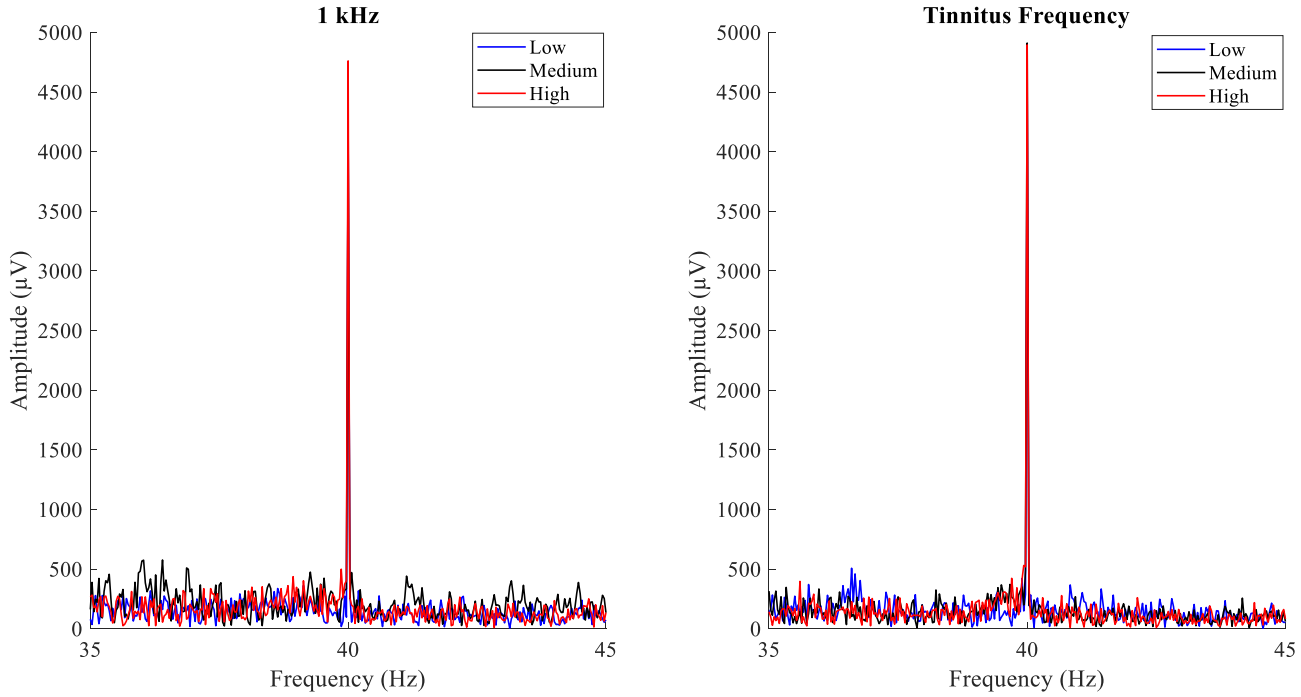


Figure 4.2: Example of an ASSR waveform for a subject. *The waveforms are recorded across different intensities and frequencies at a 40 Hz modulation rate, with the noise floor shown at neighbouring frequencies*

4.3 Results

The final demographic of participants following data quality-based rejection for ASSR slope was as follows: Acute Tinnitus (1 kHz – 39, Tinnitus Frequency – 24), Chronic Tinnitus (1 kHz – 30, Tinnitus Frequency – 12), and Controls (1 kHz – 27, Tinnitus Frequency – 12). Participants were rejected based on their overall ERP quality (both visual inspection and F statistic) due to lower signal to noise ratio for the ASSR. Eighteen Acute Tinnitus returned six months post their baseline for their re-evaluation marking their onset of their chronic stage of tinnitus (Post Acute Tinnitus). Such exclusions were performed with the researcher blind to the group to which each participant belonged.

This section is divided into six sections: 1) demographics and hearing between the groups; 2) differences in tinnitus handicap between the groups; 3) differences in hyperacusis scores between the groups; 4) differences in ASSR amplitudes between the groups; 5) differences in ASSR slope between the groups; 6) summary of results. Different statistics were run for each frequency due to the variations in sample sizes between the tinnitus frequency and 1 kHz, and these will be discussed individually.

4.3.1 Demographics and Hearing

Participant groups were attempted to be matched for age, sex, hearing, and mode of stimulus presentation. There were no significant differences across the groups for age, sex, and stimulus ear presentation for both the samples at 1 kHz and tinnitus frequency. Using the 1 kHz, 4 kHz, and 8 kHz hearing thresholds, an attempt was made to pair the groups. However, non-significant trend was present at 1 kHz ($F(2,93) = 2.724$, $p = 0.0071$) and a significant difference at 4 kHz ($F(2,50) = 3.428$ $p = 0.041$), and 8 kHz ($F(2,46) = 3.25$, $p = 0.048$), with all cases being that Chronic Tinnitus having higher thresholds than Acute Tinnitus and Controls (Table 4.1). After excluding certain subjects from each analysis, for data quality purposes, the composition of the groups including 1 kHz and tinnitus frequency analyses were slightly different, and we report each separately below. For further details please refer to table 4.1.

Subjects included in 1 kHz analysis					
Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Number of Subjects	39	30	27		
Age (Years)	M = 56.1 SD = 13.46	M = 58.67 SD = 7.78	M = 54.19 SD = 13.64	F = 1 p = 0.372	NS
Gender (M/F)	16/23	17/13	13/14	X^2 = 1.663 p = 0.435	NS
Ear presentation (U/L – B/L)	16/23	9/21	12/15	X^2 = 1.423 p = 0.491	NS
Hearing (1 kHz) dBHL	M = 13.21 SD = 9.54	M = 17.08 SD = 15.04	M = 10.09 SD = 8.67	F = 2.724 p = 0.071	Acute Tinnitus – Chronic Tinnitus: p = 0.341 Acute Tinnitus – Controls: p = 0.519 Chronic Tinnitus – Controls: p = 0.058
Subjects included in Tinnitus Frequency analysis					
Number of Subjects	24	12	12		
Age (Years)	M = 52.71 SD = 14.49	M = 54.83 SD = 9.63	M = 54.08 SD = 16.03	F = 0.104 p = 0.901	NS

Gender (M/F)	9/15	6/6	4/8	$X^2 = 0.377$ p = 0.828	NS
Ear presentation (U/L – B/L)	7/17	3/9	3/9	$X^2 = 0.784$ p = 0.676	NS
Hearing (4 kHz) dBHL	M = 22.29 SD = 13.27	M = 32.92 SD = 13.26	M = 18.75 SD = 16.18	F = 3.438 p = 0.041*	Acute Tinnitus – Chronic Tinnitus: p = 0.092 Acute Tinnitus – Controls: p = 0.757 Chronic Tinnitus – Controls: p = 0.045*
Hearing (8 kHz) dBHL	M = 32.60 SD = 20.67	M = 48.54 SD = 18.2	M = 28.08 SD = 24.94	F = 3.25 p = 0.048*	Acute Tinnitus – Chronic Tinnitus: p = 0.098 Acute Tinnitus – Controls: p = 0.812 Chronic Tinnitus – Controls: p = 0.053

Table 4.1: Participant demographics for each group.

*M denotes mean, SD denotes Standard Deviation, MD denotes Median, F statistic indicates a One Way ANOVA has been carried out, X^2 indicates a chi square goodness of fit test, NS denotes No Significant difference, * indicates presence of statistical significance at 95% confidence interval. Hearing thresholds were calculated by averaging the left and right ear values in cases of bilateral tinnitus, whereas for unilateral tinnitus, the threshold was based on the ear with tinnitus.*

4.3.2 Symptom Scores for Tinnitus Distress

With respect to symptom scores for the individuals included in 1 kHz cross-sectional comparisons between the Acute and Chronic Tinnitus for THI and TFI yielded significant differences between them, with Chronic Tinnitus having higher levels of distress than Acute Tinnitus (refer table 4.2). Comparison between the Acute and Post Acute Tinnitus group revealed a statistically significant difference with reduction over time in TFI scores with Acute Tinnitus having higher TFI scores when compared to Post Acute Tinnitus. No differences were noted between Acute and Post Acute Tinnitus for THI.

Comparable results were achieved when the identical comparisons were reiterated solely for the subjects whose data were utilised for the examination of reactions to the tinnitus frequency stimuli. There were no differences between the Acute and Chronic Tinnitus on THI, but differences were noted for TFI with Chronic Tinnitus having greater distress on TFI than Acute Tinnitus (refer table 4.3). Pairwise comparison yielded non-significant trend to significant differences between Acute and Post Acute Tinnitus group with Acute Tinnitus having higher THI and TFI scores when compared to Post Acute Tinnitus.

4.3.3 Symptom Scores for Hyperacusis Questionnaires

Significant main effects of group were observed in cross-group comparisons between Acute, Chronic, and Controls for hearing loss in participants whose data were used in the analysis of response to at 1 kHz. Tukey's post-hoc test revealed a non-significant trend between Acute Tinnitus and Chronic Tinnitus and between Acute Tinnitus and Controls, there were significant differences observed between Chronic Tinnitus and Controls. Controls had lower HQ scores compared to both Acute Tinnitus and Chronic Tinnitus. Consistent findings were obtained for IHS, and as shown by Tukey's post hoc test, Controls had lower IHS scores in

comparison to Acute Tinnitus and Chronic Tinnitus. There were statistical differences between Acute and Chronic Tinnitus too. Upon the paired longitudinal comparison, no change over time was observed for scores on either HQ or IHS.

For subjects included in the tinnitus frequency analysis, statistically significant equivalent results were obtained for both HQ and IHS across groups. Tukey's post hoc test revealed non-significant trend differences between Acute Tinnitus and Chronic Tinnitus, significant differences between Acute Tinnitus and Controls, and significant differences between Chronic Tinnitus and Controls. Controls had lower HQ scores compared to both Acute and Chronic Tinnitus respectively. In comparison to Acute Tinnitus and Chronic Tinnitus, controls yielded lower IHS scores. There were significant differences noted between Acute Tinnitus and Chronic Tinnitus. Upon the paired longitudinal comparisons, no change over time was observed for scores on either HQ or IHS. The results of the symptom questionnaires and tinnitometry for each of the three groups are shown in table 4.2.

			1 kHz		
Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Number of Subjects	39	30	27		
Tinnitus Pitch (Hz)	M = 5709.02 SD = 2064.88	M = 6775.1 SD = 1410.25	M = 5928.56 SD = 2251.04	F = 2.703 p = 0.072	NS
Tinnitus Loudness (dBSL)	M = 16.31 MD = 14.5 SD = 15.73	M = 6.46 MD = 6.17 SD = 10.05	N/A	z = 2.622 p = 0.009*	
Number of Subjects	38	24	24		

THI	M = 30.58 SD = 21.04	M = 44.33 SD = 20.68	N/A	t = - 2.524 p = 0.014*	
TFI	M = 95.6 SD = 45.79	M = 131.08 SD = 53.35	N/A	t = -2.787 p = 0.004*	
HQ	M = 12.79 SD = 7.61	M = 17.54 SD = 11.31	M = 8.13 SD = 5.62	F = 7.597 p < 0.001*	Acute Tinnitus–Control: p = 0.081
					Acute Tinnitus–Control: p = 0.088
					Chronic Tinnitus – Control: p < 0.001*
IHS	M = 41.18 SD = 12.98	M = 52.16 SD = 18.39	M = 31.79 SD = 7.58	F = 13.516 p < 0.001*	Acute Tinnitus–Chronic Tinnitus: p = 0.007*
					Acute Tinnitus–Control: p = 0.026*
					Chronic Tinnitus – Control: p < 0.001*
			Tinnitus Frequency		
Number of Subjects	24	12	12		

Tinnitus Pitch (Hz)	M = 5051.43 SD = 2266.91	M = 6686.51 SD = 2144.69	M = 4577.1 SD = 2159.78	F = 3.142 p = 0.052	NS
Tinnitus Loudness (dBSL)	M = 17.25 MD = 11.25 SD = 18.04	M = 11.35 MD = 7.83 SD = 13.06	N/A	z = 0.689 p = 0.491	
Number of Subjects	24	11	10		
THI	M = 36.25 SD = 24	M = 48.91 SD = 21.81	N/A	t = -1.535 p = 0.134	
TFI	M = 102.46 SD = 46.23	M = 148.36 SD = 59.74	N/A	t = -2.486 p = 0.018*	
HQ	M = 13.25 SD = 7.42	M = 20.27 SD = 12.41	M = 5.3 SD = 3.95	F = 8.369 p < 0.001*	Acute Tinnitus–Chronic Tinnitus: p = 0.066 Acute Tinnitus–Controls: p = 0.04* Chronic Tinnitus – Control: p < 0.001*
IHS	M = 43.12 SD = 15.75	M = 58.27 SD = 18.81	M = 28.8 SD = 3.01	F = 10.28 p < 0.001*	Acute Tinnitus–Chronic Tinnitus:

			p = 0.02*
			Acute Tinnitus– Controls: p = 0.037*
			Chronic Tinnitus – Control: p < 0.001*

Table 4.2: Participant's tinnitus characteristics for each group

*M denotes mean, SD denotes Standard Deviation, MD denotes Median, F statistic indicates a One Way ANOVA has been carried out, t statistic indicates an independent t test done, z statistic indicates a non-parametric Man-Whitney u test has been carried out, N/A- Not Applicable, NS denotes No Significance. * indicates statistically significant difference at 95% confidence interval, THI - Tinnitus Handicap Inventory, TFI – Tinnitus Functional Index, HQ – Hyperacusis Questionnaire, IHS – Inventory of Hyperacusis Symptoms*

	1 kHz		
Parameters	**Acute Tinnitus**	**Post Acute Tinnitus**	**Significance Overall**
Number of Subjects	18	18	
Tinnitus Frequency (Hz)	M = 5870.99 SD = 1791.45	M = 6603.1 SD = 1626.18	t = 1.256 p = 0.226
Tinnitus Loudness (dBSL)	M = 13.99 MD = 11.25 SD = 10.98	M = 9.08 MD = 5.33 SD = 10.11	t = -2.266 p = 0.023*

Number of Subjects	16	16	
THI	M = 31.86 SD = 20.3	M = 25.5 SD = 27.15	t = -1.626 p = 0.125
TFI	M = 101.19, SD = 43.94	M = 75.81, SD = 53.67	t = -3.418 p = 0.004*
HQ	M = 12.75 SD = 8.41	M = 13.13 SD = 8.94	t = 0.816 p = 0.237
IHS	M = 43.69 SD = 15.64	M = 43.13 SD = 18.51	t = -0.183 p = 0.857
Tinnitus Frequency			
Number of Subjects	9	9	
Tinnitus Frequency (Hz)	M = 5995 MD = 6081.49 SD = 2409.61	M = 6431.42 MD = 6507.72 SD = 1482.69	z = 0 p = 1
Tinnitus Loudness (dBSL)	M = 18 MD = 9 SD = 10.16	M = 14.037 MD = 11 SD = 9.98	z = -0.71 p = 0.359

Number of Subjects	8	8	
THI	M = 33 MD = 30 SD = 24.02	M = 25 MD = 17 SD = 29.02	z = -1.759 p = 0.079
TFI	M = 102.12 MD = 105 SD = 47.52	M = 71 MD = 68.5 SD = 52.38	z = -2.66 p = 0.008*
HQ	M = 15.13 MD = 15.5 SD = 7.53	M = 15.63 MD = 17 SD = 8.6	z = 0.339 p = 0.734
IHS	M = 44.5 MD = 43.5 SD = 17.67	M = 47 MD = 39.5 SD = 20.76	z = -0.07 p = 0.944

Table 4.3: Longitudinal changes between Acute and Post Acute Tinnitus

*The t test denotes a paired wise comparison between the Acute and Post Acute Tinnitus group and z test indicates the non-parametric Wilcoxon signed rank test. * indicates presence of statistically significant difference at 95% confidence interval, THI - Tinnitus Handicap Inventory, TFI – Tinnitus Functional Index, HQ – Hyperacusis Questionnaire, IHS – Inventory of Hyperacusis Symptoms. One person did not complete the questionnaire and hence the sample size have been reduced in both 1 kHz and tinnitus frequency analysis.*

4.3.4 ASSR Amplitude

The ASSR mean amplitude was individually evaluated among the Acute, Chronic, and Control groups for both 1 kHz and tinnitus frequencies. Before comparing amplitudes at low, medium, and high levels, we assessed the absolute stimulus presentation levels (dBSL) to establish whether possible differences in measured amplitude could be explained by stimulus presentation level. A MANOVA was carried out to establish the effect of groups (Acute,

Chronic, and Controls) on the stimulus presentation levels (low, medium, and high). intensity presentation levels.

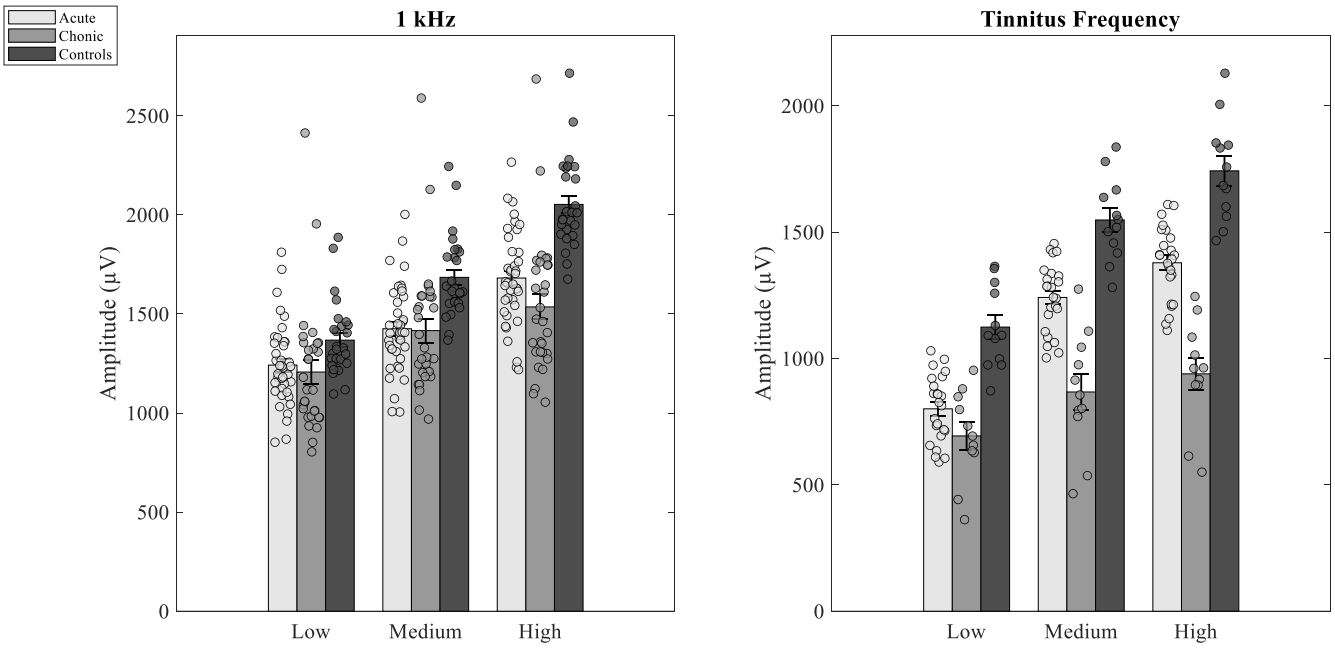


Figure 4.3: Differences in ASSR amplitude at low, medium, and high intensities between Acute, Chronic, and Controls for both 1 kHz and tinnitus frequency. *There is no statistical difference between the groups for any amplitudes across either frequencies.*

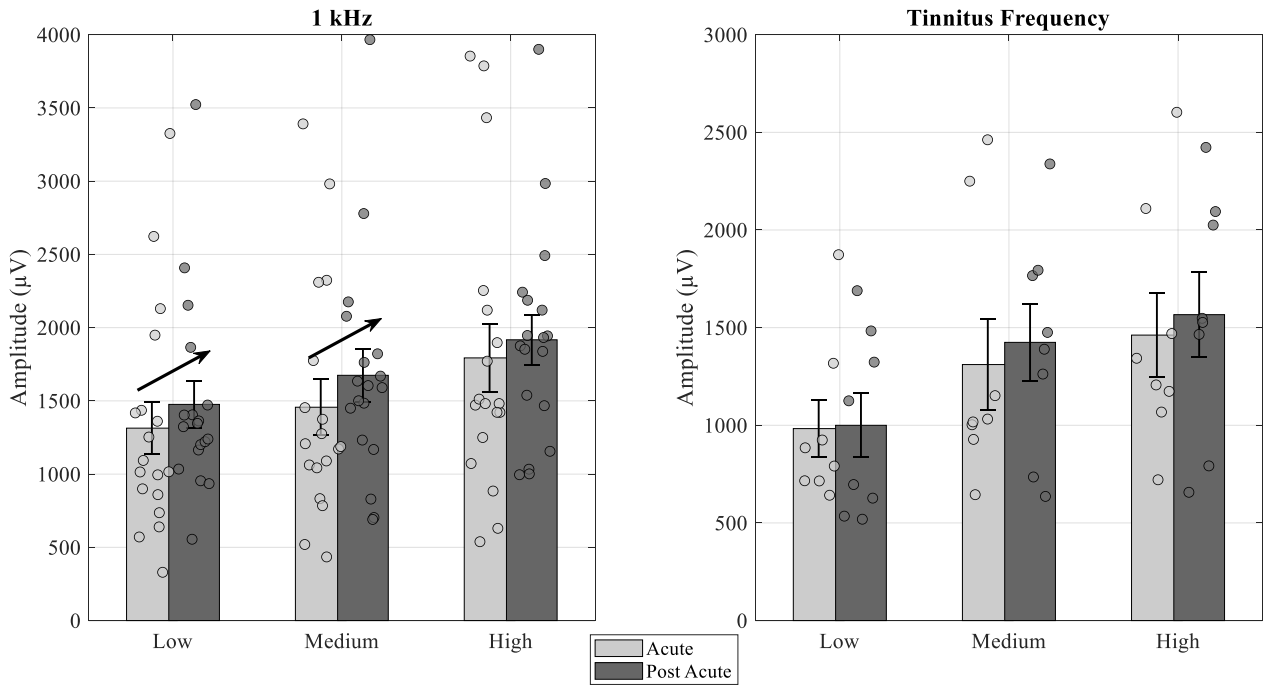


Figure 4.4: Differences in ASSR amplitude at low, medium, and high intensities between Acute and Post Acute Tinnitus for both 1 kHz and tinnitus frequency.

There is a non-significant trend (increase in ASSR amplitude indicated by arrow) between Acute and Post Acute Tinnitus for low and medium intensity at 1 kHz and none for high intensity. No differences in any loudness categories were noted for Tinnitus frequency.

1 kHz stimuli

There were statistically significant differences in the overall stimulus levels at which the ASSR stimuli were presented ($F(6,182) = 3.604$, $p = 0.002$, Wilk's $\Lambda = 0.799$, partial $\eta^2 = 0.106$). An overall main effect was seen in Low Intensity ($F(2,93) = 9.719$, $p < 0.001$, partial $\eta^2 = 0.173$), Medium ($F(2,93) = 10.045$, $p < 0.001$, partial $\eta^2 = 0.178$), and High Intensity (($F(2,93) = 10.826$, $p < 0.001$), partial $\eta^2 = 0.189$) after correction for multiple comparisons. Further Tukey's post hoc test revealed a significant difference between Acute Tinnitus and Controls, Chronic Tinnitus and Controls, and no difference between Acute Tinnitus and Chronic Tinnitus across all 3 intensities. The controls tend to have increased stimulus presentation levels compared to both the tinnitus groups. This suggested that the stimulus presentation could potentially affect the measured amplitude, and it was previously shown

that hearing varied at 1 kHz, leading to two potential confounding factors regarding the observed amplitudes.

To determine the effect of group and intensities on the ASSR amplitude, we conducted a two-way Analysis of Covariance (ANCOVA) with two factors: groups (Acute, Chronic, and Controls) and intensities (low, medium, and high), with two covariates: stimulus presentation levels (low, medium, and high), and hearing thresholds at 1 kHz. Results revealed no statistical significant main effect of intensities ($F(2,277) = 2.348$, $p = 0.097$, partial $\eta^2 = 0.017$) and statistically significant main effect of groups ($F(2,277) = 3.066$, $p = 0.048$, partial $\eta^2 = 0.022$) with near-significant difference between Chronic Tinnitus and Controls ($p = 0.055$) and no significant difference between Acute Tinnitus and Chronic Tinnitus ($p = 1$) and between Acute Tinnitus and Controls ($p = 0.142$). Controls had higher overall amplitude ($M = 1678.7$, $SE = 84.93$) when compared to Chronic Tinnitus ($M = 1388.95$, $SE = 79.62$). There was no statistically significant interaction between the groups and intensities on the amplitude ($F(4,277) = 0.279$, $p = 0.891$, partial $\eta^2 = 0.004$). With respect to the longitudinal comparison, a repeated measures 2-way ANOVA was performed to compare the effect of groups and intensities on the amplitude. Results revealed a main effect of intensity ($F(2,16) = 23.814$, $p < 0.001$, Wilk's $A = 0.251$), no main effect for group ($F(2,16) = 2.667$, $p = 0.135$, Wilk's $A = 0.873$), and no interaction effect between groups and intensities ($F(2,16) = 0.957$, $p = 0.405$, Wilk's $A = 0.893$). We however carried out a simple main effect analysis using paired t test to determine the impact of group on amplitude for individual intensities, and the results indicated presence of a non-significant trend between Acute and Post Acute Tinnitus for low intensity ($t(17) = -1.896$, $p = 0.075$) and medium intensity ($t(17) = -2.080$, $p = 0.053$) with Post Acute tinnitus (low intensity: $M = 1475.97$ $SD = 670.05$, medium intensity: $M = 1674.48$, $SD = 771.61$) having larger amplitude than Acute tinnitus (low intensity: $M = 1313.82$ $SD = 762.2$, medium intensity: $M = 1456.56$, $SD = 809.1$). No significant difference was found for the high intensity ($t(17) = -0.772$, $p = 0.451$). There was no impact of stimulus presentation levels on the obtained ASSR amplitude with no significant difference between Acute and Post Acute Tinnitus on low, mid, and high stimulus intensity presentation levels.

Tinnitus frequency

A similar analysis was performed at tinnitus frequency. A MANOVA revealed a statistically significant difference within overall stimulus presentation levels between the groups ($F(6,86)$

= 5.424, $p < 0.001$, Wilk's A = 0.526, partial $\eta^2 = 0.275$). An overall main effect was seen in Low Intensity (F (2,45) = 14.596, $p < 0.001$, partial $\eta^2 = 0.393$), Medium (F (2,45) = 12.829, $p < 0.001$, partial $\eta^2 = 0.363$), and High Intensity (F (2,45) = 11.531, $p < 0.001$, partial $\eta^2 = 0.339$) after correction for multiple comparisons. Further Tukey's post hoc test revealed a presence of significance between Acute Tinnitus and Chronic Tinnitus, Acute Tinnitus and Controls, and Chronic Tinnitus and Controls, across all 3 stimulus presentations.

Similarly, 2 way ANCOVA was carried out to establish the effect of group and intensity on the ASSR amplitude, and results revealed statistically non-significant trend of intensities ($F(2,133) = 2.75$, $p = 0.068$, partial $\eta^2 = 0.04$) with non-significant trend between low intensity and high intensity ($p = 0.073$) and no significant difference between low and medium intensity ($p = 0.287$) and between medium and high intensity ($p = 1$). High intensity tends to have increased amplitude (M = 1310.55, SE = 114.78) when compared to low intensity (M = 928.72, SD = 116.7). A statistically significant main effect was established for group ($F(2,133) = 3.162$, $p = 0.046$, partial $\eta^2 = 0.045$), with significant difference between Chronic Tinnitus and Controls ($p = 0.041$), no significant difference between Acute Tinnitus and Chronic Tinnitus ($p = 0.381$) and no significant difference between Acute Tinnitus and Controls ($p = 0.257$). Controls (M = 1417.23, SE = 134.39) had higher amplitude than Chronic Tinnitus (M = 879.65, SE = 143.22). There was no interaction effect between group and intensity for ASSR amplitude ($F(4,133) = 0.168$, $p = 0.954$, partial $\eta^2 = 0.005$). With respect to the longitudinal comparison, a Repeated Measures 2-way ANOVA (RM ANOVA) was performed to compare the effect of groups and intensities on the amplitude. Results revealed a main effect of intensities ($F(2,6) = 8.119$, $p = 0.02$, Wilk's A = 0.27), no main effect for groups ($F(2,8) = 0.276$, $p = 0.615$, Wilk's A = 0.962), and no interaction effect between groups and intensities ($F(2,8) = 1.748$, $p = 0.252$, Wilk's A = 0.632). Figure 4.3 depicts the ASSR amplitudes across groups and frequencies. We however carried out a simple main effect analysis to determine the impact of group on amplitude across intensities and the results indicated presence of no significant difference between Acute and Post Acute tinnitus for low intensity ($t(7) = 0.46$, $p = 0.919$), medium intensity ($t(7) = -0.742$, $p = 0.482$), and high intensity ($t(7) = -0.672$, $p = 0.523$) (Figure 4.4). There was no impact of stimulus presentation levels on the obtained ASSR amplitude with no significant difference between Acute and Post Acute Tinnitus on low, mid, and high stimulus

4.3.5 ASSR Slope

In analysing the variations in slope among the Acute, Chronic, and Control groups, as well as between the Acute and Post Acute Tinnitus groups, we focused on three variables: the Stimulus Dynamic Range (SDR), defined as the difference between high and low stimulus presentation; the Amplitude Dynamic Range (ADR), which represents the difference between high and low ASSR amplitude; and slope, calculated as the ratio of SDR to ADR. Determining whether SDR differed will provide insight into the possible confounding factors that might affect the obtained ADR and slope. Assessing ADR in conjunction with slope will provide a comprehensive understanding of the amplitude growth function for ASSR across different groups.

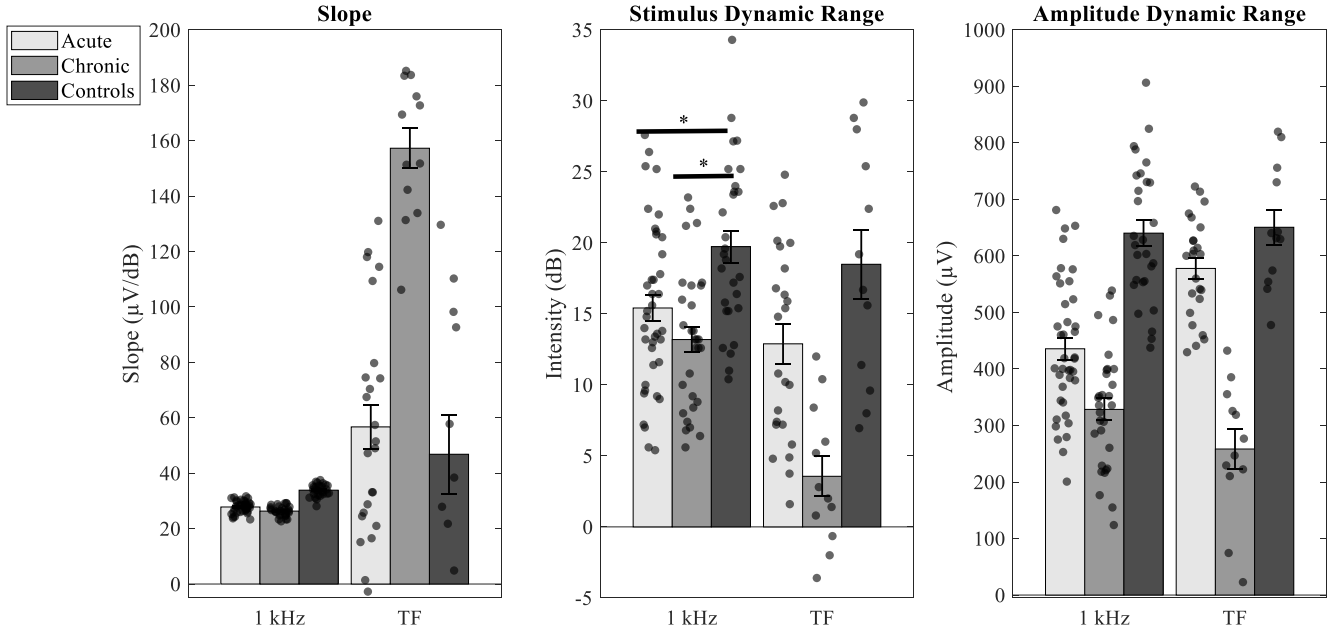


Figure 4.5: Slope, Stimulus Dynamic Range, and Amplitude Dynamic Range between Acute, Chronic, and Controls at both 1 kHz and tinnitus frequency

There is a significant difference between Acute Tinnitus and Controls and between Chronic Tinnitus and Controls for Stimulus Dynamic Range with controls having higher Stimulus Dynamic Range. No significant differences were found between other variables and groups.TF indicates Tinnitus Frequency and Asterix (*) indicates presence of significant difference

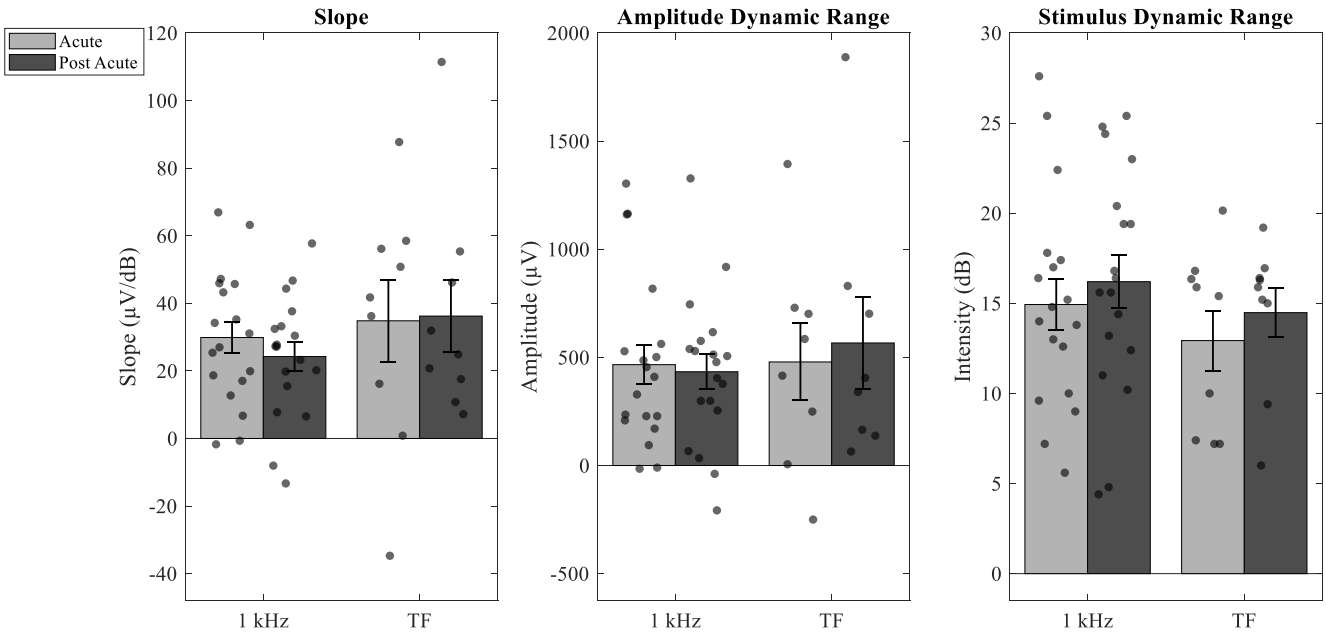


Figure 4.6: Slope, Stimulus Dynamic Range, and Amplitude Dynamic Range between Acute and Post Acute Tinnitus at both 1 kHz and tinnitus frequency. *There were no significant differences in slope, stimulus dynamic range, and amplitude dynamic range between the groups across both frequencies. TF indicates Tinnitus Frequency*

1 kHz stimuli

A one-way MANOVA was carried out to establish the effect of group on slope, SDR, and ADR; this revealed a significant difference in overall response based on groups ($F(6,184) = 3.964$, $p < 0.001$, Wilk's $\Lambda = 0.526$, partial $\eta^2 = 0.114$). An overall main effect was seen for SDR ($F(2,94) = 10.215$, $p < 0.001$, partial $\eta^2 = 0.179$) with difference between Acute Tinnitus and Controls ($p = 0.007$), Chronic Tinnitus and Controls ($p < 0.001$), and no differences between Acute Tinnitus and Chronic Tinnitus ($p = 0.317$). Controls had higher SDR ($M = 19.74$, $SD = 5.94$) when compared to Acute ($M = 15.42$, $SD = 5.81$) and Chronic Tinnitus groups ($M = 13.19$, $SD = 4.95$). There was an overall main effect of group seen for ADR ($F(2,94) = 6.346$, $p = 0.003$, partial $\eta^2 = 0.119$) with difference between Acute Tinnitus and Controls ($p = 0.049$), difference between Chronic Tinnitus and Controls ($p = 0.002$), and no difference between Acute Tinnitus and Chronic Tinnitus ($p = 0.585$). Controls ($M = 640.39$, $SD = 396.70$) had higher ADR when compared to Acute Tinnitus ($M = 435.73$, $SD = 344.19$) and Chronic Tinnitus ($M = 328.49$, $SD = 263.19$). There was, however, no main

effect of groups on slope (F (2,94) = 0.961, p = 0.386, partial η^2 = 0.02). There was an effect on SDR of group, which could also be a potential confound. This warranted us to carry out individual one-way ANCOVA to establish the effect of the group on ADR and slope individually, with covariates of SDR and hearing thresholds at 1 kHz (refer figure 4.5). With respect to Slope and ADR, there was no significant effect of group on the values of slope (F(2,92) = 1.279, p = 0.279, partial η^2 = 0.027) and there was no significant effect of group on the values of ADR (F(2,92) = 1.774, p = 0.17, partial η^2 = 0.037) (refer figure 4.5 and 4.6). Longitudinal comparisons between Acute Tinnitus and Post Acute Tinnitus revealed no significant difference in the slope values (t(17) = 0.806, p = 0.431), ADR (t(17) = 0.319, p = 0.754), and the SDR (t(17) = -1.177, p = 0.256) values respectively.

Subjects included in tinnitus frequency.

Similar analysis was carried out for the tinnitus frequency using non parametric Kruskal Wallis test to establish the effect of group on Slope, SDR, and ADR, and showed no significant effect on slope ($X^2(2)$ = 1.818, p = 0.4), non-significant trend for ADR ($X^2(2)$ = 5.286, p = 0.071), and presence of statistical significance for SDR (($X^2(2)$ = 17.806, p < 0.001). Due to possibility of SDR being a confound for slope and ADR, we further ran a Quade non-parametric ANCOVA exploring the effects of groups on slope and ADR with SDR and hearing at 8 kHz as covariates. Results reveal no statistical differences for slope ($X^2(2)$ = 0.078, p = 0.925) and for ADR ($X^2(2)$ = 0.731, p = 0.487). Longitudinal comparisons between Acute Tinnitus and Post Acute Tinnitus reveals no significant difference in the slope values upon the Wilcoxon sign ranked test (X^2 (8) = -0.059, p = 0.953) and no significant difference in the SDR (X^2 (8) = -1.007, p = 0.314) values too between the groups (refer figures 4.5 and 4.6).

4.3.6 Summary of results

In summary, there was overall significant difference in hearing at 1 kHz, 4 kHz, and 8 kHz between the groups of Acute, Chronic, and Controls. This prompted us to carry further analysis keeping these variables as potential covariates along with other potential covariates such as variations in stimulus presentation levels. Primarily the ASSR was analysed based on amplitude and slope cross sectionally between Acute, Chronic, and Controls and longitudinally between Acute and Post Acute Tinnitus. After accounting for the covariates,

the cross-sectional comparisons did not yield statistically significant group differences for either slope and amplitudes across frequencies. There were however non significant trend longitudinally between Acute and Post Acute Tinnitus with increase in low and medium amplitudes (intensities presented at low and medium) over time and no significant difference at high amplitude or for slope.

4.4 Discussion

The principal results indicate that there was a non-significant trend with an increase in the ASSR amplitude at low and mid-level intensities for Post Acute Tinnitus when compared to Acute tinnitus at 1 kHz. Tinnitus frequency did not yield any longitudinal differences. No variations in the slope were seen in either 1 kHz or tinnitus frequency. There were no cross-sectional differences across the groups in either ASSR slope or amplitude. Although the control and tinnitus groups did not differ significantly, the observed increase in amplitude may provide insight into the interpretation of findings from the Acute and Post Acute tinnitus conditions.

4.4.1 Results are not confounded by extraneous factors

Although we did not yield pure statistically significant results, it is essential to emphasise that the potential extraneous or confounding factors have either been controlled or did not impact the current results. The current study had groups that were controlled for age, sex, and stimulus ear presentation. There were differences in hearing thresholds between the groups, this was considered throughout the analysis of both amplitudes and slopes across the groups. We also found small variations in the presentation levels across the groups which was controlled too along with the variations in hearing. The degree of hyperacusis differed between the groups, and we have adjusted the individual's dynamic range for recruitment, hyperacusis/gain, and to ensure the stimulus was delivered within the participant's comfortable range. Our results were repeated using a robust longitudinal pairwise comparison design between Acute and Post Acute stages, giving these findings additional robustness to any remaining potential confounding factors in the cross-sectional comparison between Acute and Chronic Tinnitus.

4.4.2 No cross-sectional variations across the groups for Acute, Chronic, and Non-Tinnitus Controls

Our first findings indicate that there were no cross-sectional changes among the Acute, Chronic, and Non-Tinnitus Control groups, with respect to either slope and absolute amplitude indicating that the presence of tinnitus isn't associated with the ASSR amplitude. The tailored presentation level for the subjects based on their dynamic range may have influenced the outcomes of non-significant results, as the level of hyperacusis has been compensated. In Chapter 3 we observed altered CLS slope and loudness levels between Chronic Tinnitus and the other two groups which we implied might have been due to the deviations in the level of hyperacusis/distress. In this case, the tailored ASSR presentation level may have curbed the influence of hyperacusis. This was in support of the findings by Paul et al. (2014) who investigated the influence of attention on the auditory steady-state response in individuals with and without tinnitus and their results highlighted that tinnitus patients exhibited diminished attentional modulation of the 5 kHz ASSR, unlike the control group, suggesting that attentional resources may be preferentially allocated to the tinnitus percept, thus limiting further modulation. Conversely, both groups demonstrated typical attentional modulation of the ASSR at 500 Hz, indicating that the attentional alteration in tinnitus patients is frequency specific. Roberts et al. (2013) explored the role of attention and tinnitus with respect to ASSR and they observed a smaller ASSR responses in individuals with tinnitus subjects when compared to the Controls at specific frequencies. The findings by Diesch et al. (2012) further suggested that the alterations of ASSR in tinnitus individuals when compared to Controls may be partially influenced by attentional processes that imply the complex interplay between tinnitus, attention, and auditory processing. The study further emphasizes the importance of considering attentional factors when interpreting ASSR findings in tinnitus research. Sedley (2019) reviewed the role of gain in tinnitus mechanism and highlighted literature that tend to show an increased ASSR amplitudes for tinnitus individuals that is closer to the tinnitus frequency. In our case we did not obtain significant differences at the tinnitus frequency. These studies collectively contribute to our understanding of the neural mechanisms underlying tinnitus and the potential of ASSR as a tool for assessment and treatment. There is a precedent for reduced ASSR amplitude in tinnitus, and, while the reductions in our tinnitus groups are not in themselves significant, the

direction of results especially the controls could aid us in interpreting the Acute and Post Acute Tinnitus results.

4.4.3 Changes between Acute and Post Acute Tinnitus

Concerning the variations seen between Acute and Post Acute, we observed a non-significant trend with a rise in ASSR amplitude at low and medium intensity levels over time for Post Acute Tinnitus at 1 kHz when compared to Acute Tinnitus. While we acknowledge that the following results are not statistically significant, we believe they should not be entirely disregarded, particularly as they approach the threshold of significance. There is currently no clear consensus on how to handle such borderline findings, with existing studies taking varying positions. However, given the potential for these results to gain clarity when considered alongside additional data, we interpret findings with *p*-values between 0.05 and 0.1 as indicative of a potential trend and discuss them accordingly (Wood et al., 2014). In this context we treat it as a positive result and highlight the need for additional data to better know the direction of the results. We here consider this finding in the context of our hypothesis that central gain reduces over time, following the onset of tinnitus, as a regression to the mean following a period of hypersensitivity in which tinnitus first occurs. Our initial hypothesis anticipated a reduction in ASSR over time; however, in this case, we observed an increase instead. This pattern of results can still be seen as aligning with our initial hypothesis. While we originally anticipated a reduction in ASSR amplitude as an indicator of reduced central gain, the observed increase in ASSR amplitude may also reflect a reduction in central gain, depending on the interpretation. Notably, if ASSR amplitude is considered a positive indicator of central gain, then a reduction over time would support our hypothesis. However, in this case, we interpret the increased ASSR amplitude over time as a stabilizing mechanism—one that not only trends toward the control group pattern but also shows an inverse relationship with distress, reinforcing its potential relevance. Due to the absence of Acute Tinnitus studies concerning ASSR, we aim to compare the present findings with Chronic Tinnitus studies conducted before and after treatment, as well as the observed alterations in ASSR amplitude. Pantev et al. (2012) examined the effects of Tinnitus Retraining Therapy on ASSR and found significant reductions in tinnitus related ASSR following TRT. Roberts et al. (2015) demonstrated that 40-Hz frequency modulation stimulation could enhance the amplitude of the auditory steady-state response (ASSR) at 40

Hz in tinnitus as a training method. He also proposed that with the introduction of masking noise at the frequency corresponding to tinnitus pitch, the amplitude of the ASSR at 40 Hz increased for the carrier frequencies within the same range. Sadeghijam et al. (2022) explored the relationship between tinnitus distress and Auditory Steady-State Response amplitudes, and their results reveal a statistically significant negative correlation between tinnitus distress and ASSR amplitudes. They further revealed that participants with lower THI scores (indicating lower levels of tinnitus distress) exhibit a statistically significant larger ASSR amplitudes across all the carrier frequencies when compared to the participants with higher THI scores and greater tinnitus distress. Their results highlight the utility of ASSR as an objective electrophysiological measure that may be a useful indicator for subjective tinnitus distress. Sadeghijam et al. (2023) utilized binaural beats in stimulating tinnitus patients over a month, measuring the changes in ASSR amplitude between the baseline and post-stimulation. Their results indicated an increase in ASSR amplitude following binaural beat stimulation when compared to baseline, which was attributed to enhanced synchronisation from repeated exposure to binaural beats and an improved temporal map that facilitated a higher stimulation rate. The observed increase in ASSR amplitude, reflecting improved neural synchronization, supports our interpretation that Acute Tinnitus is associated with heightened central gain, increased tinnitus loudness, and greater distress. These factors likely reduce neural synchrony to external amplitude-modulated sounds. Over time, as habituation occurs and these contributing factors diminish, the resulting increase in ASSR amplitude suggests improved synchrony with the incoming stimulus. Furthermore, the direction of this change aligns more closely with the control group pattern, reinforcing our interpretation of increased ASSR amplitude as a marker of recovery or stabilization.

4.4.4 Changes in neural synchrony and adaptation

We consider our longitudinal design to be more robust due to which we interpret the current findings based on the longitudinal designs. The generation of ASSR is thought to be a superposition of the Middle Latency Response and Auditory Brainstem Response with a predominant generation at the level of Heschl's gyrus (primary auditory cortex), and possibility of contributions from the auditory belt regions, which are secondary auditory cortical areas (Bohórquez & Özdamar, 2008). The generation of AM tones throughout the auditory pathway is heavily dependent on mechanisms such as phase locking (synchronized

firing), temporal envelope coding (tracking the overall shape of the AM response), rate coding (increased spikes per second for increase in intensity), and population coding (group of neurons firing for different modulation frequencies). These vary along the auditory pathway, with lesser dependence of mechanisms like phase locking at the level of auditory cortex (Louage et al., 2004). When it comes to tinnitus, it has been posited that consequently to decreased input due to hearing loss, there is aberrant (increased) neural synchrony specifically in the delta band oscillations that manifests as tinnitus (Eggermont & Tass, 2015). The abnormal increase in neural synchrony of spontaneous activity within the cortical system may have an impact on the incoming acoustic signals, where the neural synchrony is reduced to incoming sounds due to a lack of phase locking, which is essential for the formation of auditory steady-state responses (ASSR). A study by Motomura et al. (2024) investigated the impact of abrupt sound pressure changes on the 40-Hz auditory steady-state response. Transient frequency analysis was employed to examine the relationship between the magnitude of sound pressure change and both the amplitude and inter-trial phase coherence of the 40-Hz ASSR. The ASSR was elicited by a click train with a fixed inter-click interval, and an abrupt change in sound pressure was introduced midway through the train. The results demonstrated that the abrupt change caused desynchronization of the 40-Hz ASSR, and the degree of desynchronization was dependent on the magnitude of the sound pressure change. In our case, we attribute that the tinnitus is an ongoing neural response that increases the desynchronization causing reduction of the magnitude of ASSR. It is due to this why there was a non-significant trend with an increase in ASSR amplitude between Acute and Post Acute Tinnitus, indicating a possible reduction of the tinnitus activity over time from the onset of tinnitus which is accompanied by significant reductions in both tinnitus distress and tinnitus loudness.

Despite observing a notable increase in low and mid-intensity levels of the ASSR amplitude between Acute and Post-Acute Tinnitus, the question emerges as to why ASSR levels do not exhibit a significant increase for high-intensity stimulation. Is this attributable to a reduced sample size, which could be increased to amplify the effect size to reveal statistical variations, or is it a characteristic of high-intensity signals that typically do not demonstrate significant changes in the presence of tinnitus? The other factor to consider is that there remains to be a tendency to have a steeper slope value when the amplitude differences tend to

be large and by lowering the amplitude at low and mid intensity, there tends to be a steeper slope. Thus, if high intensity increases, the slope remains unchanged; conversely, if high intensity remains constant but only low to mid intensities change, the slope will decrease thus reducing the amplitude growth and further central gain. There was a lack of clarity as to whether the slope, amplitude, or both changes longitudinally due to the above-mentioned limitations, and this further warrants an area of study to resolve in relation to the intensity dependence of ASSR. As outlined in section 4.1.2, the slope is a possible metric of central gain. Does central gain remain constant in tinnitus, as indicated by Zeng (2013), who suggested that tinnitus induces a baseline shift, signifying central noise? Alternatively, does central gain diminish over time, aligning with our hypothesis? Studies of Auditory Evoked Potentials in tinnitus indicate prolonged latencies of their corresponding peaks in affected patients. Kehrle et al. (2008) discovered that the latency of the Auditory Brainstem Response is often prolonged in individuals with tinnitus, suggesting a potential delay in conduction within the brainstem attributable to the presence of tinnitus at that level. Most tinnitus studies report increased latency, which is often attributed to enhanced neural adaptation in response to the persistent tinnitus signal. In our case, regardless of whether tinnitus intensity increases or decreases, it is plausible that the interaction between a loud external sound and the ongoing tinnitus signal leads to greater neural adaptation. This may explain the stronger adaptation observed at low and mid intensity levels, offering an alternative interpretation of our findings

4.4.5 Role of attention and distress

ASSR can be modulated by changes in attention too (Matulyte et al., 2024). Mahajan et al. (2014) explored the role of selective sustained attention on auditory steady-state responses using a frequency tagging paradigm. They compared ipsilateral and contralateral activations in terms of possible presence of attentional modulation towards ASSR. Their findings revealed that attention modulates ASSR amplitude which is generally observed in the contralateral hemisphere. Skosnik et al. (2007) further explored the relationship between effects of selective attention and the auditory steady-state response, particularly in the gamma frequency band not phase locked to stimulus. They utilized an oddball paradigm presenting click trains at different frequencies and the participants were expected to discriminate the target and non-target stimuli. Their findings reveal that when attending to a specific click frequency, the ASSR became enhanced corresponding to that frequency, especially in the

gamma band. This further suggests that ASSR can be modulated by selective attention further highlighting that the gamma band oscillatory activity plays a significant role in auditory attention and discrimination. This implies that attentional modulation of ASSR could be applicable to the tinnitus findings too. A study by Paul et al. (2014) demonstrated that individuals with tinnitus exhibit impaired attentional modulation of the 40 Hz auditory steady-state response specifically within their tinnitus frequency range. The controls tend to show increased ASSR amplitude with attention at both 500 Hz and 5 kHz, but the tinnitus individuals tend to show attention modulation at 500 Hz (below their tinnitus frequency) and not 5 kHz. Roberts et al. (2013) in their investigations towards the role of attention in tinnitus, revealed that tinnitus individuals tend to have a frequency dependent effect on the ASSR amplitude by having a reduced ASSR amplitude at 5 kHz (closer to the tinnitus frequency) when compared to controls further suggesting a possibility of a "busy line" phenomenon, where the ongoing tinnitus-related neural activity interferes with the processing of external stimuli. Alternatively, the reduced ASSR amplitude at the tinnitus frequency could reflect the reduction of thalamocortical synapses or hyperpolarization of thalamic sources subsequent to deafferentation.

In our case, the observed increase in ASSR amplitude over time from Acute to Post Acute Tinnitus may be attributed to heightened attention to the tinnitus during its onset, which enhances the "busy line" phenomena, hence diminishing the amplitude of the ASSR during the onset. It is to note that we did not conduct an attention based ASSR study targeting on the frequency dependence of the stimulus. We solely rely on passive attention processing that may impact the overall auditory processing for individuals with Acute Tinnitus due to the ongoing "busy line" phenomena. It is to be further noted that we did not see this difference at the tinnitus frequency which could be highly attributed to the poor sensitivity of ASSR at high frequencies above 4 kHz and all the groups had a mean tinnitus frequency above 4 kHz (Petitot et al., 2005). With respect to the increased ASSR amplitude at 1 kHz, Chapter 2 elucidates that the distress associated with tinnitus is heightened during its start due to intensified attention and hypervigilance towards the condition, which may subsequently influence the amplitude of Auditory Steady-State Responses (ASSR).

The Auditory Steady-State Response (ASSR) can be influenced by distress; in cases of tinnitus, increased distress associated with tinnitus may affect the ASSR. Sadeghijam et al.

(2022) suggests a relationship between tinnitus distress, attention networks, and ASSR amplitudes. Specifically, they propose that tinnitus individuals with high distress tend to have an increased activity around the prefrontal regions (which is strongly associated with attention and distress networks) and thereby leading to lower ASSR amplitudes. The reduction in ASSR amplitudes can also be due to the overlap between these attention and distress networks with the ASSR generating networks, potentially causing interference. On the other hand, those with lower tinnitus distress may exhibit less prefrontal involvement and thereby a higher ASSR amplitudes due to reduced inhibition or reduced overlapping between the distress networks and ASSR generating network. The article also further highlights that the generation of an ASSR and the robustness of it is based on the combination of the activity of auditory and prefrontal network regions, and individuals with Chronic Tinnitus with severe distress tend have abnormal ASSR amplitude due to inhibitory dysfunction. The article by Moossavi et al. (2019) explores the relationship between Auditory Steady-State Response amplitudes and tinnitus distress levels through a brief pilot study. The levels of tinnitus distress were divided based on the THI scores: a high-stress group and a low-stress group. The researchers measured the amplitudes of the 40 Hz ASSR in both the stress groups and their findings reveal that individuals that individuals with low distress tend to have increased ASSR when compared to individuals with high distress and predominantly involved the antero-frontal and right auditory regions for the differences across all carrier frequencies. The authors conclude that there is a correlation between ASSR amplitude and the degree of tinnitus distress, as measured by the THI. In summary, elevated distress may reduce ASSR amplitude and Chapter 2 explicitly demonstrates that heightened distress correlates with the onset of tinnitus, which subsequently reduces over time. This phenomenon may have contributed to the reduction of ASSR during the initial onset of tinnitus (due to high distress), which tends to increase over time due to reduction in distress.

4.4.6 Gamma Amino-Butyric Acid (GABA) and Tinnitus

Gamma-Aminobutyric acid (GABA) is an inhibitory neurotransmitter in the central nervous system that is an amino acid which is non-proteinogenic in nature (Hepsomali et al., 2020). GABAergic neurons releases GABA that plays a significant function of regulating neuronal excitability throughout the nervous system (Zhou & Danbolt, 2013). The amplitude of ASSR has been associated with changes in GABA. Sugiyama et al. (2021) found that boosting

GABAergic transmission via lorazepam, a benzodiazepine, increased the 40 Hz ASSR in the early auditory cortex. The study by Toso et al. (2024) examined the roles of GABAergic and NMDA receptor-mediated synaptic interactions in generating the 40 Hz auditory steady-state response (ASSR) in the human auditory cortex. Using MEG and placebo-controlled pharmacological interventions, healthy participants received lorazepam (a GABAA_AA receptor agonist) and memantine (an NMDA receptor antagonist). Lorazepam significantly increased 40 Hz ASSR amplitude, indicating a key role for GABAergic inhibition in modulating this response. In contrast, memantine had no effect, suggesting NMDA receptor-mediated excitation may be less critical. These findings support the use of 40 Hz ASSR as a non-invasive marker of cortical circuit function, relevant to disorders like schizophrenia.

GABA has been linked to tinnitus, and Chapter 1 elaborates on its role in this condition. A prevailing theory is that the reduced GABAergic inhibition in the auditory pathway may contribute to the onset of tinnitus due to increase in hyperexcitability and this has reflected with the presence of lower levels of GABA at the level of the auditory cortex for tinnitus individuals (Sedley et al., 2015; Isler et al., 2022). As Toso et al. (2024), emphasized the association between GABAergic activity and ASSR amplitude variations, the reduced ASSR amplitudes observed during the Acute Tinnitus condition in our study may suggest a potential link with GABAergic mechanisms considering there were reduced ASSR amplitudes which could be framed as hypotheses that might inform future studies through GABA-sensitive techniques such as magnetic resonance spectroscopy (MRS) (Sedley et al., 2015).

4.4.7 Limitations

The current study has specific limitations that can be addressed in future research. Initially, Chronic Tinnitus was inadequately matched with Controls, and although hearing was considered in the study, a more substantial finding may have been achieved if hearing across frequencies had been controlled**.** The longitudinal design included a restricted number of patients, which could be increased in future studies to increase statistical power, especially regarding changes at high intensity levels and changes in slope. No responses were detected at the tinnitus frequency, maybe attributable to the sensitivity of Auditory Steady-State Responses (ASSR) at elevated frequencies and the necessity for larger sample sizes to address the anomalous deviations in values.

4.4.8 Summary

The current study indicates that tinnitus-related alterations in ASSR (reduction of 1 kHz amplitude compared to controls) peak at onset and thereafter diminishes, possibly impacted by alterations in neural synchronisation, tinnitus-related distress, GABAergic inhibition and/or attentional modulation. This may or may not suggest a shift in neural activity like central gain, given there were no cross-sectional differences between the groups, particularly between Acute Tinnitus and Controls. The longitudinal variations, particularly at low and medium intensity levels, suggest that the tinnitus activity in isolation diminish over time, that may or may not affect central gain. Figure 4.7 integrates the above-mentioned arguments and depicts the decline of neural responses linked to tinnitus with time, attributable to enhanced neural synchronisation. This activity represents a continuous stimulation that diminishes phase locking to the incoming signal, while a reduction in tinnitus enhances phase locking to the incoming signal.

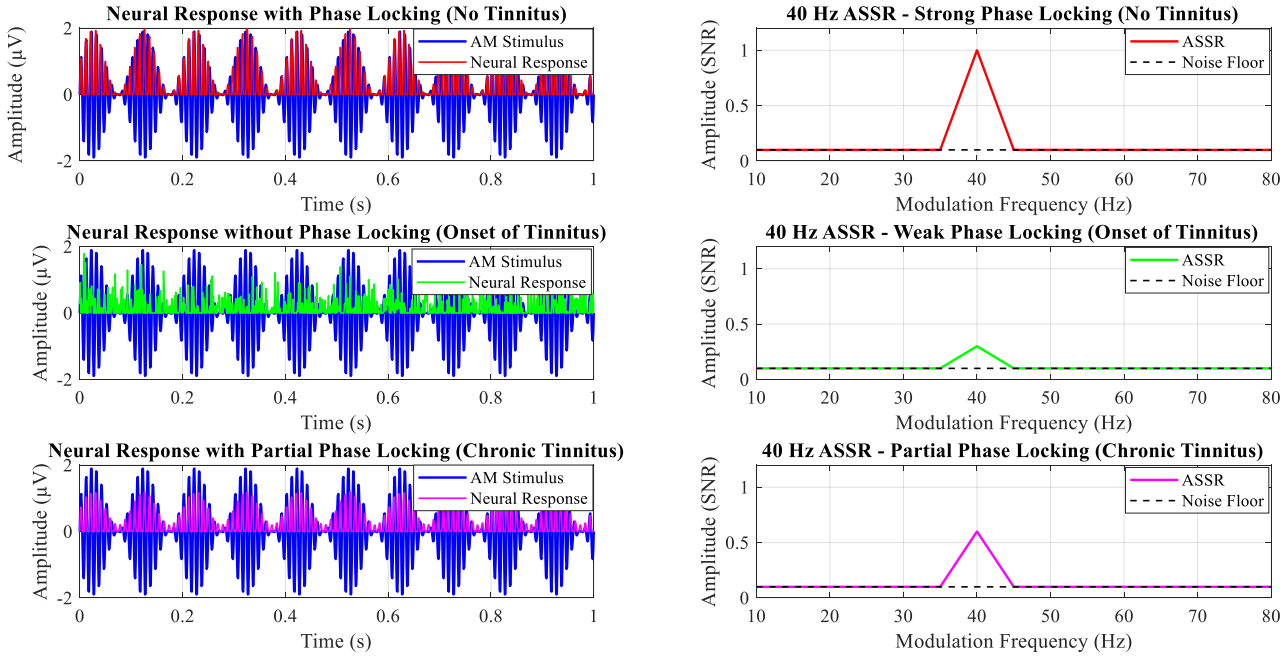


Figure 4.7: Differences in neural response of AM tones across 3 conditions of No Tinnitus, Acute Tinnitus, and Chronic Tinnitus.

In the absence of tinnitus, there exists sufficient neuronal synchrony (phase locking), allowing the neurons to respond appropriately to the amplitude-modulated stimuli. The onset

of tinnitus diminishes neural synchronisation as the neurons that should respond to the amplitude-modulated tones now produce a continuous ongoing stimulation (spontaneous activity), hence decreasing phase locking to the incoming signal. In the chronic stages of tinnitus, the tinnitus activity diminishes, leading to a reduction in abnormal neural synchronisation and an enhancement of the auditory steady-state response (ASSR) amplitude to a nearly normal level.

Chapter 5 The role of tinnitus in mitigating central variance (imprecision) revealed through the natural history of intensity dependence of the auditory evoked potential (IDAEP) from acute to chronic stages.

5.1 Introduction

5.1.1 Summary of previous chapters

Chapters 2, 3, and 4 examined various metrics of neural responses associated with tinnitus, beginning with the alterations in distress, which typically reduce over time. This reduction suggests that the novelty of tinnitus as a stimulus initially heightens focused attention at the onset, resulting in increased distress, which subsequently decreases as signs of habituation and adaptation emerge. The CLS, which assessed sound sensitivity subjectively, tapped into the mechanisms of central gain. Our findings indicated no alterations in the measures of slope and individual loudness categories longitudinally between Acute and Post Acute Tinnitus. Furthermore, there were no differences between Acute Tinnitus and Controls, with only the Chronic Tinnitus group exhibiting increased slope and loudness, typically associated with heightened levels of distress and hyperacusis. The ASSR on the other hand which not only tapped into the mechanisms of central gain but also into other neural responses like neural synchrony tend to show restoration of neural synchrony over time.

5.1.2 Need for further electrophysiological measurements (especially evoked potentials measures)

Considering how mechanisms of tinnitus largely revolve around alterations in neural response properties such as gain, a complementary approach to existing resting state EEG/imaging measures in Acute Tinnitus involves employing evoked potential measurements to investigate variations in response amplitudes to auditory stimuli associated with tinnitus, which can allow inference about underlying alterations in sound processing mechanisms. Auditory evoked potential measures encompass subcortical assessments like the auditory brainstem response and auditory steady state response at high amplitude modulation rates, as well as early cortical measures such as the mid latency response, auditory steady state response (Chapter 4) at mid-to-low amplitude modulation rates, cortical auditory evoked potentials, mismatch negativity, and higher cortical measures like the P300 (Fan & Li, 2022).

The N1-P2 complex of the auditory evoked potential has been widely studied in tinnitus, as well as basic auditory research, as an objective measure of sound processing. N1-P2 indicates the detection of changes in the auditory environment, representing a late stage of cortical processing, sensory gating, sound change detection, and attention allocation, with the majority of the contribution coming from the primary and secondary auditory cortices. When measuring inhibitory functions in tinnitus, repetition suppression of N1 and N1-P2 has been utilised as a marker, particularly when measuring sensory gating by paired click/tone stimulation. The reduction of amplitude in the second tone when compared to the first tone is attributed as normal inhibitory function and an attenuation of this reduction has been found in individuals with tinnitus and with multiple studies implicating reduction in sensory gating functions in individuals with tinnitus (Mohebbi et al., 2019; Spielmann Moura et al., 2010).

With respect to N1 and N1-P2 alone in tinnitus, several studies show that thalamocortical circuit hyperexcitability, maladaptive neural synchrony, and aberrant central gain result in an increased N1 amplitude and a decreased N1-P2 latency (Fan & Li, 2022). A further application of the N1 response (or N1-P2 complex) is the Intensity Dependence of Auditory Evoked Potentials (IDAEP), which is also sometimes termed Loudness Dependence of the Auditory Evoked Potential (LDAEP). The Intensity Dependence of Auditory Evoked Potential (IDAEP) has been validated as a reliable tool for assessing serotonergic function, showing a positive correlation with serotonin levels. This method calculates a single slope value by measuring N1-P2 amplitude changes across varying intensity levels. A steep IDAEP slope suggests reduced serotonin levels or action, and increased hypervigilance or hyperreactivity to sensory stimuli, whereas a shallow slope indicates the opposite (Carrillo-de-la-Peña et al., 2006).The slope of the IDAEP can be used to comment on the excitatory function of neural response such as central gain, which is also a factor of key interest in our current investigation, as serotonin modulates the inhibitory function. Ko et al. (2016) carried out a study measuring the spike probability of axon initial segment through hyperpolarization-activated nucleotide-gated (HCN) channels in the auditory system and established the role of Serotonin especially 5 HT_{IA} in the regulating the activation range of these HCN in the auditory brainstem. This shows that hyperexcitability of auditory neurons can also be directly linked to serotonergic function. With an IDAEP paradigm providing a measure of the central serotonergic system, we could interpret it as a particular kind of

hyperexcitability or even central gain function as well. Interestingly Juckel et al. (1996) tested IDAEP in cats with normal hearing, modifying central serotonergic activity using serotonin agonist and antagonist agents, and found that in early potentials, there is a gradual increase in amplitude at low levels of stimulus intensity, and a constant or decreasing amplitude at high levels of intensity during increased serotonergic activity highlighting a possible phenomenon of augmenting, where amplitude increases with intensity, or reduces, where amplitude levels off or decreases as intensity increases (Prescott et al., 1984). There has been a small number of studies of IDAEP in chronic tinnitus. In a study by Norena et al. (1999) the use of large interindividual Late Auditory Evoked Responses (LAER) was examined by comparing 25 tinnitus patients with 13 controls.
When compared to controls, patients with unilateral tinnitus showed a greater N1-P2 amplitude and intensity dependence, whereas subjects with bilateral tinnitus showed a gre ater N1-P2 intensity dependence but a shorter N1 latency. However, another study by Lee et al. (2007) established differences between tinnitus and non-tinnitus controls and associated tinnitus with weaker, rather than stronger, IDAEP. Findings by Cartocci et al. (2012) reported increased IDAEP slope at N1-P2 and N1 in individuals with Tinnitus when compared to controls. Given the inconsistencies across studies, it is important to replicate similar research in tinnitus, particularly focusing on the natural history or natural course of the condition. This could provide valuable insights into a better understanding of tinnitus.

Outside the context of IDAEP in relation to hypersensitivity, IDAEP also has strong associations with serotonergic and inhibitory function based on its slope values, serotonin has been widely discussed in literature with reports of association between tinnitus and variations in serotonin levels (Knipper et al., 2010; Fornaro & Martino, 2010b). A study by Pillai et al. (2020) investigated the relationship between the loudness dependence of auditory evoked potentials (LDAEP) and the serotonin system using positron emission tomography (PET) and electroencephalography (EEG). The researchers measured serotonin-1A (5-HT1A) receptor and serotonin transporter (5-HTT) binding in the brain of healthy controls and patients with unipolar and bipolar depression. They found that LDAEP was significantly correlated with 5-HT1A positively and 5-HTT negatively in the temporal cortex, but not in the midbrain or raphe nucleus. This suggests that LDAEP may reflect central serotonin transmission, particularly in the temporal cortex. The study supports the hypothesis that LDAEP is

influenced by serotonergic activity which would further validate or utility of the tool. Serotonin plays an important role in auditory regulation and is an important component of modulatory circuitry. Its principal pathway begins in the raphe nuclei and projects widely throughout the brain (Simpson & Davies, 2000). Although, the pathway is mostly extrinsic, there are multiple projections to the auditory system, which heighten on specific tasks or commands. $5HT_{IA}$ and $5HT_2$ have found to be present in Cochlear Nucleus, Inferior Colliculus, and Auditory Cortex (Simpson & Davies, 2000). Serotonin modulates glutamate and GABA, which are significant excitatory and inhibitory transmitters and of primary interest in tinnitus literature, and it is this reason as such is important to consider alongside investigating major neurotransmitters (Sedley et al., 2015).

Serotonin levels and tinnitus distress have largely been linked, with research suggesting that those with high levels of distress may have abnormal serotonin levels. According to other research, serotonin is crucial for sensory gating, and people with tinnitus have problems with gating mechanisms as a result of low serotonin levels. Some also speculate a role of serotonin in driving tinnitus and causing its persistence; Sun et al. (2007) highlighted the possible role of Serotonin in tinnitus persistence as individuals with onset of tinnitus having heightened stress and anxiety due to hypervigilance; individuals who are under stress may have reduced serotonergic function, whilst selective serotonin reuptake inhibitors (SSRIs) can have a role in reducing tinnitus distress in the context of associated anxiety and/or depression. The production and chronification of tinnitus may also be influenced by serotonin because hearing impairment results in a significant amount of gain at the auditory central level (Salvinelli et al., 2003). Serotonin can help reduce tinnitus, however research on animals with salicylate-induced tinnitus have showed that serotonin levels are elevated during the beginning of the condition. This may be the reason why extrapolating from some studies indicates that using SSRIs might sometimes make tinnitus worse.

The conflicting findings from research suggesting possible beneficial and detrimental effects of serotonin in tinnitus underscore the ambiguity regarding the precise function, or combination of functions, of serotonin in tinnitus, and a need for more direct research (Folmer & Shi, 2004; Robinson et al., 2007; Oishi et al., 2010). An additional concern with the research on Serotonin and tinnitus is the dependence on peripheral serotonin levels obtained from blood tests, which are mostly independent of brain serotonergic function. The

latter should be the primary focus of investigation but are challenging to quantify directly. An inherent benefit of IDAEP is its capacity to serve as a correlate to central serotonergic activity where potential link between IDAEP slope and central serotonergic activity can be established. Furthermore, it is important to consider that studies on humans using serotonin have not been able to conclusively establish a causal relationship between changes in serotonin levels and tinnitus i.e. are serotonergic alterations a cause or consequence of tinnitus.

5.1.3 Aims of the present study

Here, we seek to understand the neurobiological alterations associated with tinnitus, from its initial onset to its subsequent chronification, which presently has very limited evidence regarding its neural correlates (Vanneste et al., 2024). We focus on IDAEP, given its relevance to neural responses such as central gain, sensory gating, and serotonergic function, all of which are strongly theoretically implicated in tinnitus but with conflicting findings in the chronic stages. We hypothesised decreased inhibitory function, sensory gating and/or serotonergic function around the time of onset of tinnitus stemming from our principal hypothesis (Chapter 1), as factors likely to allow spontaneous auditory pathway activity (which we have previously referred to as a 'tinnitus precursor') to cross the threshold into conscious perception. We also hypothesised that this activity would fully or completely normalise as tinnitus becomes chronic, either because of regression to the mean, or because of compensatory processes associated with chronic tinnitus. This will be important in understanding the full picture of the brain mechanisms of tinnitus and their time course. In addition to relying on cross-sectional comparisons between groups stratified for the tinnitus characteristics of interest (in this case, duration of tinnitus from its onset), which present a high risk of confounding with other group differences, we additionally repeated the testing of participants with acute tinnitus at a later stage once their tinnitus had become chronic. Our major research measure was IDAEP at 1 kHz since it is a well-established indicator of inhibitory measurement and serotonergic function. Our principal hypothesis was that IDAEP would be increased in the acute tinnitus group, most specifically compared to the same individuals in the subsequent chronic stage of their tinnitus, and possibly compared to those with chronic tinnitus and healthy controls (though these cross-sectional comparisons might be subject to more potential confounds).

However, tinnitus mostly occurs at high frequencies, distant from 1 kHz. The lack of previous studies on IDAEP at high frequencies, including those linked to tinnitus, is noteworthy. We think it is important to address whether the phenomenon of IDAEP exists at high frequencies, and, if so, whether it shows different alterations to the more familiar 1 kHz IDAEP. This analysis was exploratory rather than hypothesis-driven, as the characteristics of IDAEP at high frequencies are presently unknown.

5.2 Methods

5.2.1 Participants

We studied 29 participants with Acute Tinnitus (which we defined as duration 3 days to 6 weeks), with mean tinnitus duration 3.93 weeks (SD 2 weeks) with one outlier with a duration of 12 weeks, 25 participants with Chronic Tinnitus (defined as duration more than 6 months), with median duration 5 years, and 18 hearing-matched (to the acute tinnitus group) non-tinnitus controls. Participants were recruited through community advertising on Google Ads, and internally within Newcastle University's research volunteer pool. The 29 Acute Tinnitus participants were invited for reassessment after a minimum of 6 months from tinnitus onset (which we took to indicate their chronic stage), and of them 15 volunteered for and completed this further testing. We refer to this as the 'Post Acute' group, to distinguish it from the group recruited during the chronic stage of tinnitus. Please refer to Chapter 3, section 3.2.1 for inclusion and exclusion criteria. The Control participants were matched to the acute tinnitus group for age, sex, hearing, and stimulus frequency and presentation ear. Details are displayed in Table 1.

5.2.2 Audiological Assessment

Please refer to Chapter 3, section 3.2.2

5.2.3 Tinnitometry

Please refer to Chapter 3, section 3.2.3

5.2.4 IDAEP Stimuli

With a total of 200 stimuli per frequency and a 25-minute total experiment length, the IDAEP stimuli consisted of pure tones at 1 kHz or the individual's matched tinnitus frequency (or, for controls, the matched tinnitus participant's tinnitus frequency), with a duration of 100 ms,

onset/offset ramp of 10 msec, and an interstimulus interval randomized between 1 sec and 1.5 sec. For every frequency, three different intensities were presented. Stimuli were presented in one block, with intensities and frequencies fully randomised within that block. To account for hearing loss, loudness recruitment and hyperacusis, whilst keeping the stimuli clearly audible yet comfortable, stimulus intensities were presented in accordance with each participant's dynamic range, i.e. the range between their hearing threshold and uncomfortable loudness level, which were established for the specific experimental stimuli at both 1 kHz and the tinnitus frequency. The hearing thresholds were established using an ascending-descending run of 3 dB steps until at least 50% positive response was obtained (i.e. 2 correct responses out of 4 trials) (refer Chapter 3, section 3.2.2). The uncomfortable loudness level was established by presenting an ascending run of 3 dB step size from the threshold until the participant just perceived the tone to be uncomfortably loud or showed signs of discomfort ('Recommended Procedure for Determination of Uncomfortable Loudness Levels', 2022). Dynamic range was defined as uncomfortable loudness level (ULL) minus hearing threshold. Stimulus intensities were determined as follows:

A. Low Intensity: hearing threshold plus 60% of Dynamic Range
B. High Intensity: ULL – 10 dB
C. Mid Intensity: Mean of Low and High Intensity

Subjects with a difference of less than 5 dB difference between Low and High stimulus intensities as determined by this method were excluded, on account of having insufficient dynamic range to yield adequate signal-to-noise ratio in their IDAEP slope estimates.
For those with unilateral tinnitus, stimuli were only presented in the tinnitus ear; for those with bilateral tinnitus, they were presented in both ears. Participants with acute tinnitus were matched individually with controls who shared the same age, sex, and hearing. Control participants were presented with stimuli that matched the frequency and presentation ear(s) of their matched tinnitus participant but were individualised in intensity to the control participant based on the same dynamic range procedure. Table 1 shows the ear presentations for each group.

5.2.5 IDAEP EEG Recording

EEG was recorded using a 64-channel Active Two system (Biosemi) in a soundproof room. Electrode offset was kept at the manufacturer's recommended limits of ±10mv with a

sampling rate of 256Hz. The experiment was a passive task, during which the participants watched a silent subtitled movie of their choosing. All stimuli were generated and presented using the Matrix Laboratory (MATLAB) version R2019a, using the Psych toolbox (Pelli, 1997).

5.2.6 EEG Pre Processing

Data were processed in MATLAB, version R2019a, using the EEGlab toolbox (Delorme & Makeig, 2004) and customised code. Data were re-referenced to the P9/P10 (Linked mastoids) montage. The data were bandpass filtered between 1 Hz and 30 Hz using a non-phase-distorting (zero-phase) filter to preserve the temporal characteristics of the cortical evoked potentials. Channels judged to contain excessive noise were removed and reconstructed by interpolation from neighbouring channels. Channel rejection was based on visual inspection and keeping 0.8 as the accepted correlation between channels. This was followed by epoching between - 0.1 and 0.5 s from stimulus onset. Independent Component Analysis (ICA) was performed, and components capturing predominantly eye blinks or eye movements were removed from the data. Subsequent to ICA, artifact rejection was also carried out using individual component rejection where components are auto rejected keeping a probability of 5 and Kurtosis of 8 as the threshold. Baseline correction was performed based on the time period of -100 to 0 msec.

5.2.7 Data Analysis

After pre-processing, we used customised code to perform automatic peak detection to determine the N1 and P2 for each subject. We did this by utilising the active electrode as FCz to measure the most negative peak amplitude for N1 between latency 90 and 200 msec and the most positive peak amplitude for P2 between latency 100 and 250 msec. The peak-to-peak amplitude between N1 and P2 in μV was taken as the variable of interest. Each subject had peak to peak amplitude calculated for 3 intensities across 2 frequencies of 1 kHz and tinnitus frequency. With these, the slope function at each frequency was calculated as the slope is a key component of the IDAEP function and the slope was calculated as the quotient of the Amplitude Dynamic Range (ADR: relative difference of the high and low N1-P2 amplitude) and Stimulus Dynamic Range (SDR: relative difference of the high and low stimulus intensity).

$$\frac{\text{High intensity response amplitude} - \text{Low intensity response amplitude}}{\text{High intensity stimulus intensity} - \text{Low intensity stimulus intensity}}$$

Units of the IDAEP slope are thus μV/dB. Note that the mid-intensity stimuli were not used for analysis but were judged important to include in the paradigm for their influence on the overall statistics of presented stimuli. Statistical analysis was performed using the Statistical Package for Social Science (SPSS). Based on the Shapiro-Wilk test of normality, the data was normally distributed across the three groups for 1 kHz, and hence parametric statistics were used. However, the data were not normally distributed for tinnitus frequency, and hence a non-parametric statistic was used for these. The statistical analysis was done in three stages.

In the initial analysis, the N1-P2 values obtained at different intensities and frequencies were compared between groups both cross-sectionally and longitudinally. Individual MANOVA (Multivariate Analysis of Variance) was performed at a frequency of 1 kHz and the tinnitus frequency. If a significant effect was found, a Tukey's post hoc test was implemented.
As a second analysis, the IDAEP slope values (for N1-P2) for each frequency were compared between the groups. Since there existed sample size inequality between the stimulus frequency groups, statistical analysis was conducted independently for each frequency instead of a factorial analysis with group and frequency. Significant differences across the groups for 1 kHz were determined using a one-way ANOVA (Analysis of Variance), and for the tinnitus frequency groups, the Non-parametric Kruskal Wallis test was used. To compare the groups between the Acute phase and the follow-up post-acute phase for both frequencies, a paired t test was employed. Because the subject numbers were lower for this analysis (given the extreme challenges of recruiting and retaining this group), and we had a very clear prior hypothesis, we used one-tailed statistics to only seek a decrease in IDAEP slope over time. For all other analyses, two-tailed statistics were used.

Additionally, using groups with both Acute and Chronic Tinnitus combined, we conducted an exploratory analysis to investigate the relationship between the IDAEP slope and the duration of tinnitus. This instance involved the use of a Pearson correlation. We also carried out a comparable exploratory analysis to investigate the relationship between SDR and IDAEP slope, in case differences in SDR might partially explain or obscure between-group

differences in IDAEP slope. We ran a Pearson's correlation test for each of the three groups independently to ascertain the direction of the correlations between the slope and SDR. The effect of SDR on slope for each of the three groups independently at both frequencies was then examined using linear regression. The regression slopes for the three groups were then compared using and Kruskal Wallis for regression slopes.
With respect to the longitudinal comparisons between the groups, all 3 variables of N1-P2, IDAEP slope, and regression slopes were compared using a either a paired t test or Wilcoxon sign ranked test based on the distribution.

5.3 Results

Participant groups comprised 29 Acute Tinnitus, 25 Chronic Tinnitus, and 18 Controls. Participants were rejected if they had insufficient stimulus dynamic range (criteria of minimum 5 dB was set based on overall distribution of data) or overall ERP quality. Such exclusions were performed with the researcher blind to the group to which each participant belonged. ERP quality was judged subjectively, and the minimum acceptable SDR was based on inspection of a funnel plot of SDR against IDAEP slope estimate. For 1 kHz, a total of 2 participants were rejected for Acute Tinnitus, 5 for Chronic, and 1 for Control. For Tinnitus Frequency, 4 were rejected for Acute Tinnitus, 12 for Chronic and 3 for Controls.

This section is divided into five sub sections: 1) demographics and hearing between the groups; 2) differences in tinnitus distress between the groups; 3) differences in N1-P2 amplitudes between the groups 4) differences in IDAEP slope between the groups; 5) The relationship between IDAEP slope and stimulus dynamic range. Different statistics were run for each frequency due to the variations in sample sizes between the tinnitus frequency and 1 kHz, and these will be discussed individually.

5.3.1 Demographics and Hearing

Participant groups were attempted to be matched for age, sex, hearing, and mode of stimulus presentation. There were no significant differences across the groups for age, sex, and stimulus ear presentation for both the samples at 1 kHz and tinnitus frequency. Using the 1 kHz, 4 kHz, and 8 kHz hearing thresholds, an attempt was made to pair the groups. For unilateral tinnitus, the groups were matched at the tinnitus ear, and for bilateral tinnitus, at the right and left ears individually. There were no significant differences between subject groups

at either 1 kHz or 4 kHz for both unilateral and bilateral tinnitus. The only significant difference was found at 8 kHz between the Acute Tinnitus and Chronic Tinnitus groups for bilateral tinnitus in the left ear (p = 0.05), with the Chronic Tinnitus group having a higher hearing threshold than Acute Tinnitus. For further details please refer to Table 5.1.

Subjects included in 1 kHz analysis					
Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Number of Subjects	27	20	17		
Age (Years)	M = 52.89 SD = 13.56	M = 54 SD = 9.5	M = 53.53 SD = 15.54	F = 0.43 p = 0.958	NS
Gender (M/F)	5/22	9/11	3/14	X^2 = 5.737 p = 0.079	NS
Ear presentation (U/L – B/L)	12/15	7/13	6/11	X^2 = 0.569 p = 0.752	NS
Hearing (1 kHz) dBHL	M = 12.31 SD = 8.20	M = 15.5 SD = 15.87	M = 10.75 SD = 11.48	F = 0.791 p = 0.458	NS

Subjects included in Tinnitus Frequency					
Number of Subjects	25	13	15		
Age (Years)	N = 25 M = 51.8 SD = 13.59	N = 13 M = 54.38 SD = 9.94	N = 15 M = 51.87 SD = 15.17	F = 0.184 p = 0.833	NS
Gender (M/F)	5/20	4/9	2/13	X^2 = 1.304 p = 0.521	NS
Ear presentation (U/L – B/L)	10/15	5/8	5/10	X^2 = 0.181 p = 0.913	NS

Hearing (4 kHz) dBHL	M = 20.6 SD = 14.83	M = 25 SD = 13.42	M = 16.33 SD = 13.69	F = 1.301 p = 0.281	NS
Hearing (8 kHz) dBHL	M = 25.2 SD = 17.73	M = 461.15 SD = 16.15	M = 28.17 SD = 23.87	F = 3.005 p = 0.059	Acute Tinnitus – Chronic Tinnitus: p = 0.05*

Table 5.1: The participants demographics for each group are shown in Table 1. *N denotes number of samples, M denotes mean, SD denotes Standard Deviation, MD denotes Median, F statistic indicates a One Way ANOVA has been carried out, H statistic with X^2 indicates a non-parametric Kruskal Wallis test, X^2 indicates a chi square goodness of fit test, NS denotes No Significance. * indicates presence of statistical significance at 95% confidence interval. Hearing thresholds were calculated by averaging the left and right ear values in cases of bilateral tinnitus, whereas for unilateral tinnitus, the threshold was based on the ear with tinnitus.*

5.3.2 Symptom Scores for Tinnitus Distress

After excluding certain subjects from each analysis, for data quality purposes, the composition of the groups included in 1 kHz and tinnitus frequency analyses were slightly different, and we report each separately below.

1 kHz

For subjects whose data were included in analysis of responses to 1 kHz stimuli, cross-sectional comparisons between the Acute and Chronic Tinnitus for THI and TFI yielded no significant differences (THI: t = -1.125, p = 0.148, TFI: t = -1.814, p = 0.076). A paired comparison between the Acute and Post-Acute Tinnitus group revealed a significant reduction over time in THI score (t(11) = -2.549, p = 0.0027), TFI score (t(11) = -4.282, p = 0.013 with Acute Tinnitus having higher THI (M = 24.5, SD = 11.88) and TFI(M = 83.08, SD = 32.62) scores when compared to Post-Acute Tinnitus (THI - M = 17.33, SD = 13.08, TFI – M = 83.08, SD = 30.63.

Tinnitus Frequency

Similar results were obtained when the same comparisons were repeated for just the participants whose data were used for analysis of responses to tinnitus frequency stimuli. These comprised: between Acute and Chronic ($t = 0.485$, $p = 0.631$, $t = -0.22$, $p = 0.827$) and the same for the pairwise comparison between Acute and Post Acute Tinnitus group (THI: $t(9) = -2.106$, $p = 0.064$, TFI: $t(9) = -3.913$, $p = 0.003$) with Acute Tinnitus having higher THI ($M = 25.8$, $SD = 12.38$) and TFI ($M = 88.5$, $SD = 29.62$) scores when compared to Post-Acute Tinnitus (THI - $M = 19$, $SD = 13.8$, TFI – $M = 59.6$, $SD = 28.95$).

5.3.3 Symptom Scores for Hyperacusis Questionnaires

1 kHz

Significant main effects of group were observed in cross-group comparisons between acute, chronic, and non-tinnitus controls for hearing loss in participants whose data were used in the analysis of response to at 1 kHz $F(2,63) = 7.132$, $p = 0.002$).

Tukey's post-hoc test revealed significant differences between controls and Acute Tinnitus ($p = 0.02$) and chronic Tinnitus ($p = 0.002$). Controls had lower HQ scores ($M = 6.35$, $SD = 4.6$) compared to both Acute Tinnitus ($M = 14.07$, $SD = 5.96$) and Chronic Tinnitus ($M = 16.44$, $SD = 11.34$). Consistent findings were obtained for IHS, $F(2,63) = 6.442$, $p = 0.003$ and as shown by Tukey's post hoc test, controls had lower IHS scores ($M = 31.28$, $SD = 6.8$) in comparison to Acute Tinnitus ($M = 44.11$, $SD = 12.4$, $p = 0.014$) and Chronic Tinnitus ($M = 49.35$, $SD = 18.1$, $p = 0.001$). Upon the paired longitudinal comparison, no change over time was observed for scores on either HQ ($t(12) = 0.04$, $p = 0.966$) and IHS ($t(12) = -0.326$, $p = 0.75$).

Tinnitus Frequency

For subjects included in the tinnitus frequency analysis, statistically significant equivalent results were obtained for both HQ ($F(2,46) = 9.575$, $p < 0.001$*) and IHS ($F(2,46)$ 5.849, $p = 0.006$*) across groups. Tukey's post hoc test revealed significant differences between controls with Acute Tinnitus ($p = 0.007$) and chronic Tinnitus ($p < 0.001$). Controls had lower HQ scores ($M = 6.31$, $SD = 4.79$) compared to both Acute Tinnitus ($M = 14.04$, $SD = 6.18$) and Chronic Tinnitus ($M = 19$, $SD = 10.97$) respectively. In comparison to Acute Tinnitus ($M = 43.72$, $SD = 12.8$, $p = 0.011$) and Chronic Tinnitus ($M = 46.2$, $SD = 14.3$, $p = 0.013$), controls

(M = 31.38, SD = 7.08) yielded lower IHS scores. The results of the symptom questionnaires and Tinnitometry for each of the three groups are shown in Table 5.2.

Subjects included in 1 kHz					
Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Number of Subjects	27	20	17		
Tinnitus Pitch (Hz)	M = 5419.39 SD = 2699.77	M = 7201.98 SD = 1946.4	M = 5505.03 SD = 2313.81	F = 3.705 p = 0.03*	Acute Tinnitus–Chronic Tinnitus; p = 0.037* Chronic Tinnitus – Control; p = 0.087
Tinnitus Loudness (dB)	M = 17.66 SD = 17.04	M = 8.89 SD = 12.18	N/A	t = 1.958 p = 0.056	
Number of Subjects	27	17	14		
THI	M = 35.19 SD = 21.26	M = 42.71 SD = 22.11	N/A	t = -1.125 p = 0.148	
TFI	M = 100.88 SD = 45.24	M = 129.76 SD = 60.10	N/A	t = -1.814 p = 0.076	
HQ	M = 14.07 SD = 5.96	M = 17.41 SD = 10.9	M = 6.35 SD = 4.6	F = 8.676 p < 0.001*	Acute Tinnitus–Control;

					p = 0.008*
					Chronic Tinnitus – Control; p < 0.001*
IHS	M = 44.11 SD = 12.4	M = 49.35 SD = 18.1	M = 31.28 SD = 6.8	F = 7.345 p = 0.002*	Acute Tinnitus– Control; p = 0.014*
					Chronic Tinnitus – Control; p = 0.001*
Subjects included in Tinnitus Frequency					
Number of Subjects	25	10	15		
Tinnitus Pitch (Hz)	M = 5283.93 SD = 2749.27	M = 6992.63 SD = 1353.69	M = 5232.53 SD = 2272.44	F = 2.658 p = 0.079	NS
Tinnitus Loudness (dB)	M = 17.79 MD = 16.5 SD = 17.73	M = 6.62 MD = 3.67 SD = 11.59	N/A	z = 1.955 p = 0.05	
Number of Subjects	25	13	15		
THI	M = 36.46 SD = 21.45	M = 32.8 SD = 18.65	N/A	t = 0.485 p = 0.631	

TFI	M = 102.84 SD = 46.4	M = 107.1 SD = 63.7	N/A	t = -0.22 p = 0.827	
HQ	M = 14.04 SD = 6.18	M = 19 SD = 10.97	M = 6.31 SD = 4.79	F = 9.575 p < 0.001*	Acute Tinnitus–Controls; p = 0.007*
					Chronic Tinnitus – Controls; p < 0.001*
IHS	M = 43.72 SD = 12.8	M = 46.2 SD = 14.3	M = 31.38 SD = 7.08	F = 5.849 p = 0.006*	Acute Tinnitus–Controls; p = 0.011*
					Chronic Tinnitus – Controls; p = 0.013*

Table 5.2. The participant's tinnitus distress and Tinnitometry related data for each group are shown in Table 5.2

*N denotes number of samples, M denotes mean, SD denotes Standard Deviation, F statistic indicates a One Way ANOVA has been carried out, t statistic indicates and independent t test done, N/A- Not Applicable, NS denotes No Significance. * indicates statistical significance at p < 0.05, THI - Tinnitus Handicap Inventory, TFI – Tinnitus Functional Index, HQ – Hyperacusis Questionnaire, IHS – Inventory of Hyperacusis Symptoms. It is important to highlight that there is an asymmetry in the sample sizes between the questionnaires and the original sample, resulting from the exclusion of participant questionnaires with incomplete responses.*

5.3.4 N1-P2 amplitude

1 kHz

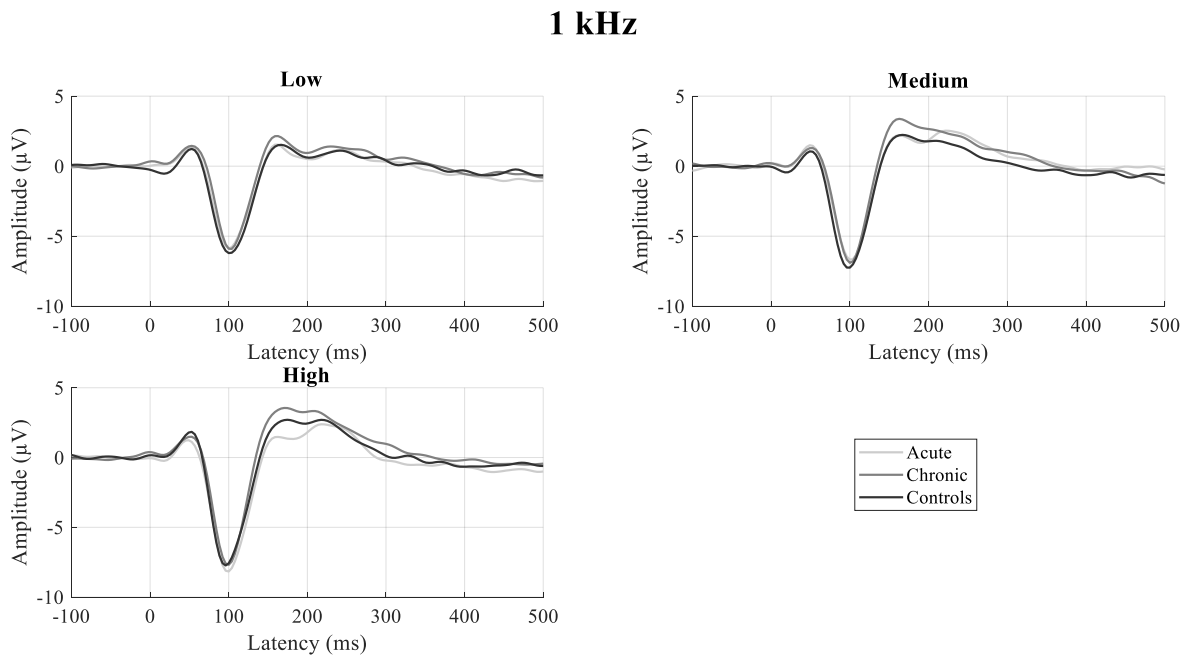


Figure 5.1: The N1-P2 waveforms across groups for 1 kHz is illustrated in figure 5.1. *Graph indicates low, medium, and high intensities with Acute, Chronic, and Controls depicted in each intensity.*

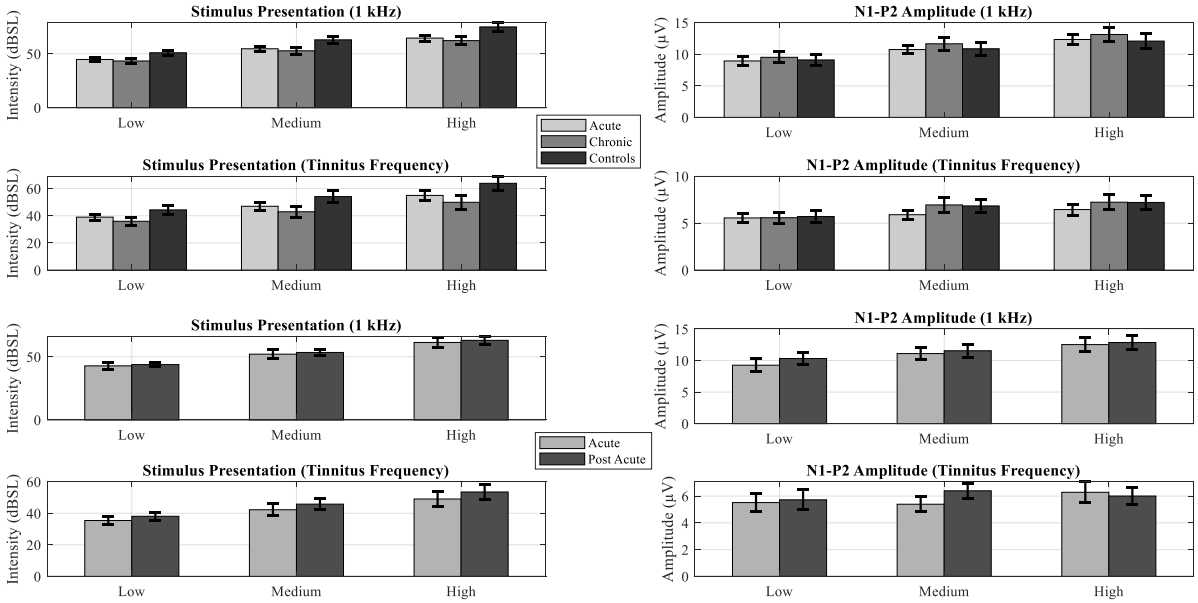


Figure 5.2: Stimulus presentation levels and corresponding N1-P2 responses are illustrated across groups for low, medium, and high intensity conditions. *Results as depicted in section 5.3.4 indicate a significant effect with controls having high stimulus presentation levels when compared to the other groups of tinnitus and no significant effect of N1-P2 amplitudes across intensities for both 1 kHz and tinnitus frequency. Error bars indicate standard error for mean.*

Before comparing absolute N1-P2 amplitudes among groups, we assessed the stimulus presentation levels to ensure that the results are not influenced by varying stimulus presentation levels. Upon carrying a one-way ANOVA, there was an overall significant effect for low ($F(2,61) = 3.346$, $p = 0.042$), medium ($F(2,61) = 3.346$, $p = 0.042$), and high ($F(2,61) = 3.346$, $p = 0.042$). In all three intensity levels, there were significant differences between Chronic Tinnitus and Controls for low ($p = 0.046$), medium ($p = 0.046$), and high ($p = 0.046$) with Controls having higher stimulus presentation levels when compared to Chronic Tinnitus. Figure 5.1 depicts the N1-P2 waveforms across conditions and groups for 1 kHz and figure 5.2 illustrates the stimulus presentation levels across the groups.

A 2-way ANOVA was conducted to examine the effect of group and stimulus intensity category (high, medium, and low) on the IDAEP amplitude and results revealed no presence of statistically significant interaction effects ($F(4,183) = 0.034$, $p = 0.998$). We further validated the results with stimulus presentation level as a covariate and reran with a 2-way Analysis of Covariance and similar results were obtained with no significant interaction effects ($F(4,183) = 0.073$, $p = 0.990$). A paired t test showed that there were near-significant longitudinal differences between Acute and Post Acute Tinnitus groups for low intensity N1-P2 ($t(12) = -2.008$, $p = 0.068$). Post Acute Tinnitus had a higher amplitude ($M = 10.32$, $SD = 3.15$) compared to Acute Tinnitus ($M = 9.26$, $SD = 3.55$). Additional, other intensity indicators for N1 showed no statistically significant variation between the groups. Figure 5.2 depicts the stimulus intensity presentation levels and N1-P2 amplitude across gorups.

Tinnitus Frequency

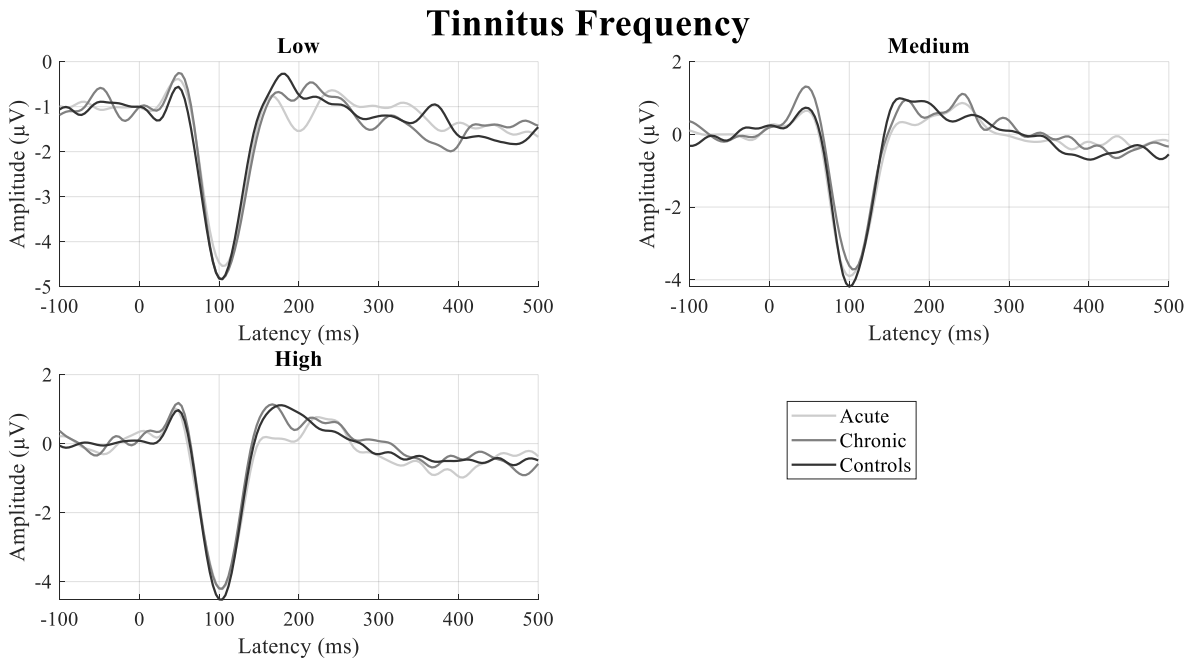


Figure 5.3: The N1-P2 waveforms across groups for tinnitus frequency is illustrated in figure 5.3. *Graph indicates low, medium, and high intensities with Acute, Chronic, and Controls depicted in each intensity.*

There were no statistically significant effects on stimulus presentation levels across groups for low ($F(2,50) = 2.141$, $p = 0.128$), medium ($F(2,50) = 2.141$, $p = 0.128$), and high ($F(2,50) = 2.141$, $p = 0.128$). Similar results were obtained for the tinnitus frequency pertaining to amplitude changes. A 2-way ANOVA was conducted to examine the effect of groups and levels of amplitude (high, medium, and low) on the IDAEP amplitude, and results revealed no statistically significant interaction effects ($F(4,149) = 0.219$, $p = 0.928$).
Regarding the comparative longitudinal differences between the Acute and Post Acute tinnitus groups, the cross-sectional results were reproduced and no statistically significant differences between the groups across low, medium, or high levels was obtained. Figure 5.3 depicts N1-P2 waveforms for tinnitus frequency across the groups for low, medium, and high intensities.

5.3.5 IDAEP Slope

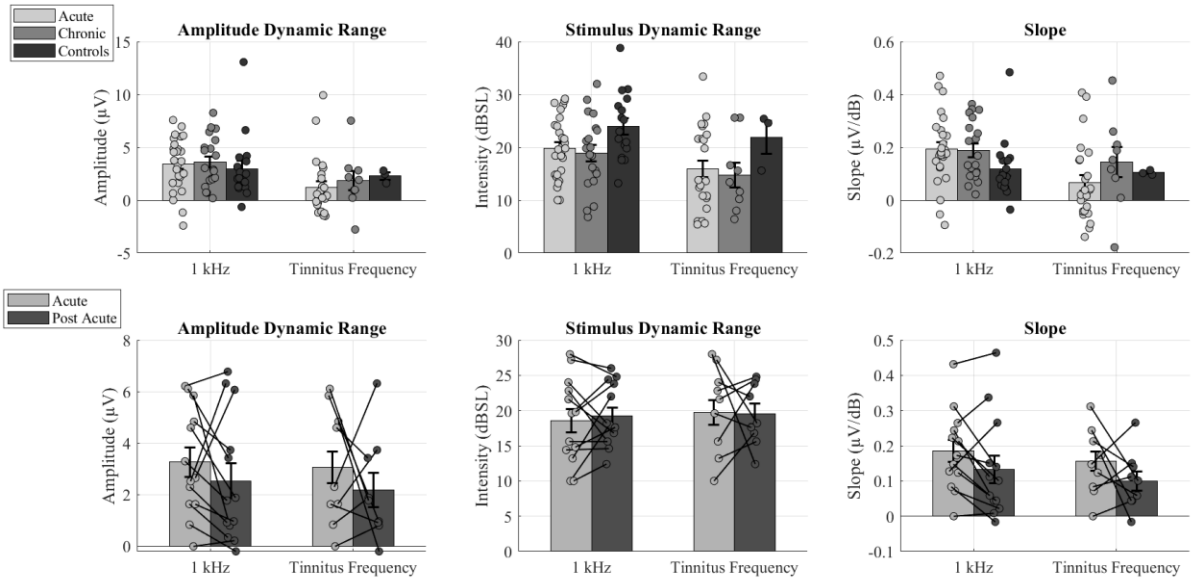


Figure 5.4. Variations in Amplitude Dynamic Range, Stimulus Dynamic Range, and Slope (Amplitude Dynamic Range/Stimulus Dynamic Range) between groups across frequencies. *Error bars indicate standard error for mean and TF indicates Tinnitus Frequency. A statistically significant difference was found between Controls and Acute Tinnitus, Controls and Chronic Tinnitus for Stimulus Dynamic Range at 1 kHz. A statistically significant difference was noted between Acute and Post Acute Tinnitus for Slope at 1 kHz. Other variables yielded no significant differences.*

1 kHz

There was no significant effect of subject groups on IDAEP slope ($F(2,61) = 2.194$, $p = 0.120$) and on ADR $F(2,61) = 0.274$, $p = 0.761$). However, there was significant effect on SDR $F(2,61) = 3.346$, $p = 0.042$) with a statistically significant difference present between Chronic Tinnitus and Controls ($p = 0.046$) with Controls having higher SDR ($M = 23.98$, $SD = 6.37$) when compared to Chronic Tinnitus ($M = 18.89$, $SD = 6.92$). It is due to the significant effect noted in SDR between groups, we decided to further validate our results with a covariate analysis, keeping SDR as covariate and measuring the effects of groups in Slope and ADR with a one-way ANCOVA. Similar results were replicated after controlling for SDR in slope $F(2,61) = 1.608$, $p = 0.209$) and ADR $F(2,61) = 0.903$, $p = 0.411$). Based on our primary hypothesis, a one-tailed t test was employed (seeking only decreases over time)

regarding the longitudinal change in IDAEP slope between Acute (M = 0.186, SD = 0.11) and Post Acute Tinnitus (M = 0.133, SD = 0.14), which revealed a significant difference with a longitudinal decrease in IDAEP slope over time (t (12) = -1.919, p = 0.04). Figure 5.4 illustrates the SDR, ADR, and slope values respectively across groups (cross sectional and longitudinal) for both 1 kHz and tinnitus frequency.

Tinnitus Frequency

At the tinnitus frequency, there was no significant difference in both IDAEP slope ($X^2(2)$ = 3.601, p = 0.165) and ADR ($X^2(2)$ = 2.212, p = 0.331) across groups. Further, there was no significant effect for SDR (F(2,50) = 2.141, p = 0.128). Similarly, there was no significant longitudinal change in IDAEP slope over time (t = 0.74, p = 0.477). However, the more striking observation was that IDAEP slope values were all close to zero, despite robust ERPs being obtained (Acute Tinnitus: M – 0.059, SD – 0.147, Chronic Tinnitus: M – 0.122, SD – 0.17, Controls: M – 0.034, SD – 0.149, Acute Tinnitus: M – 0.05, SD – 0.13, Post Acute Tinnitus Phase: M – 0.28, SD – 0.97).

5.3.6 Relationship between IDAEP slope and stimulus dynamic range

We chose to conduct an additional exploratory analysis at 1 kHz and tinnitus frequency to determine the association between Stimulus Dynamic Range (SDR) and IDAEP slope. This was partly as a control analysis, to ensure that the considerable difference observed in slope between acute and chronic tinnitus was not due to differences in mean stimulus dynamic range between groups, on account of ensuing dynamic range adaptation. Furthermore, we were interested to see whether different stages of tinnitus were associated with differences in dynamic range adaptation for IDAEP, which might manifest as different relationships between dynamic range and IDAEP slope.

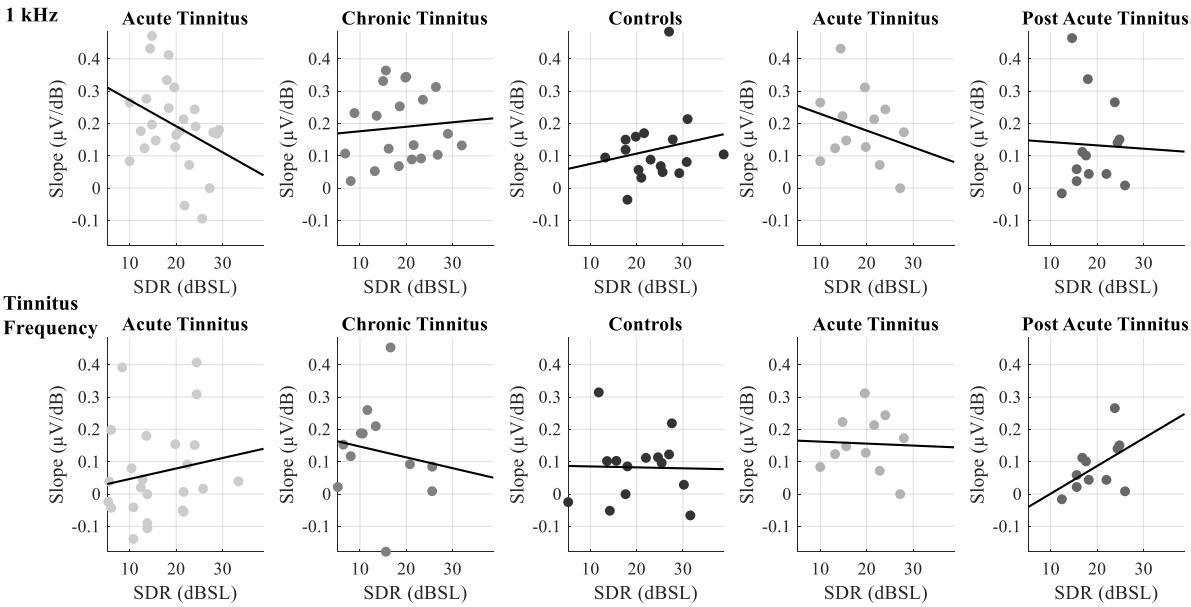


Figure 5.5: Represents the relationship between slope and stimulus dynamic range across groups and frequencies. *SDR indicates Stimulus Dynamic Range. A non-significant negative trend was noted for Acute Tinnitus at 1 kHz.*

1 kHz

Results revealed a mild negative correlation which was a non-significant trend for the acute tinnitus group (correlation: r(25) = -0.356, p = 0.068, regression: b = -0.008, SE = 0.004, t(25) = -1.91, p = 0.068, R^2 - 0.127), no correlation for the chronic tinnitus group (correlation: r(18) = 0.086, p = 0.717, regression: b = 0.001, SE = 0.004, t(18) = 0.368, p = 0.717, R^2 - 0.07), and no correlation for the non-tinnitus controls (Correlation: r(15) = 0.182, p = 0.485, regression: b = 0.003, SE = 0.005, t(15) = 0.61, p = 0.549). The longitudinal analysis reveals no correlation for the Acute Tinnitus (correlation: r(11) = 0.265, p = 0.382, regression: b = -0.004, SE = 0.005, t(11) = -0.91, p = 0.381, R^2 - 0.07) and no correlation for the Post Acute Tinnitus between the stimulus dynamic range and the slope (corelation - r(11) = -0.032, p = 0.917, regression: b = -0.001, SE = 0.009, t(11) = -0.11, p = 0.91, R^2 - 0.001). Figure 5.5 depicts the relationship between SDR and slope for cross sectional comparisons and longitudinal comparisons respectively.

Tinnitus Frequency

For the tinnitus frequency, between IDAEP slope and SDR, there was no correlation for Controls (correlation – r(13) = 0.005, p = 0.94, regression - b = 0.002, SE = 0.004; t(13) =

2.43; p = 0.03; R2 - 0.312) and no correlation for both Acute Tinnitus group (correlation – r(23) = 0.158, p = 0.452; regression - b = 0.003, SE = 0.004; t(23) = 0.77; p = 0.45; R^2 - 0.148), and Chronic Tinnitus group (correlation – r(11) = -0.127, p = 0.679; regression - b = 0.003, SE = 0.007; t(11) = -0.424; p = 0.679; R^2 - 0.127).

The longitudinal analysis reveals for the acute tinnitus to have no correlation between stimulus dynamic range and the slope (correlation – r(9) = 0.311, p = 0.352, regression - b = -0.0068: SE = 0.0069, t(9) = -0.98, p = 0.352, R^2 - 0.096) and for the Post-Acute Tinnitus, there was no correlation between the stimulus dynamic range and the slope (correlation = r(9) = 0.941, p = 0.783, regression; b = -0.001; SE = 0.0036, t(9) = -0.283, p = 0.783, R^2 - 0.008). Figures 5.5 depict the relationship between SDR and slope for both cross sectional and longitudinal comparisons respectively.

5.4 Discussion

5.4.1 Summary of main findings

There were significant differences in the IDAEP slope at 1 kHz between Acute and Post Acute Tinnitus, longitudinally, with increased slope values around the onset of tinnitus. Additionally, there was a non-significant trend in correlations between SDR and IDAEP slope, with Acute Tinnitus showing a trend in negative correlation, and both Chronic Tinnitus and Controls showing no correlation.

5.4.2 Results cannot be explained by hearing thresholds, distress, stimulus, or early auditory processing differences.

It is to be noted that the current results were not confounded by hearing, as there were no significant differences across groups at 1 kHz. In addition, absolute N1-P2 amplitudes were relatively consistent across the subject groups, with only a near-significant trend towards higher amplitude in the Post Acute compared to Acute stages observed at low intensity for 1 kHz. Therefore, the results appear specific to the processes of IDAEP itself, rather being consequences of upstream differences in auditory processing.

It should also be mentioned that we used three different intensities, each tailored to the subject's dynamic range, and that the subject's ULL and threshold determined the presentation level. Through this approach, we managed to account for variables including recruitment, hearing impairment, and even hyperacusis-related gain to a certain degree. Importantly, this

approach kept stimuli within a similar range of perceived intensities and individual reactions to stimuli, and ensured that no stimuli were perceived as aversive, which might have recruited various secondary neural processes that can affect sensory processing. This individualised dynamic range approach resulted in stimulus sensation levels (i.e. stimulus intensity minus hearing threshold), which did not differ significantly between Acute and Chronic Tinnitus groups, or longitudinally between Acute and Post Acute Tinnitus, hence the mean group results cannot be trivially explained by the tailored stimulus presentation. Whilst tinnitus distress did reduce over time in the Acute to Post-Acute transition, there was no cross-sectional difference in tinnitus distress between Acute and Chronic groups, so the combination of comparisons rules out this potential confound of tinnitus-related distress being a more trivial explanation for the findings. There were no confounding variables because all other variables, including age, sex, and mode of presentation (U/L or B/L), were matched between groups. Our results were repeated using a robust pairwise comparison design between Acute and Post Acute, giving validity to our overall findings even in the event that there might be a confounding factor in the cross-sectional comparison between Acute and Chronic Tinnitus.

5.4.3 IDAEP is increased in the acute stages of tinnitus

The interpretation of this result is in line with our hypothesis motivated by the sensory precision model (Sedley et al., 2016), which postulates an increase in auditory (or even more generalised sensory) hyperexcitability and/or hypervigilance associated with the onset of tinnitus, with subsequent full or partial normalisation over time. We suggested that the onset of tinnitus occurs because this state of heightened sensory sensitivity increases the precision of the prediction error arising from spontaneous auditory pathway noise, leading to overcoming of the default auditory prediction of ‘silence’ (in the absence of an incoming stimulus). This prediction error is at least partially resolved by predictions updating to accept tinnitus as a sound source, and further reductions in the prediction error arise when transient factors causing the auditory hypersensitivity ease. Whilst not uniquely attributable to this account of tinnitus, the finding of transiently increased IDAEP specifically around tinnitus onset is at least supportive of this model and helps to specify the type of auditory hypersensitivity implicated in tinnitus onset, which could in turn lead to interventions targeted specifically to the acute stage of tinnitus.

The fact that the IDAEP slope reduces over time might also, or alternatively, indicate that tinnitus itself exerts a progressive or long-term stabilising effect on auditory hyper-reactivity, either due to its perceptual presence directly, or by the shifting of auditory predictions, and how these shape the perception of external sounds. In the broadest sense, having some stable, enduring predictions might help constrain the range of posterior perceptual representations.

5.4.4 Lack of IDAEP at high frequencies

The tinnitus frequency did not exhibit a mean IDAEP slope effect at all, perhaps because of the individuals' lower stimulus dynamic ranges at the tinnitus frequency, due to the presence of hearing loss in all our groups, or simply because the N1-P2 complex is not intensity dependent at high frequencies (as such stimuli have been conspicuously absent from prior IDAEP literature). Due to the limits of sound intensities possible with our equipment at high frequencies affected by hearing loss, there were some subjects in whom we could not determine their ULL at the tinnitus frequency; stimulus frequency in these subjects was thus limited by technical possibility rather than ULL. However, there was a significant positive correlation for controls, near-significant negative correlation for the chronic tinnitus group, and no correlation for the acute tinnitus groups between IDAEP slope and SDR for stimuli at the tinnitus frequency. This indicates some similar relative differences between groups to those seen at 1 kHz.

5.4.5 Findings may be missed if only chronic tinnitus is studied.

Many reports have been made on sound-evoked changes in chronic tinnitus, often with negative, inconsistent or non-replicable results. In our results, there was no significant difference in mean IDAEP slope between tinnitus groups and controls, though both showed non-significantly steeper slopes, whereas similar studies of IDAEP in chronic tinnitus have shown both larger and smaller slope values than controls (Norena et al., 1999; Lee et al., 2007; Cartocci et al., 2012). Whilst this could be the result of differences in sample size, aversiveness of stimuli used, and/or the clinical characteristics of the tinnitus groups, our study provides a case in point that the study of acute tinnitus specifically can provide a clearer and more robust picture of the processes that cause tinnitus and be less susceptible to more subtle factors that are contingent upon specific details rather than tinnitus itself. It may be that, when removing the confound of hyperacusis by providing stimuli tailored to the

individual's tolerable dynamic range, there is no major difference between non-tinnitus control, acute, and chronic tinnitus groups.

The discrepancies in findings, with some emphasising tinnitus as excessive excitation and inhibitory dysfunction while others emphasise reduced amplitude responses, have made the mechanisms of tinnitus somewhat ambiguous. Therefore, including the natural history of tinnitus, including acute tinnitus, would enhance our comprehension of the wider tinnitus mechanisms. Taking a step back, our findings also suggest that tinnitus is not simply caused and maintained through persistent changes in auditory gain or hypersensitivity, but also other processes, including more nuanced differences in adaptation.

5.4.6 Tinnitus is related to the whole auditory domain, rather than just specific frequencies.

It is equally important to recognise that both acute and chronic tinnitus exhibit a substantial effect at non-tinnitus frequencies, highlighting how it is related (perhaps in cause and effect) to domain-wide changes in auditory processing, rather than simply effects limited to the deafferented frequency ranges. In many studies, the tinnitus frequency is treated as the area of interest, distant frequencies treated as a control condition (Goodwin & Johnson, 1980; Hébert et al., 2013). Conversely, other studies have reported tinnitus-related effects on auditory evoked potentials at low and mid frequency. For instance, tinnitus groups fairly reliably show reduced amplitude mismatch negativity (MMN) and P300 responses at frequencies around 1 kHz (Yukhnovich et al., 2024). These have been interpreted as indicating tinnitus interfering with attentional mechanisms, potentially involving frontal, temporo-parietal and memory areas (Husain & Schmidt, 2014).

5.4.7 IDAEP in tinnitus shows increased Dynamic Range Adaptation

The fact that tinnitus may be at least partly independent and distinct from gain has been highlighted in recent research (Sedley, 2019; Knipper et al., 2013). Our exploratory investigation of the relationship between IDAEP slope and stimulus dynamic range suggests a more nuanced relationship. To distinguish it from the familiar IDAEP slope discussed so far, we introduce the term ‘dynamic range related IDAEP’ (drIDAEP) to refer to the regression coefficient between IDAEP slope and stimulus dynamic range. drIDAEP at 1 kHz was not present in non-tinnitus Controls and Chronic Tinnitus but showed a trend in Acute Tinnitus. It is to note that even though we did not yield statistically significant correlation

between SDR and slope for Acute Tinnitus, we are still interpreting the trend as a possible negative correlation to build our model. The trend in negative value of drIDAEP in the Acute Tinnitus indicated that, as stimulus dynamic range increased, IDAEP reduced, presumably via a process related to dynamic range adaptation. Put another way, the Acute Tinnitus tended to maintain an invariant absolute difference in neuronal responses to 'high' and 'low' intensity stimuli, irrespective of the intensity difference between those stimuli, whilst the Chronic Tinnitus and Control group's response difference increased as the stimulus difference did. At frequencies related to tinnitus, and affected by hearing loss, none of the groups showed patterns of correlation, indicating normal dynamic range adaptation at high frequencies. Whilst further study should aim to examine dynamic range adaptation (and drIDAEP) directly in tinnitus, by using experimental sessions of different stimulus dynamic ranges, the present results show a pattern indicating relatively preferential reactivity, in tinnitus, to smaller sensory changes over larger ones. In some sense, this could be considered an adaptive state when faced with hearing loss and/or auditory hyper-reactivity: reducing excessive responses to large stimulus changes (akin to ameliorating hyperacusis), whilst retaining sensitivity towards subtle changes in the auditory environment. The heightened dynamic range adaptation observed in Acute Tinnitus may signify the onset of tinnitus, while both Chronic Tinnitus and Post Acute Tinnitus typically exhibit diminished dynamic range adaptation, suggesting an adaptation of hypersensitive neural responses over time.

Consistent with our findings, Diesch & Hassel-Adwan (2017) explored dynamic range adaptation in their study, where they used a varied amplitude modulation tones in both ascending and descending run to compare tinnitus with controls. They suggested that tinnitus individuals had stronger undershoot (strong decrease in amplitude with decrease in stimulus) and weaker overshoot (small increase in amplitude with increase in stimulus). In keeping with this, Yukhnovich et al. (2024) also observed that, once hyperacusis had been excluded, tinnitus was associated with larger MMN responses to downward intensity deviants.

Very limited literature has directly searched for the presence of dynamic range adaptation in tinnitus, and our results indicate that this area should further be explored as a matter of priority. Zeng (2020) introduced the concept of 'central variance' to tinnitus, which increases sharply and nonlinearly with increasing central gain, but does not change significantly with increasing central noise. Here in our interpretation, we employ the term variance to refer to

cortical level responses to a normal array of environmental sounds. Tinnitus, as central noise, or as a pervasive constant auditory prediction, mitigates central variance by reducing specific aspects of central gain. We consider whether tinnitus itself has a broader role in moderating excess hypersensitivity in light of our study's findings that both the tinnitus and the controls exhibit evidence of dynamic range adaptation that the controls do not. i.e. tinnitus, and its different stages, might encompass distinct and separable effects on central gain and central variance. We would like to emphasize that two mechanisms appear to be occurring simultaneously based on our data. The first is a clear reduction in the slope of the input-output function over time, indicative of a decrease in auditory sensitivity. The second is a potential trend toward increased dynamic range adaptation, although this observation did not reach statistical significance in our current analysis. Despite only observing a trend, this pattern of dynamic range adaptation may provide important support for the mechanism of sensitivity reduction. Specifically, an expansion of the dynamic range could serve to normalize auditory processing by accommodating a broader range of sound intensities, thereby mitigating the heightened neural responses associated with tinnitus and auditory hypersensitivity. Together, these findings suggest that while the primary driver may be a reduction in auditory sensitivity, dynamic range adaptation could play a complementary role in modulating the auditory system’s response over time. Future studies with larger sample sizes or more sensitive measures will be necessary to conclusively establish the contribution of dynamic range adaptation in the recovery process.

5.4.8 Role of Tinnitus as a stabilizing mechanism to mitigate gain (invariance to gain)

Our findings are in contradiction to most of the tinnitus literature as we highlight a potential trend of the presence of adaptive consequences of tinnitus, as opposed to the more classical arguments of processes predisposing to tinnitus being adaptive but the tinnitus itself being purely maladaptive. It has previously been argued that forming a prediction to account for noise (i.e. error) in the auditory system reduces sensory uncertainty (i.e. imprecision, or variance), and our present results showing indicators of increased sensory uncertainty at the time of tinnitus onset is entirely compatible with this (De Ridder et al., 2014; Sedley et al., 2016). However, this is not the only way in which developing a stable and pervasive sensory prediction could be an adaptive method of lowering sensory uncertainty. We consider that the process that tinnitus is trying to correct is the one responsible for increased IDAEP slope in

general, namely increased high-level cortical reactivity to auditory changes, resulting in excessive variance in auditory signals at a cortical level in response to a normal range of environmental sounds. This places additional demands on higher perceptual and cognitive networks, which must react to these changes. Because auditory representations are the interplay of sensory inputs and prior predictions, forming a stable 'moderate' prior would be expected to draw posterior representations (i.e. inferred sounds) closer toward this prior, essentially reducing variance or uncertainty (i.e. more intense stimuli are interpreted as relatively quieter, and less intense or non-existent stimuli as relatively louder). Thus, tinnitus may provide a form of gain control that mitigates increased central variance or promotes 'central invariance' to gain. As we have highlighted earlier, the onset of tinnitus seems to be associated with relative preservation of reactivity to small auditory changes alongside suppression of larger changes, which may indicate why it typically exists alongside a degree of hyperacusis, as a complementary and partially mitigating process. Based on the drIDAEP findings, Figure 5.6 presents a suggested model for the time course of tinnitus across different groups. An individual with hearing loss would have a normal slope function (appropriate increased gain) in accordance with their degree of hearing loss. If the central gain increase is excessive, there would be a high steep slope which initiates the onset of tinnitus, which would attenuate the slope function through increased dynamic range adaptation. Over time due to adaptation, there would be further reduction of central gain moving towards a near normal baseline (variance) further propagating the extent of dynamic range adaptation to reduce proportional to the optimization of variance.

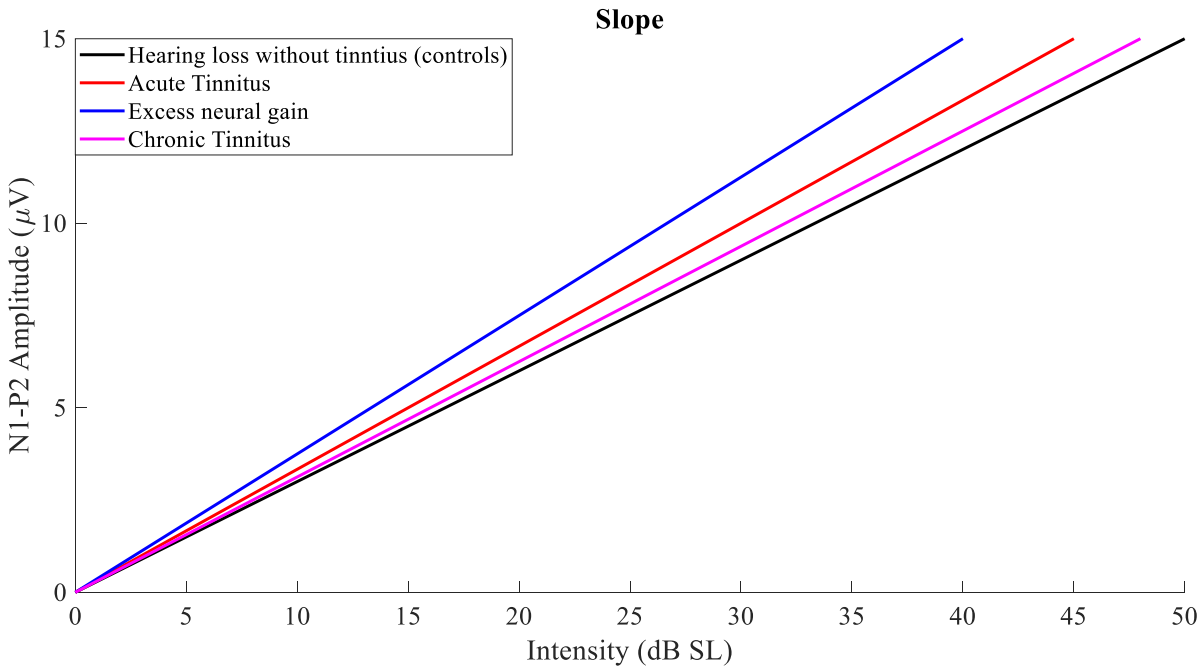


Figure 5.6. Time course of tinnitus from its onset until its subsequent chronification through changes in slope (input-output function). *The hearing loss without tinnitus (black line; normal variance) exhibits a suitable input-output function relative to the deafferentation rate. In instances where the gain surpasses the homeostatic threshold (blue line; excess variance), the input-output function (slope) increases, triggering the onset of tinnitus (red line; normalization of excess variance), which subsequently reduces the excess steepness through enhanced dynamic range adaptation, ultimately (pink line; further normalization of excess variance) diminishing it further towards a non-tinnitus state (black; normal variance).*

5.4.9 Correlation between IDAEP slope and Serotonin

The IDAEP slope has been established by previous literature as a potential correlate for serotonergic modulations with a steep slope indicating a reduction in serotonin and a shallow slope indicating increase in serotonin especially 5-HT1 (Carrillo-de-la-Peña et al., 2006; Pillai et al., 2020). Hyperacusis, the heightened sensitivity to everyday sounds, is thought to arise from central gain enhancement following reduced auditory input, mediated by homeostatic plasticity, altered inhibitory control, and descending pathway dysfunction (Brotherton et al., 2015; Pienkowski et al., 2014). Serotonin plays a complex, region-specific role in modulating auditory sensitivity, with studies reporting both elevated and depleted levels in hyperacusis. Elevated serotonin during development, for example, may reduce

GABAergic inhibition, increasing auditory cortex excitability—as seen in autism-associated hyperacusis (Sato et al., 2023). Conversely, serotonin depletion in the nucleus accumbens of rodents induces hyperacusis-like behaviour, marked by exaggerated startle responses in quiet conditions (Sato et al., 2023). With tinnitus and hyperacusis being commonly present in the same individuals and argued to have shared underlying mechanisms of auditory hyperactivity and based on our various models postulating tinnitus to have increased neural activity during its onset due to excess central gain, a strong hyposerotonergic state, as evidenced by a significant increase in the IDAEP slope during the acute phase should be the potential finding which is exactly what we have established in our result.

We couldn't find much supporting literature related to serotonin and acute tinnitus due to the scarcity of acute tinnitus literature. Rest literature focuses on chronic cases and measuring serotonin levels in whole blood rather than directly within the central nervous system (Sachanska, 1999). The findings from these studies are inconsistent: while some report elevated serotonin levels in tinnitus patients (Sachanska, 1999), (Liu et al., 2003)(Clewes, 2012) conversely, evidence also supports a serotonergic deficiency in tinnitus. A post-mortem study revealed a significant reduction in serotonergic neurons within the dorsal and obscurus raphe nuclei of tinnitus patients, highlighting the potential involvement of the brainstem serotonergic system in tinnitus pathology (Almasabi et al., 2022). Although direct tinnitus-specific studies on serotonin are limited, previous research suggests that lower central serotonin levels are associated with heightened auditory sensitivity, as demonstrated in the Davis et al. (1980). Model which is in support to our current findings. Even though we did not directly measure serotonin levels in our tinnitus study, this finding through IDAEP (which has been established as a strong indicator of serotonergic variations) would aid us in generating a potential directional hypothesis for future studies in acute tinnitus and serotonergic variations.

Another potential link between serotonin and acute tinnitus in this current study is the medical history provided by our participants as highlighted in Chapter 2 section 2.2 which reveal 8 out of the 23 participants who provided their medical history were under SSRI which is almost 34% of the participants who provided their medical history and 15% out of the total Acute Tinnitus population which is highly significant. Studies have highlighted SSRI to

alleviate tinnitus (Sachanska, 1999) and it would be interesting to explore the potential role of serotonin receptor levels during the onset of tinnitus.

5.4.10 Avenues for future studies

We would further like to highlight that so far, we have considered only the physiological origin of the tinnitus sound, and not perceptual, psychological, autonomic or cognitive reactions, which are important properties of tinnitus, and may have their own effects on responses to auditory stimuli. IDAEP not only involves gain in the auditory pathway, but the influence of higher cortical structures and neuromodulatory centres. Because IDAEP is closely related to serotonergic function, we believe that there might be central serotonergic changes around the time of tinnitus onset (i.e. a relative serotonergic deficiency). Even though some participants mentioned their use of SSRI, it is unclear from them as to the dosage and the duration of their medication which needs to be kept in mind for future research. It remains to be determined what factors, or combinations of factors, are responsible for the initial side in IDAEP associated with tinnitus onset. However, it remains to be established that the onset due to increased gain and Selective Serotonergic Reuptake Inhibitors (SSRI) can be attempted for acute tinnitus individuals. It is also to be noted that stress is well recognised to exert a substantial influence on the neurotransmitter and neuromodulator systems of the brain, especially serotonin, which is essential for regulating mood and maintaining emotional stability. Suppressed serotonin levels resulting from chronic stress can contribute to the onset of mood disorders including depression and anxiety. The process initiates with the stimulation of the hypothalamic-pituitary-adrenal (HPA) axis in reaction to stress, leading to the hormonal secretion of cortisol. Extended exposure to elevated serum cortisol levels can interfere with the production, secretion, and activity of serotonin. Depletion of tryptophan, the amino acid precursor to serotonin, and changes in the activation of serotonin receptors are among the processes by which this disturbance may occur (Young, 2007; Jenkins et al., 2016).

The role of abnormal physiological stress responses in the development and persistence of tinnitus symptoms has been extensively studied and shown in several studies. Consequently, there exists a correlation between the type of stress and the onset and severity of tinnitus, as attributed by Ciminelli et al. (2018) and Sahley & Nodar (2001) and, moreover, the relationship between serotonin levels and tinnitus discomfort has been extensively

established, with studies indicating that those experiencing severe distress may exhibit atypical serotonin levels. There is uncertainty over which of the factors of psychologic, cognitive, or autonomic or their combination leads to the alterations in cortical processing that enable the development of tinnitus, either in general or in specific individuals.
In addition to being possible aetiologies of tinnitus, these factors may also influence tinnitus outcomes as discussed previously. Although the study does not appear to be influenced by tinnitus distress, it is possible that responses to tinnitus could still impact IDAEP. Therefore, it is crucial to investigate the ways in which IDAEP is affected by these processes, both inside and beyond the framework of tinnitus. Importantly, we have inferred drIDAEP from between-subject correlations, and there is a priority need for studies examining this through within-subject contrasts and also the need to run dynamic range adaptation paradigm.

5.5 Conclusion

We report the novel finding that during acute tinnitus, IDAEP—an inverse correlate of serotonin-mediated habituation to sensory stimulation—is increased, and then returns to normal during the chronic stages of tinnitus. Moreover, adaptation of the IDAEP slope to stimulus dynamic range is linked to tinnitus onset that later regresses over time. We argue that tinnitus is not gain nor noise but more of an alternative gain controller as an invariance to central gain. Future research should consider measuring dynamic range adaptation through specific paradigms tapping into the mechanism of dynamic range adaptation such as measuring the slope differences across different stimulus dynamic range.

Chapter 6 Stimulus novelty of Tinnitus a causative factor for its persistence. A novelty based P300 paradigm on Acute, Chronic, and Non-Tinnitus Controls

6.1 Introduction

6.1.1 Resting State EEG preliminary results

We carried out a resting state EEG test aiming to measure brain activity in Acute Tinnitus (N = 46), Post Acute Tinnitus (N = 26), and Control (N = 30) groups using a 64-channel Active Two Biosemi system in a soundproof room. These data are not sufficiently finalised in their analysis to include as a full thesis chapter, but the summary is included as the preliminary results motivated the experiment described in this chapter. Data were recorded at a sampling rate of 256 Hz with a low-cut filter of 0.5 Hz. Participants were instructed to remain seated with eyes open and focus on a fixed cross for a period of 5 minutes while minimising movements throughout the recording session. Preprocessing was done using EEGLAB toolbox of MATLAB where steps for artifice removal (channel rejection and independent component analysis) were carried out to eliminate artifacts such as muscle activity and eyeblinks. Data were re-referenced to the common average. The pre-processed data were transformed into the frequency domain, and source localization analysis was performed using sLORETA (Standardized Low Resolution Electromagnetic Tomography) to estimate current density in specific brain regions across frequency bands of delta, theta, alpha, beta, and gamma. The resulting source activity was used to compute resting state oscillatory activity for subsequent statistical analysis (paired t test between Acute and Post Acute Tinnitus and independent t test between Acute and Chronic Tinnitus). The longitudinal follow-up from the Acute to Post Acute Tinnitus were associated with a reduction in activity (delta oscillation magnitude) in right Inferior Parietal Cortex (IPC; Figure 6.1A). The cross-sectional comparison of people with Acute Tinnitus with an age/sex/hearing/distress-matched Chronic Tinnitus group shows greater resting-state delta activity in the Anterior Cingulate Cortex (ACC) in the Acute Tinnitus group (Figure 6.1B). However, this did not change between the Acute and Post Acute Tinnitus longitudinally.

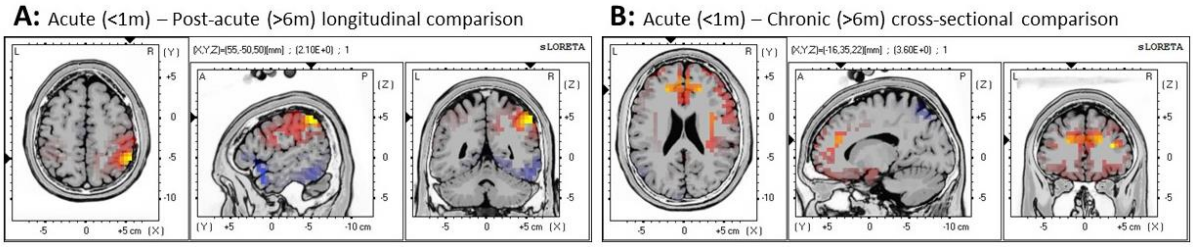


Figure 6.1: Resting state brain activity distinguishing acute from chronic tinnitus. ***A:*** *Greater delta (1-4 Hz) power in inferior parietal cortex in the acute stage (<1 month from onset) of tinnitus compared to the chronic (>6 months) stage of the same individuals followed longitudinally.* ***B:*** *Greater delta power in anterior cingulate cortex of a group of individuals in the acute stage of tinnitus compared cross-sectionally to an age/sex/hearing/distress-matched group with chronic tinnitus (>6 months, and years in most cases).*

6.1.2 Background

Revisiting our introduction section (Chapter 1), tinnitus, an auditory phantom perception, has a remission rate of just 15%, which is considerably lower than that of other disorders (Gopinath et al., 2010). This indicates that for most individuals acquiring Tinnitus, there is an early transition from the onset of tinnitus to its chronic phase, which generally persists over time. Additional research indicates that tinnitus lasting over four weeks, in the absence of Acute external or middle ear diseases, is likely to continue long-term in at least 80% of persons (Vielsmeier et al., 2020). However, the question remains as to why tinnitus predominantly remains chronic and does not resolve, despite the brain's intricate systems designed to achieve homeostasis via different gain control mechanisms. In earlier chapters, we thoroughly examined the alterations in central gain from the onset to the chronic stages of tinnitus. We found that, although reductions in hypersensitivity are observed through ASSR and IDAEP, the gain associated with subjective sound sensitivity remains unchanged over time. This implies that the mechanisms contributing to the chronicity of tinnitus may commence early due to no variations seen between the onset and chronic stages of tinnitus. This was supported by the opinions of both Hullfish et al. (2019) and Noreña & Farley (2013) where both highlight that changes related to the early chronification of tinnitus begin soon after the onset of tinnitus with even Hullfish et al. (2019) further highlighting that acute stages of tinnitus may be less than 4 weeks. It is also further to note that the processes that initiate tinnitus are not necessarily the same as those that make it chronic as tinnitus may be

initiated by temporary events like noise exposure, but its persistence involves complex changes in the brain's auditory pathways, leading to a chronic state (Sedley et al., 2016; Kaltenbach, 2007; Kaltenbach & Manz, 2012). Our previous chapters (Chapters 3, 4, and 5) have emphasized that the purpose of the onset of tinnitus is to regulate gain through possible mechanisms of increased dynamic range adaptation (refer Chapter 5) but however the chapters does not elaborate into the mechanisms behind the chronification of tinnitus. It is further to note that the homeostatic processes in the brain may mask tinnitus-related changes over time as highlighted by Yang et al. (2011) and Roberts et al. (2013) where they further highlight that there are mechanisms of adaptation following the onset of tinnitus which can be evidently seen why tinnitus severity does not always correlate with hearing loss. In addition to alterations in homeostatic processes from the onset of tinnitus to its chronic state, numerous changes associated with chronic tinnitus may result from the condition itself rather than the causal mechanisms as discussed in Chapter 1 where increased distress at the time of tinnitus onset may contribute to its chronification, primarily relating to the reactivity to tinnitus rather than its underlying cause.

In summary, further exploration is warranted of potential mechanisms contributing to the persistence of tinnitus, which might involve wider networks than just the auditory pathway. Investigating alterations in brain activity along the development of tinnitus and its subsequent persistence is crucial to understanding the low remission rate of tinnitus. A limited number of source power or functional connectivity studies have compared acute and chronic tinnitus, using resting state EEG/fMRI measurements. Lan et al. (2021) identified a notable enhancement in power within the middle frontal gyrus and parietal cortex in the gamma band for acute tinnitus in comparison to the chronic tinnitus group. Cai et al. (2020) conducted a functional connectivity study that demonstrated an enhancement in both functional and causal connectivity for the superior temporal gyrus, amygdala, and nucleus accumbens in acute tinnitus group relative to persons without tinnitus. Both these studies compared separate acute and chronic tinnitus groups cross-sectionally. Extrapolating from research findings not directly examining acute tinnitus, Vanneste et al. (2011) proposed that patients with newly developed tinnitus exhibit heightened activity in the auditory cortex, supplementary motor area, dorsal anterior cingulate cortex, and insula. De Ridder et al. (2022) proposed a triple network model for tinnitus, suggesting that persons with Acute Tinnitus exhibit an anti-

correlation between the default mode network and the central executive network, which facilitates the propagation of tinnitus distress through the salience network. To summarise the limited findings, electrophysiological and imaging results indicate an increase in activity in non-auditory regions around the start of tinnitus compared to its chronic stages. This may manifest as, or result from, heightened distress or increased focus of attention on the tinnitus at its onset. Much current tinnitus theory emphasises the involvement of the pregenual or dorsal/lateral anterior cingulate cortex in the onset of tinnitus, either by activating tinnitus or by exhibiting heightened activity associated with newly developed tinnitus characterised by distress (Song et al., 2015; Vanneste et al., 2024; Hullfish et al., 2019). Other literature emphasises the significance of insular activity during acute phases of tinnitus. Chen et al. (2023) demonstrated atrophy of the ventral anterior insula in chronic tinnitus compared to acute tinnitus groups and concluded that the generation and persistence of tinnitus result from the aberrant structural and functional reorganisation of the insula. Araneda et al. (2018) emphasised the significance of the prefrontal cortex in the onset and maintenance of tinnitus, attributing to it deficiencies in the executive functions and inadequate inhibitory regulation. Regarding the generation and maintenance of tinnitus, our preliminary resting state results (figure 6.1) have highlighted increased oscillatory activity in the inferior parietal lobe and anterior cingulate cortex during acute tinnitus when compared to chronic tinnitus longitudinally and cross sectionally. Furthermore, acute Tinnitus literature have highlighted in common increased oscillatory activity to be around regions like the anterior cingulate, insula, and prefrontal cortex. All of these regions together are essential in constituting the salience network.

Both salience (prominence/significance) and novelty (newness or unexpectedness) might be strongly associated with acute tinnitus due to prominence and newness the tinnitus has during its onset. In the present study, therefore, we sought to assess the role of the salience network, to potentially explain the onset of tinnitus. However, another question pertains to whether the factors of salience or novelty processing contribute to the persistence and non-remission of tinnitus. Hence, to commence an investigation into the mechanisms underlying the persistence of tinnitus, it would be prudent to examine the involvement of the salience network in relation to the salience or novelty of tinnitus. One way would be to assess novelty mechanisms of tinnitus through an electrophysiological P300 paradigm, where the

spatiotemporal dynamics are centred around the anterior cingulate and the temporoparietal networks (Yago et al., 2003). The P3a, or novelty-driven P300, along with the P3b, engages the ventral frontal/parietal attention network respectively, with the former designed to detect salient changes and the latter focused on attention orientation, both elicited by the ACC and temporo-parietal regions (Kim, 2014). Based on the aforementioned literature reporting increased activity of non-auditory regions in the prefrontal cortex regions during acute stages of tinnitus, we hypothesize that the components of salience or novelty would be heightened during the acute phases, subsequently diminishing and stabilising throughout the ensuing chronic stage and this follows with the other components of P3b too. We further hypothesize that the enhanced salience during the onset of tinnitus may further result in activating networks that might result in the persistence of tinnitus as few studies highlight the role of attentional networks towards the tinnitus to contribute to the persistence of tinnitus (Haider et al., 2018). The preceding chapters have thoroughly examined the mechanistic variations associated with hypersensitivity/central gain from the onset of tinnitus to its chronic state, while the current chapter elucidates the various networks that may facilitate the persistence of tinnitus.

6.2 Methods

6.2.1 Participants

A total of 23 participants with Acute Tinnitus (symptoms lasting for less than 4 to 6 weeks) were subsequently followed up 6 months later as they transitioned into the Chronic stage. For convenience, this group is referred to as the Post Acute Tinnitus group to distinguish it from the actual Chronic Tinnitus data collected cross-sectionally in this study,
27 participants with Chronic Tinnitus (symptoms persisting for more than 6 months), and 19 non-Tinnitus participants (no Tinnitus symptoms) participated in the current study, recruited as a community sample through advertisements on Google adverts and internally at Newcastle University. Please refer Chapter 3, section 3.2.1 for inclusion and exclusion criteria.

6.2.2 Audiological Assessment

Refer Chapter 3, section 3.2.2.

6.2.3 Tinnitometry

Refer Chapter 3, section 3.2.3.

6.2.4 P300 Stimulus

Four categories of stimuli based on different acoustical frequencies were delivered for the P300 study. The High Frequency Deviance (HFD) which is a pure tone stimulus presented at the participant's tinnitus frequency, Standard Stimuli (SS) which is a pure tone stimulus presented at one octave below the tinnitus frequency, Low Frequency Deviance (LFD) which is a pure tone stimulus presented at one octave below the standard stimuli, and the Novel Stimuli (NS) which is comprised of a collection of random ambient sounds (e.g., dog bark, cat meow, car honk), with each sound given singularly to maintain novelty, and all sounds were specifically chosen to ensure neutral valence (emotional neutrality in response to the stimulus such as white noise, bird chirps, water droplets, etc). All four stimuli were presented at a 60% level relative to the differences between the Uncomfortable Loudness Level (ULL) and the threshold Dynamic Range (DR) with NS priorly undergoing an RMS match to the average of the first three categories before the ULL and DR were established.
Both the threshold and ULL were determined via an ascending procedure with the threshold established at 50% response based on the number of trials and the ULL established independently to prevent the presentation of multiple sounds at potentially uncomfortable elevated intensities (Chapters 4 and 5). For the controls, an individual was matched to the Acute Tinnitus group where the respective frequency of that person was taken as HFD, SS, and LFD. The dynamic range of those frequencies was later calculated for the controls in the same way as for Tinnitus participants.

6.2.5 P300 Stimulus Parameters and Recording Parameters

EEG was recorded using a 64-channel Active Two Biosemi system in a soundproof room. These 64 channels were sufficient to remove ocular artifacts and an additional ocular channel was not used. Electrode offset was kept at the manufacturer's recommended limits of ±10 mv. A total of 1200 standards and 50 deviants of each type were presented in a randomized order with 5 to 11 standards between deviants. The HFD, SS, NS, and LFD all had a 10 msec Onset/Offset Ramp and 300 msec duration of presentation and across the four Stimulus, an 800 msec Interstimulus Interval was maintained. With respect to the intensity presentation of

NS, the intensity of the novel stimulus was calibrated to correspond with the average RMS (Root Mean Square) across all the NS based on a sampled loudness matched stimulus. By standardising the RMS, all stimuli were delivered at a uniform loudness level, facilitating a clear comparison of the P300 response to novelty devoid of intensity-related fluctuations. The experiment was a Go-No-Go paradigm where the participants were asked to positively respond to the HFD and LFD stimuli only, by pressing the spacebar button every time they heard either of these, and to ignore the SS and NS stimuli. A fixation cross (+) that changes into a X symbol every time they pressed the spacebar was provided as visual feedback for their response. All stimuli were generated and presented in Matrix Laboratory (MATLAB) version R2019a. The total duration of the experiment was approximately 25 minutes.

6.2.6 EEG Pre Processing

All EEG data underwent preprocessing in MATLAB version R2019a EEGlab toolbox. Firstly, re-referencing at P9/P10 (Linked mastoids) was carried out and later filtering was performed with a high pass of 1 Hz and low pass of 30 Hz. This was followed by epoching between - 0.1 and 1 s and channel rejection based on visual inspection and keeping 0.8 as the accepted correlation between channels. Artifact rejection was done using the Independent Component Analysis (ICA) where artifact-containing components like an eye blink, eye movements, muscle artifacts, and other electrical artifacts, were removed. Apart from ICA, artifact rejection was also carried out using individual trial rejection where components are auto rejected keeping a probability of 5 and Kurtosis of 8 as the threshold. Baseline correction was further done from -100 to 0 ms from stimulus onset.

6.2.7 Data analysis

The P300 component was produced and evaluated from the respective epochs for the Acute, Post Acute, Chronic Tinnitus, and Control groups. The total number of trials was averaged for each condition, and the P300 response was measured at a post-stimulus interval of a time window between 250 ms to 400 ms for LFD and HFD, and between 220 ms to 400 ms for NS, specifically at the Pz electrode for LFD and HFD, and at FCz for NS.
Automatic peak height detection was conducted for the aforementioned time windows corresponding to the respective P300 groups. A visual inspection of each waveform under every situation was conducted to identify a suitable P300 response and subjects were rejected

if the P300 peak/waveform was not visually present at the above-mentioned latency windows for each condition.

6.2.8 Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) with descriptives and inferential statistics calculated. Upon the Shapiro-Wilk test of normality, the data was normally distributed across the three groups, and hence parametric statistics were used. We used P300 relative peak amplitudes for our statistical analysis. Relative peak amplitude was defined by calculating the average amplitude across three conditions and then normalizing each condition by dividing its amplitude by this average to obtain the relative amplitude for each condition. The rationale for this is the presence of subject-specific variations in amplitude across all conditions. Examining the relative differences in salience and attentional processing of novel, non-tinnitus-related, and tinnitus-related stimuli aids in mitigating these absolute differences. For the within-group Acute-Post Acute comparison, we employed a method to establish the relative amplitude by calculating the average amplitude for all three conditions in each subject for both time points combined and then normalizing each condition by dividing its amplitude by this average. This was to give a more equal weighting to each subject, considering the large between-subject differences in overall P300 amplitude.

We conducted a One-Way ANOVA with subject groups as the independent variable and amplitudes across conditions (HFD, LFD, and NS) as the dependent variable to determine the main effects of the groups and to analyse within-group differences; a Tukey's post hoc test was subsequently performed. A paired t test was conducted to determine the significant difference between the paired groups of Acute and Post-Acute Tinnitus.

6.2.9 Source reconstruction

Source reconstruction was performed across groups and conditions for the P300 amplitude. Relative amplitudes were employed in the statistical analysis, necessitating normalisation for the source analysis as well. As a part of normalization, for each subject, the trial-averaged time series for every electrode was divided by the mean P300 amplitude value across conditions and subjects within that group for the FCz electrode between time point 220ms and 400 ms for NS and Pz electrode between 250 ms and 400 ms for both LFD and HFD.

Source reconstruction was conducted initially only on group comparisons that demonstrated statistically significant difference in sensor space (differences in relative amplitude), i.e. source reconstruction was for localisation purposes, of between-group differences already found to be significant.

To compare source-localized P300 activity among Acute Tinnitus, Chronic Tinnitus, and Control groups, we first identified statistically significant differences at the sensor level—i.e., the scalp-recorded event related P300 signals. Upon detecting such significance, we employed standardized low-resolution brain electromagnetic tomography (sLORETA) to perform voxel-by-voxel independent t-tests, enabling the localization of neural generators underlying the observed P300 components. The analysis was done sing SLORETA's built-in statistical tools individually between the groups (Pascual-Marqui et al., 1994). These tests identified regions where cortical activation differed significantly between groups.

Additionally, to the targeted source analyses based on significant sensor-level differences, paired t tests were conducted for within-group comparisons across different stimulus conditions (LFD, HFD, and novelty) to assess condition-specific differences in P300 source activation in case there was a paired differences at source space (not evident in sensor space). Non-parametric randomization tests (Nichols & Holmes, 2002), using 5000 permutations, were applied to control for multiple comparisons across voxels. Statistical maps were established regardless of significant difference (as significant differences have already been established at sensor space) and significant voxels were grouped based on their corresponding brain regions, including cortical lobes and Brodmann areas. It is further to note that maximum t statistics levels within the time series during group comparisons were considered as the region of cortical activation for that group comparison.

6.3 Results

Out of the total participants enrolled (Acute Tinnitus – 23, Chronic Tinnitus – 27, Controls – 19), five individuals were omitted owing to data quality: one from the Acute group, three from the Chronic group, and one from the Post Acute group. The final participant composition consisted of 22 individuals with Acute Tinnitus (mean tinnitus duration: 4.27 weeks, median: 4 weeks, SD: 2.45 weeks), of which 4 participants exhibited a tinnitus duration ranging from 7 to 10 weeks and were classified as outliers. Among the 22 individuals with Acute Tinnitus, 9 returned after 6 months to form the Post Acute Tinnitus

group, 24 with Chronic Tinnitus (mean tinnitus duration: 8.73 years, median – 4 years, SD – 9.14 years) and 19 Controls.

6.3.1 Demographics and Hearing

Efforts were made to match participant groups based on age, sex, and hearing ability. Participants were matched for age ($F(2,62) = 1.514$, $p = 0.228$), sex ($X^2 = 1.422$, $p = 0.491$) and with respect to hearing, no significant main effect was observed for hearing thresholds at 1 kHz ($F(2,62) = 0.593$, $p = 0.556$). A significant main effect was present at 4 kHz ($F(2,62) = 7.778$, $p < 0.001$), with a notable difference between Acute Tinnitus and Chronic Tinnitus ($p = 0.019$) and between Chronic Tinnitus and Controls ($p = 0.001$). Chronic Tinnitus exhibited higher thresholds ($M = 38.75$, $SD = 14.52$) compared to Acute Tinnitus ($M = 25.68$, $SD = 16.33$) and Controls ($M = 21.31$, $SD = 15.03$). No differences were noted between Acute Tinnitus and Controls ($p = 0.64$) Comparable outcomes were observed at 8 kHz, revealing a significant main effect ($F(2,62) = 7.173$, $p = 0.001$), with notable differences solely between Chronic Tinnitus and Controls ($p = 0.001$). Chronic Tinnitus exhibited a higher threshold ($M = 49.79$, $SD = 19.22$) compared to Controls ($M = 27.5$, $SD = 18.54$). No significant changes were noted between Acute Tinnitus and Chronic Tinnitus ($p = 0.169$), nor between Acute Tinnitus and Controls ($p = 0.126$). Figure 6.2 illustrates the hearing thresholds, while Table 6.1 provides information regarding the participants' demographics.

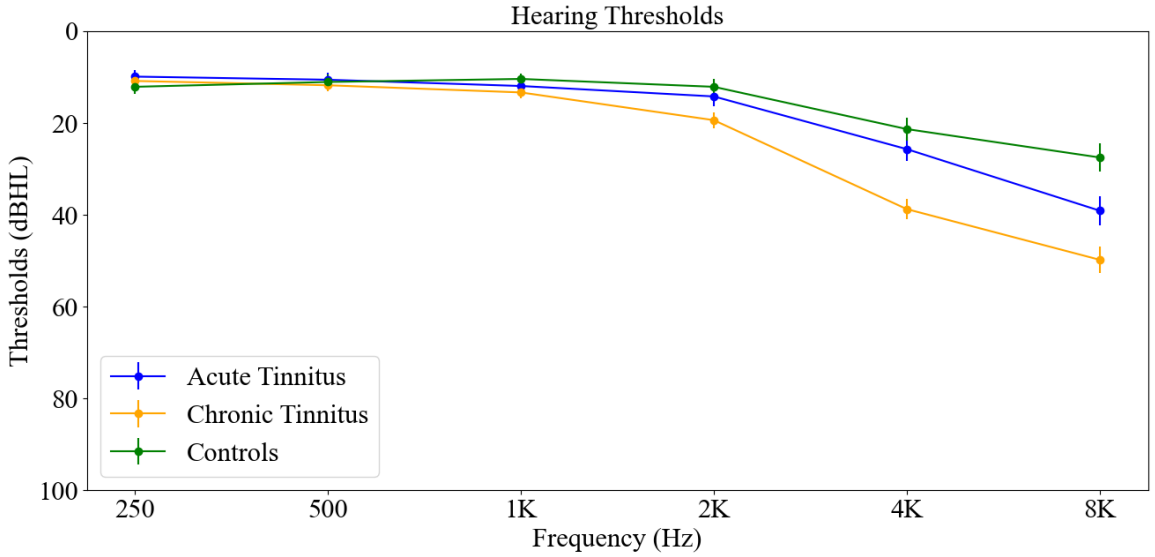


Figure 6.2: Combined average hearing threshold for both ears across Acute, Chronic, and Non-Tinnitus Controls. *Acute Tinnitus and Controls were matched for hearing at 1 kHz, 4 kHz, and 8 kHz. However, Chronic Tinnitus was not matched for 4 kHz and 8 kHz (refer table 6.1)*

Parameters	**Acute Tinnitus**	**Chronic Tinnitus**	**Controls**	**Significance Overall**	**Post hoc Significance**
Age (Years)	N = 22 M = 55.36 SD = 13.65	N = 24 M = 60.54 SD = 8.63	N = 19 M = 56.26 SD = 9.23	F = 0.228 p = 1.514	NS
Gender (M/F)	15/7	18/6	11/8	X^2 = 1.422 p = 0.491	NS
Hearing (1 kHz) dBHL	M = 11.93 SD = 11.15	M = 13.33 SD = 7.89	M = 10.39 SD = 6.47	F = 0.593 p = 0.556	NS
Hearing (4 kHz) dBHL	M = 25.68 SD = 16.33	M = 38.75 SD = 14.52	M = 21.32 SD = 15.03	F = 7.776 p < 0.001*	Acute – Chronic; p = 0.014* Chronic – Control; p = 0.001*
Hearing (8 kHz) dBHL	M = 39.09 SD = 19.67	M = 49.79 SD = 19.22	M = 27.5 SD = 18.54	F = 7.173 p = 0.001*	Chronic = Control; p = 0.001*
Tinnitus Pitch (Hz)	M = 6823.65 SD = 1459.67	M = 6865.27 SD = 1989.12	M = 6476.5 SD = 1206.27	F = 0.354 p = 0.703	NS
Tinnitus Loudness (dBSL)	M = 11.46 MD – 8.33 SD = 12.33	M = 5.34 MD – 4.5 SD = 10.59	N/A	z = 1.617 p = 0.106	N/A

Table 6.1: Participants' demographics for each group. *N denotes number of samples, M denotes mean, SD denotes Standard Deviation, MD denotes Median, F statistic indicates a One Way ANOVA has been carried out, X^2 indicates a chi-square goodness of fit test, z test indicates non parametric Man-Whitney U test, NS denotes No Significance. * indicates presence of statistical significance at $p < 0.05$. Hearing thresholds were calculated by averaging the left and right ear values.*

6.3.2 Symptom scores

Symptom scores for tinnitus and hyperacusis were obtained and calculated cross sectionally across the groups. With respect to the tinnitus symptom scores, no significant differences were obtained between the Acute and Chronic Tinnitus for THI ($t(40) = -1.254$, $p = 0.217$) and for TFI ($t(40) = -1.091$, $p = 0.282$). Regarding the hyperacusis questionnaires, there were no significant differences observed for the HQ questionnaire scores across the Acute, Chronic, and Control groups. ($F(2,57) = 2.877$, $p = 0.064$. However, a significant effect was obtained for IHS ($F(2,57) = 7.177$, $p = 0.001$) across the groups with a significant difference between Chronic Tinnitus and Controls ($p = 0.001$) with Chronic Tinnitus ($M = 50.45$, $SD = 17.35$) having increased hyperacusis scores when compared to controls ($M = 33.11$, $SD = 8.27$). No differences were obtained between Acute Tinnitus and Chronic Tinnitus ($p = 0.188$) and between Acute Tinnitus and Controls ($p = 0.116$).

For the longitudinal changes, there were no significant differences between the Acute and Post Acute Tinnitus for THI ($z = -1.5$, $p = 0.136$), TFI ($z = -1.23$, $p = 0.219$), HQ ($t(5) = -0.169$, $p = 0.873$), and IHS ($z = -0.315$, $p = 0.753$). Refer table 6.2 for further details.

Parameters	Acute Tinnitus	Chronic Tinnitus	Controls	Significance Overall	Post hoc Significance
THI	M = 32.7 SD = 21.33	M = 41.36 SD = 23.26	N/A	t = -1.25 p = 0.217	N/A
TFI	M = 105.7 SD = 49.05	M = 123.55	N/A	t = -1.091 p = 0.282	N/A

		SD = 56.19			
HQ	M = 12.3 SD = 8.89	M = 16.36 SD = 10.72	M = 9.67 SD = 6.08	F = 2.88 p = 0.064	Chronic Tinnitus – Control; p = 0.055
IHS	M = 42.35 SD = 15.11	M = 50.45 SD = 17.35	M = 33.11 SD = 8.27	F = 7.177 p = 0.002*	Chronic Tinnitus – Control; p = 0.001*

Table 6.2: The participant's symptom scores for both tinnitus and hyperacusis.

THI - Tinnitus Handicap Inventory, TFI – Tinnitus Functional Index, HQ – Hyperacusis Questionnaire, IHS – Inventory of Hyperacusis Symptoms

6.3.3 P300 Amplitude changes between Acute, Chronic, and Control. Groups

For the relative P300 amplitude (defined by calculating the average amplitude across three conditions and then normalizing each condition by dividing its amplitude by this average to obtain the relative amplitude for each condition), no significant effect was seen for HFD F((2,62) = 0.184, p = 0.832) and NS (F(2,62) = 0.779, p = 0.463), but an effect was seen for LFD (F(2,62) = 3.121, p = 0.05) with a significant difference between Acute Tinnitus and Controls (p = 0.04) with Controls (M = 1, SD = 0.38) having a higher amplitude when compared to Acute Tinnitus (M = 0.75, SD = 0.33). Refer Figure 6.3 for further information of P300 amplitudes and Figure 6.4 for individual waveforms across groups and conditions.

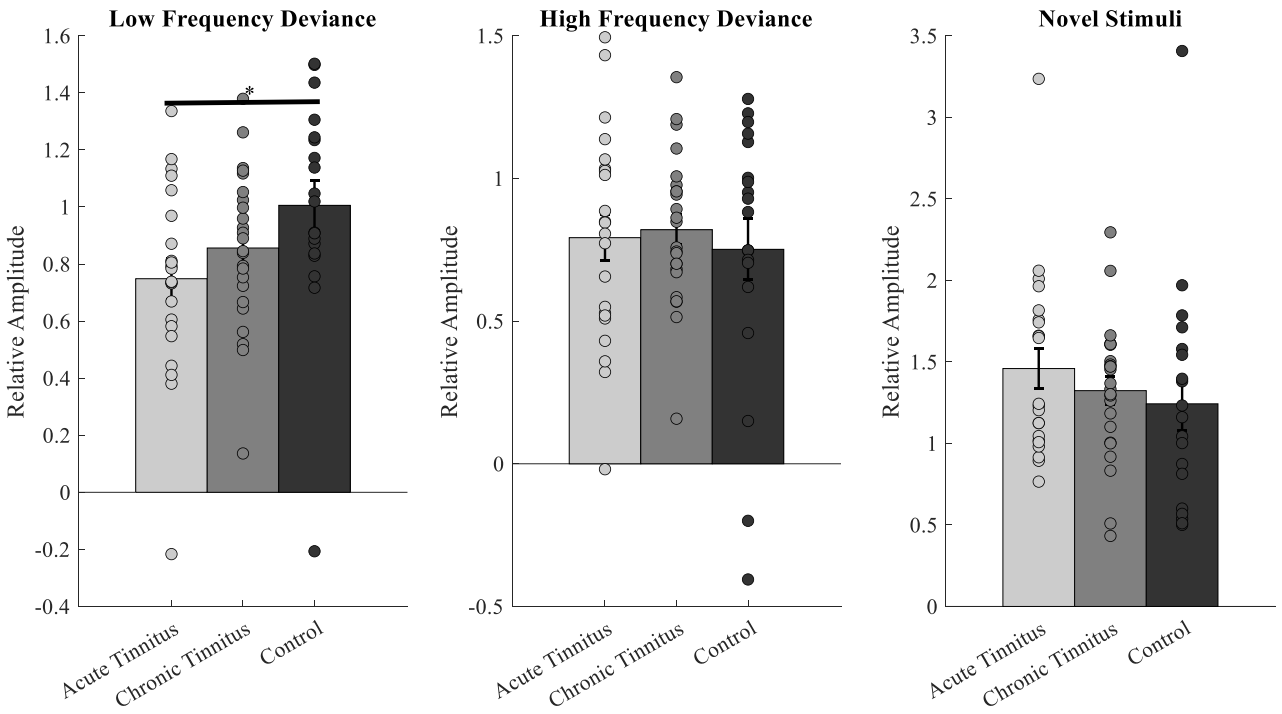
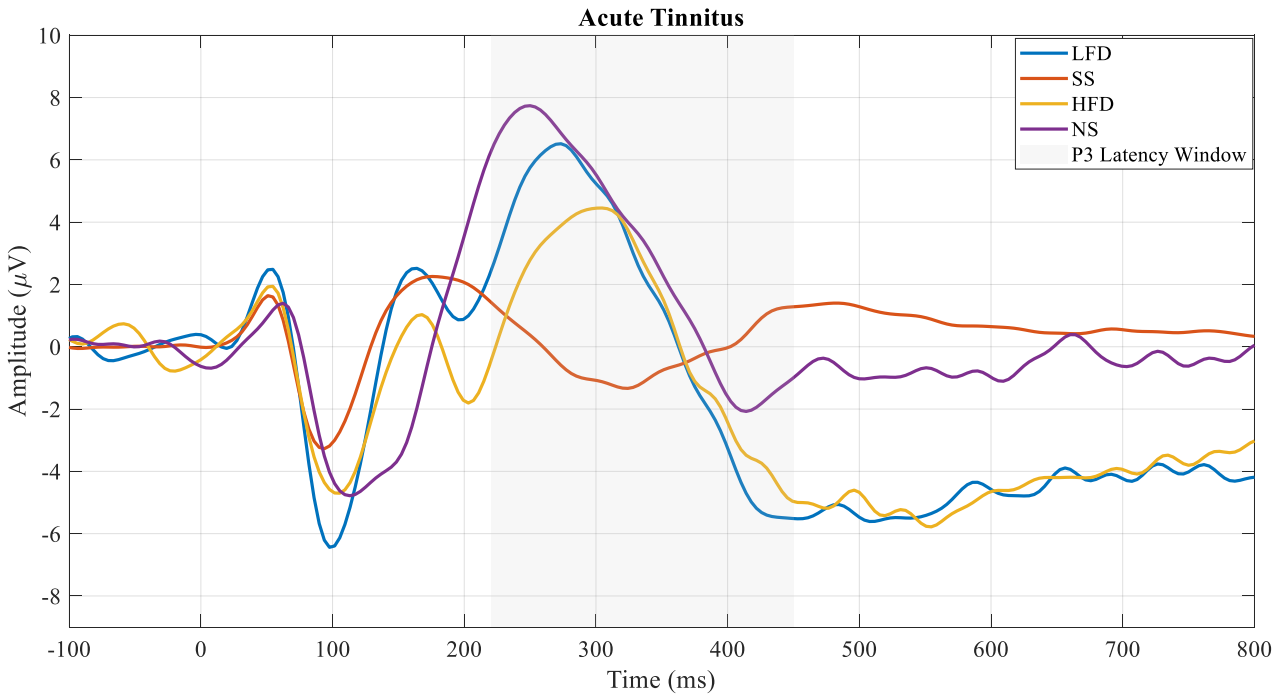
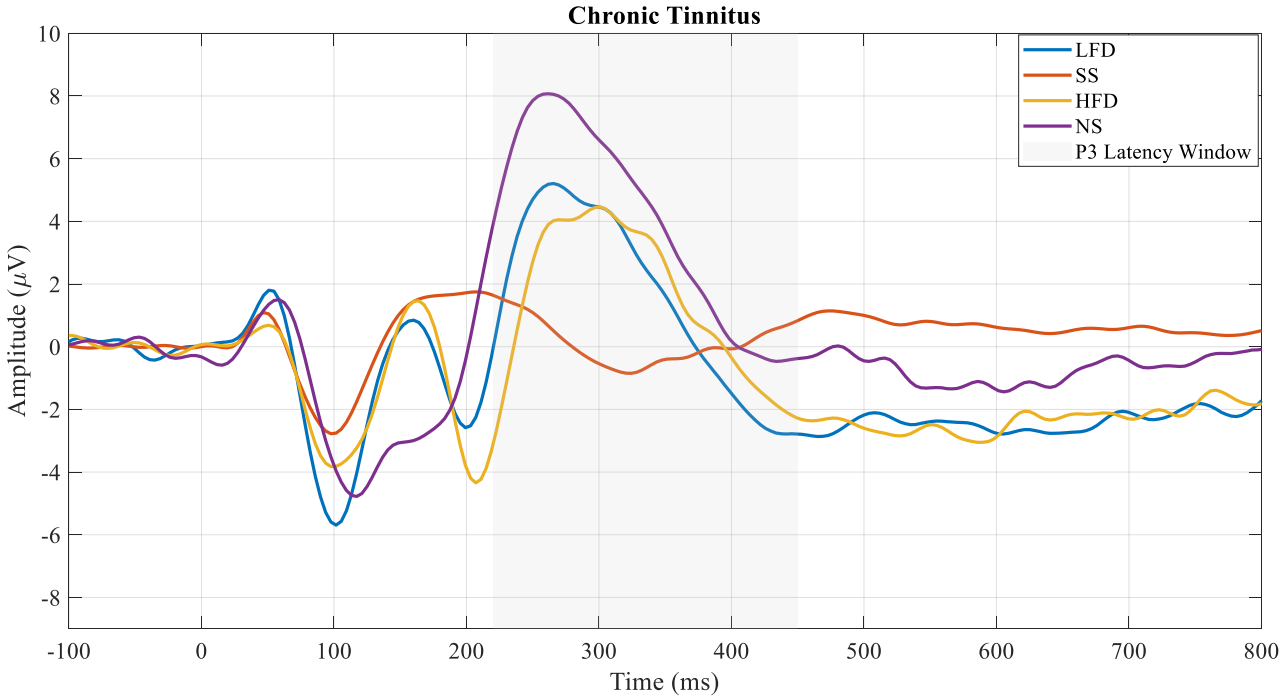


Figure 6.3. Relative P3 amplitude differences between Acute, Chronic, and Controls across conditions. *The figures reveal the presence of significant difference between Acute Tinnitus and Controls for LFD and no significant differences across the groups for HFD and NS. The Asterix (*) indicates the presence of significant difference*


Acute Tinnitus
LFD
SS
HFD
NS
P3 Latency Window
Amplitude (μV)
Time (ms)
10
8
6
4
2
0
-2
-4
-6
-8
-100
0
100
200
300
400
500
600
700
800


Chronic Tinnitus
LFD
SS
HFD
NS
P3 Latency Window
Amplitude (μV)
Time (ms)
10
8
6
4
2
0
-2
-4
-6
-8
-100
0
100
200
300
400
500
600
700
800

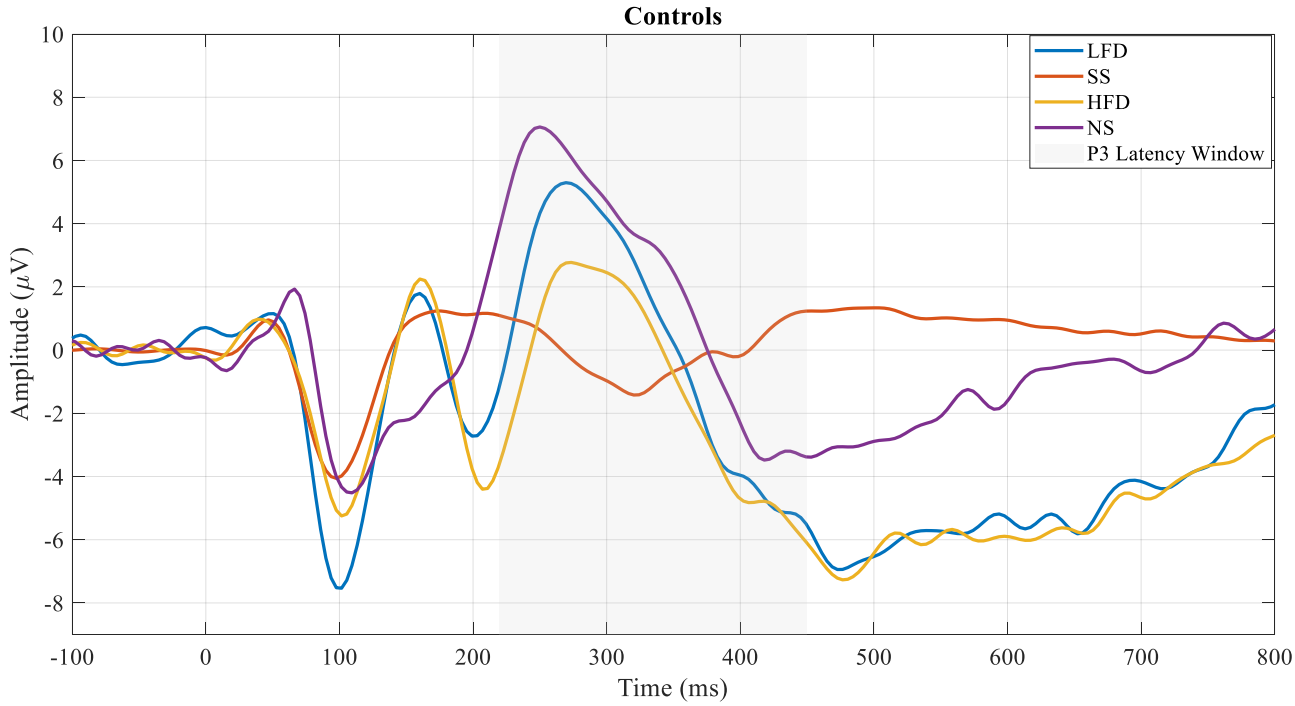


Figure 6.4: P300 waveforms between groups and across conditions. *LFD: Low Frequency Deviance, SS: Standard Stimuli, HFD: High Frequency Deviance, NS: Novel Stimuli*

6.3.4 P300 Amplitude changes between Acute Tinnitus and Post Acute Tinnitus

Upon paired longitudinal comparisons between the Acute and Post Acute group for the P300 amplitude, there was a non-significant trend for LFD ($t(8) = 2.038$, $p = 0.076$), a significant difference for HFD ($t(8) = 3.47$, $p = 0.008$), and a non-significant trend for NS ($t(8) = 1.867$, $p = 0.099$) with greater amplitude for Acute Tinnitus (LFD: $M = 0.86$, $SD = 0.56$, HFD: $M = 0.73$, $SD = 0.38$, novelty: $M = 1.75$, $SD = 0.81$) when compared to Post Acute Tinnitus (LFD: $M = 0.62$, $SD = 0.4$, HFD: $M = 0.5$, $SD = 0.39$, novelty: $M = 1.54$, $SD = 0.8$). Figure 6.5 illustrates the relative amplitude changes between Acute and Post Acute Tinnitus.

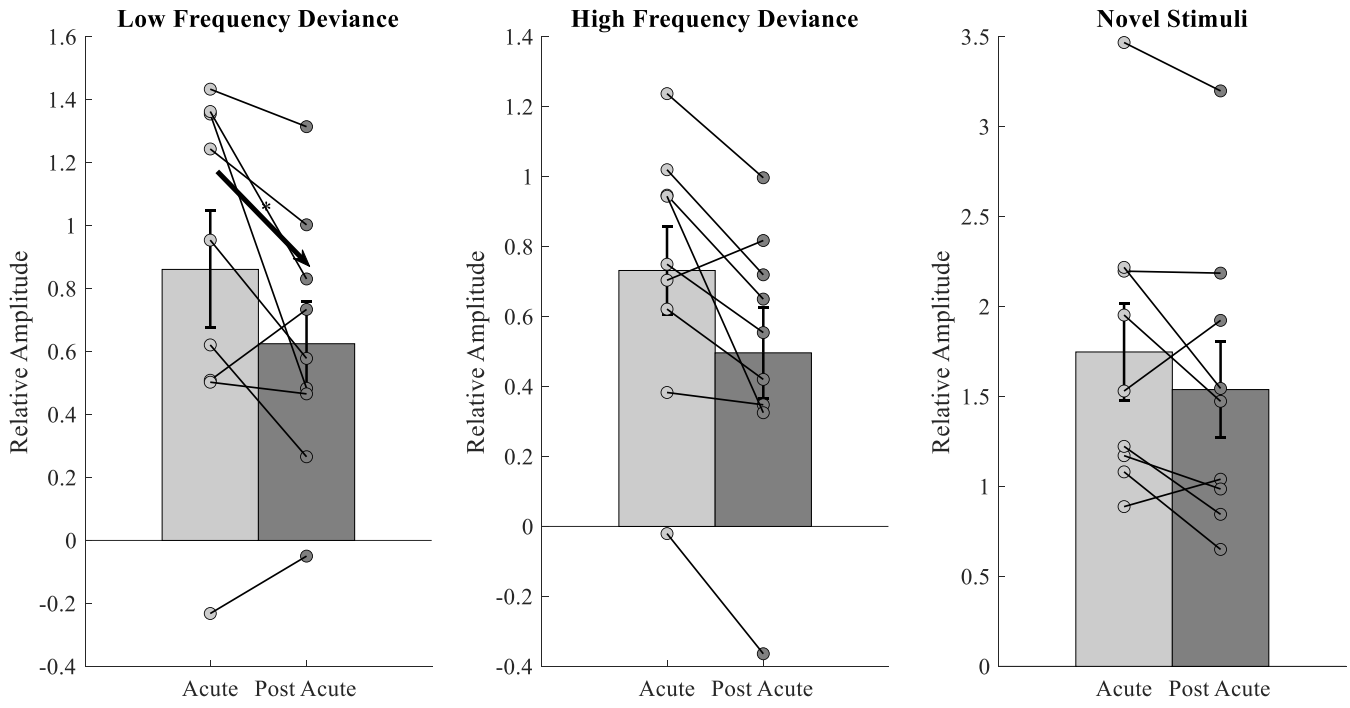
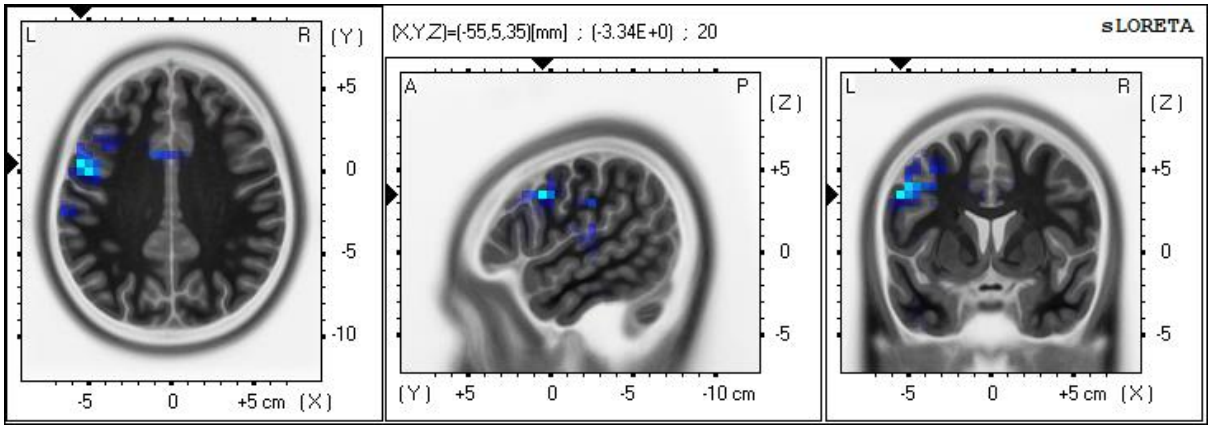


Figure 6.5: Relative P3 amplitude differences between Acute and Post Acute Tinnitus across conditions. *Significant difference noted for LFD and non-significant trend noted for HFD and NS*

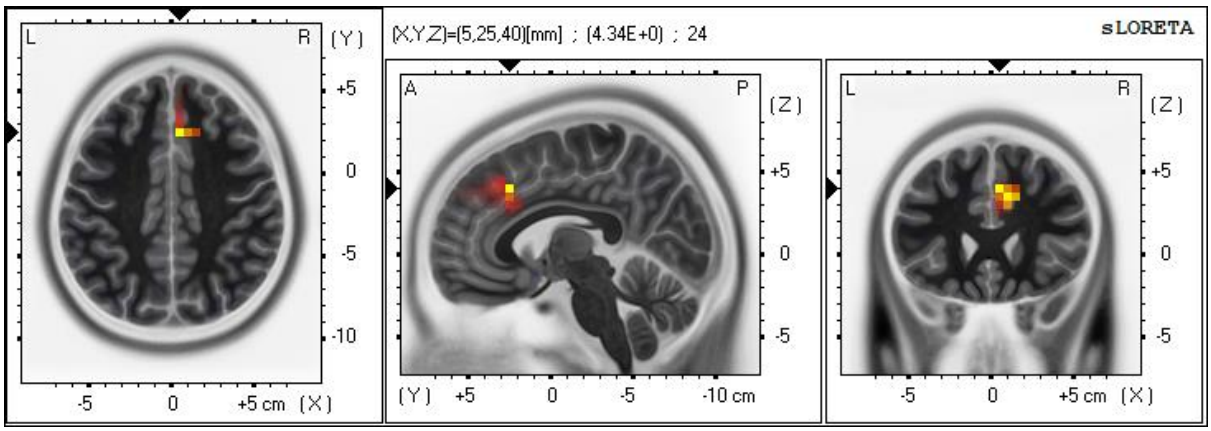
6.3.5 Source reconstruction.

An examination of source localisation was performed for both independent and matched groups. Currently, we are presenting solely the maximum or minimum t-statistics among the groups. As stated in the preceding section, we are exclusively reporting the source localisation activity for the groups that achieved statistical significance in the sensor space, specifically in the cross-sectional comparison between Acute Tinnitus and Controls for LFD, and the paired non-significant trend to significant differences between Acute and Post Acute Tinnitus for LFD, HFD, and NS. Between the Acute Tinnitus and Controls for LFD, the sources were localized in the inferior frontal gyrus. Concerning the longitudinal changes between Acute and Post Acute Tinnitus, the sources were localized in the anterior cingulate gyrus, inferior parietal lobe, and cingulate gyrus for HFD, LFD, and NS respectively. Figure 6.6 illustrates the source spaces for the above-mentioned group differences.

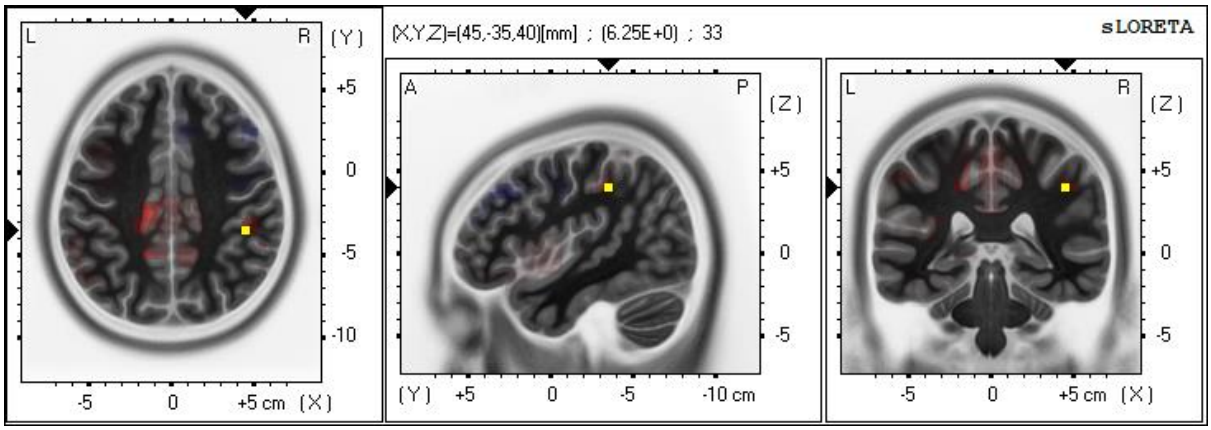
a.


(X,Y,Z)=(-55,5,35)[mm] ; (-3.34E+0) ; 20
sLORETA
L
R
(Y)
+5
0
-5
-10
-5
0
+5 cm (X)
A
P
(Z)
+5
0
-5
(Y) +5 0 -5 -10 cm
L
R
(Z)
+5
0
-5
-5 0 +5 cm (X)

b.


(X,Y,Z)=(5,25,40)[mm] ; (4.34E+0) ; 24
sLORETA
L
R
(Y)
+5
0
-5
-10
-5
0
+5 cm (X)
A
P
(Z)
+5
0
-5
(Y) +5 0 -5 -10 cm
L
R
(Z)
+5
0
-5
-5 0 +5 cm (X)

c.


(X,Y,Z)=(45,-35,40)[mm] ; (6.25E+0) ; 33
sLORETA
L
R
(Y)
+5
0
-5
-10
-5
0
+5 cm (X)
A
P
(Z)
+5
0
-5
(Y) +5 0 -5 -10 cm
L
R
(Z)
+5
0
-5
-5 0 +5 cm (X)

d.

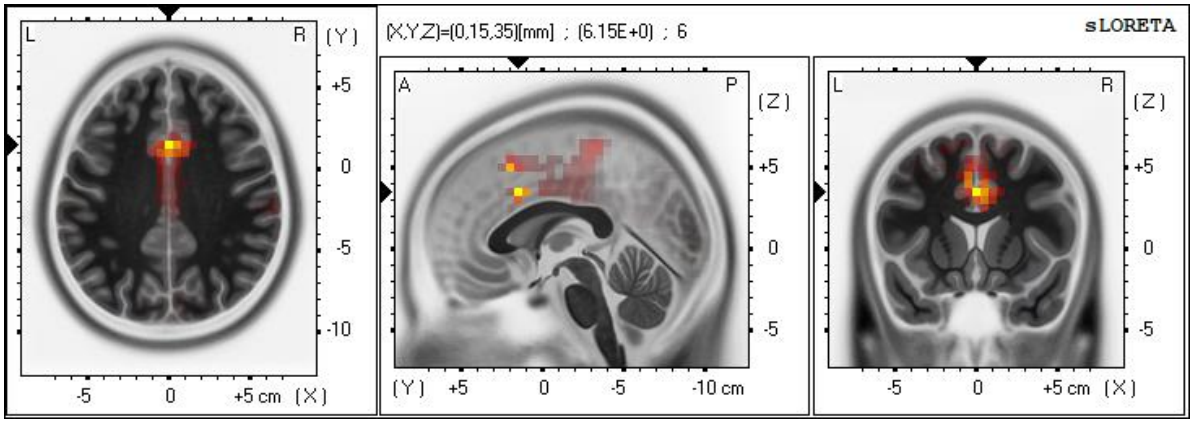


Figure 6.6: Source localization between group comparisons using sLORETA for peak P300 amplitude across conditions. *The above illustrated sources indicate maximum t statistics. a. Source localized in the inferior frontal gyrus between Acute Tinnitus and Control for LFD. b. Source localized in the anterior cingulate cortex between Acute Tinnitus and Post Acute Tinnitus for HFD. c. Source localized in the inferior parietal lobe between Acute Tinnitus and Post Acute Tinnitus for LFD. d. Source localized in the anterior cingulate cortex between Acute Tinnitus and Post Acute Tinnitus for Novelty.*

6.4 Discussion

The primary findings of our data include alterations in P300 amplitude for LFD between Acute and Control as well as a general trend of decrease in P300 amplitude in Post Acute Tinnitus compared to Acute Tinnitus across the three conditions.

6.4.1 Amplitude changes between Acute Tinnitus, Chronic Tinnitus, and Controls

Regarding P300 investigations in tinnitus, there is a paucity of literature in this domain, prompting us to draw inferences from the extensively researched Mismatch Negativity (MMN) studies related to tinnitus. The limited P300 studies, and more numerous MMN studies, in tinnitus have consistently shown reduced amplitudes of responses, at least to stimuli remote from the tinnitus frequency. With respect to the frequency deviants, the reduction in amplitude in Acute Tinnitus relative to Controls for LFD is concordant with previous P300 studies comparing Chronic Tinnitus with Controls at frequencies away from the tinnitus frequency. Gabr et al. (2022) conducted a study with the deviant stimuli being around 1050 Hz and the standard stimuli being around 1000Hz, they attributed the reduced P300 amplitude and prolonged latency to cognitive impairment and further implied attentional deficits in tinnitus due to possible involvement of the dorsolateral prefrontal

cortex. A study by Shalaby et al. (2022) reported reduced P300 amplitude and prolonged latencies in individuals with tinnitus especially at a deviant frequency of 2 kHz. They attribute that the reason might be due to the impairment of central auditory processing and selective attention to the stimulus as a consequence of tinnitus. We further highlight MMN studies that have conducted deviance detection in relation to tinnitus due to the scarcity of literature related to P300 and tinnitus. Most of the MMN studies in tinnitus highlight the reduction of attention away from the tinnitus frequency when compared to at the tinnitus frequency (Yukhnovich et al., 2024).

In our study, we obtained a non-significant increase in P300 amplitude for Acute Tinnitus for novelty processing when compared to Controls, which we attribute to heightened salience (increased focussed attention) and distress associated with Acute Tinnitus during its initial phases. It is crucial to acknowledge that all existing implications stem from Chronic Tinnitus research, and to our knowledge, none have been documented in Acute Tinnitus. The sole MMN study in Acute Tinnitus by (Sedley et al., 2016) indicated that there were no significant differences between Acute and Chronic Tinnitus in intensity deviance detection, which is on par with our current findings from a cross-sectional comparison of both conditions, potentially attributing this to an absence of differences in attention allocation between the groups.

The source reconstruction revealed a reduction in the inferior frontal gyrus regions for Acute Tinnitus compared to Controls, for LFD. This has been attributed to overall reduction in distress when there is increased activity in the frontal regions which can be implicated with controls having reduced distress (Carpenter-Thompson et al., 2015). Furthermore, Lan et al. (2021) highlighted the presence of heightened attention towards the tinnitus in the acute stages of tinnitus when compared to the chronic stage which is due to the potential role of fronto-parietal attentional network. In our case, a similar argument can be considered possible for the Post Acute Tinnitus group as there is reduction of activity around the inferior parietal lobe in this group when compared to the Acute Tinnitus group for an incoming acoustic signal. This further implies that there is a high possibility of increased orientation of inferior parietal lobe activity to the tinnitus in the Post Acute Tinnitus group indicating the potential involvement of fronto-parietal attentional network during Post Acute Tinnitus (onset of chronification of tinnitus) facilitating its persistence . Another potential explanation

associated with the function of the inferior frontal gyrus pertains to the prediction update mechanism. Todd & Robinson (2010) investigated the brain's capacity to utilise contextual information for updating predictions and reducing prediction error. In an MMN study conducted by them, which sought to identify the differences in the deviance response between randomly generated deviance and linked deviance (non-randomized deviant stimulus generation), the results indicated a decrease in MMN amplitude to the linked deviance. This reduction may be attributed to successful anticipation of the linked deviance stimuli, thereby diminishing prediction errors and the MMN response. This anticipatory behaviour, according to them, is closely associated with the inferior frontal gyrus. Individuals with Acute Tinnitus may experience heightened attention and distress towards the tinnitus during its onset, which can diminish their focus on stimuli outside the tinnitus frequency. This reduction impairs the predictive update mechanism to LFD, leading to an altered amplitude in comparison to Controls.

6.4.2 Post-Acute Tinnitus has a decreasing trend of P300 amplitude when compared to Acute Tinnitus

All three scenarios demonstrate either a reduced or trend in reduced P300 amplitudes in Post Acute Tinnitus relative to Acute Tinnitus, to all deviant types. The decrease in Post Acute Tinnitus relative to Acute Tinnitus corroborates our hypothesis that salience or novelty components are amplified during acute phases, subsequently decreasing and stabilising in the chronic stage. This pattern also aligns with frequency deviance, and our results emphasise the relatively intensified processing of novelty in contrast to the relatively reduced novelty and frequency deviance processing during the post-acute stages of tinnitus. The source reconstruction indicates a nearly significant elevation of activity in the Cingulate gyrus for both HFD and NS, as well as in the inferior parietal lobe for LFD, during Acute Tinnitus compared to the Post Acute Tinnitus groups.

Novelty changes between Acute and Post Acute Tinnitus

Regarding novel stimulus responses, we interpret the current findings as an increased novelty-related activity in the Acute stage rather than a diminished activity in the Post Acute stage, given the direction of cross-sectional comparisons between Acute Tinnitus, Chronic Tinnitus, and Controls. The Controls tend to have a non-significant reduction of novelty when compared to both Acute Tinnitus and Chronic Tinnitus. Although the results were not

statistically significant, we considered the direction of the amplitude changes across groups in the cross-sectional analysis to help interpret the patterns observed in the longitudinal comparisons. The aforementioned results regarding novelty may be attributed to heightened tinnitus-related distress during the acute phase, with the activity of cingulate gyrus strongly associated with distress. Vanneste et al. (2010) emphasised the potential role of the cingulate gyrus in tinnitus-related distress, given its crucial function within the limbic system. Regarding the alleviation of tinnitus-related distress over time were corroborated by Vielsmeier et al. (2020), who emphasised that although the characteristics of tinnitus remained unchanged, there was a moderate decrease in tinnitus-related distress over time. Research conducted by Wallhäusser-Franke et al. (2017) revealed no alterations in distress and perceived loudness among persons with elevated distress at onset; however, a decrease in distress was noted in the low-level distress group, aligning with our present findings.

In addition to the cingulate cortex being linked to distress, it is also a crucial element of the attentional networks, which have been extensively related with tinnitus. Simonetti & Oiticica (2014) hypothesized increased activities in the regions of cingulate gyrus along with prefrontal cortex and insula which correlates with improved cognition regarding focused attention. The article emphasizes that individuals with tinnitus tend to show increased cingulate gyrus activity, indicating that their focused attention is predominantly directed towards tinnitus, similar to the attention tasks non-tinnitus individuals tend to focus on. A recent study by Vanneste et al. (2024) highlighted the presence of an on-and-off mechanism for individuals with intermittent tinnitus that is influenced by the pregenual anterior cingulate cortex. The onset of tinnitus is associated with increased theta activity and reduced functional connectivity with the auditory cortex, while the offset of tinnitus is facilitated by an increased alpha activity in the pregenual anterior cingulate cortex. Apart from these, the anterior cingulate cortex has also been associated with error detection or conflict monitoring as reported by Dali et al. (2022). The increased salience activity at the commencement of tinnitus can be ascribed to its novelty, as tinnitus serves as a novel stimulus that elicits a top-down response from the anterior cingulate cortex, functioning as a conflict detector. The heightened awareness of novel tinnitus during its onset correlates with an increased response to externally presented novel stimuli, as evidenced by a recent study on pain conducted by Hubbard et al. (2020a). This study identified an aberrant increase in the salience network in

response to pain stimuli in fibromyalgia patients, suggesting a generalised hypervigilance to salient stimuli. This phenomenon is similarly observed in our Acute Tinnitus patients, who can either exhibit a generalised increase in hypervigilance towards any external salient/novel stimuli due to their heightened awareness or may even be the potential cause of the emergence of tinnitus.

The anterior cingulate cortex's error detection property resembles the Bayesian predictive coding phenomenon, wherein Bayesian coding theory asserts that the brain probabilistically encodes sensory information through probability distributions. The brain constructs an internal model and juxtaposes it with external sensory input; greater similarity between the sensory input and the internal model results in reduced prediction error, while lesser similarity leads to increased prediction error (Knill & Pouget, 2004). The same has been applied to tinnitus research as well with predictive coding models formulated by De Ridder et al. (2014) and Sedley et al. (2016) where they state that the absence of auditory input from hearing loss prior to its onset creates a discrepancy between the predicted and the actual input, leading to a prediction error. To mitigate prediction error resulting from deafferentation, the brain often compensates for absent information, which may manifest as tinnitus. We wish to emphasise that the anterior cingulate cortex's role in error detection for Acute Tinnitus may occur even during the precursor phase of tinnitus amidst hearing loss, which subsequently diminishes over time following the onset of tinnitus. These further associates the anterior cingulate cortex and novelty with the generation of tinnitus, aiming to minimise the prediction error between actual reduced input and expected input, in alignment with the model proposed by De Ridder et al. (2014). An alternative hypothesis suggests that the anterior cingulate cortex/salience may play a heightened role following the onset of tinnitus, wherein the tinnitus signal could induce an elevation in prediction error due to the discrepancy between anticipated sensory input (silence) and actual input (augmented spontaneous activity). This prediction error is interpreted as tinnitus, which diminishes over time as the internal model transitions from a default state of silence to increased spontaneous activity, as noted by Sedley et al. (2016). Our findings of reduced amplitude for novelty over a period of time suggests a decrease in the prediction error, accompanied by a decline in the activity of the anterior cingulate cortex during the same period further supports the model by Sedley et al. (2016) and in accordance with our principal hypothesis.

LFD and HFD changes between Acute and Post Acute Tinnitus

The amplitudes for both LFD and HFD were showed either reduction or trend in reduction in the Post Acute stages of tinnitus. In contrast to the interpretation indicating possible adaptation to novelty in the post-acute phase, it has been demonstrated that P300 amplitudes for LFD were diminished in Acute Tinnitus compared to Controls cross sectionally which is in the opposite direction if we were to keep the cross-sectional comparison to indicate the direction of results. Thus, the observed direction diverges from that associated with novelty, necessitating the interpretation that both LFD and HFD exhibit reduced activity over time. Furthermore, the additional decrease in Post Acute Tinnitus relative to Acute Tinnitus may be ascribed to attention reallocations between frequencies. In this instance, contrary to other studies, we are not directly linking reduced cognitive performance or attention deficiencies in patients with tinnitus Gabr et al. (2022) but rather suggesting a pre-conscious diversion of attention from external stimuli due to an internal concentration on the tinnitus. The inferior parietal cortex that was visualized in the source space for LFD with reduced inferior parietal activity over time is associated with the fronto-parietal attentional network, where recent research emphasises the heightened focused attention on tinnitus, hence increasing the fronto-parietal attentional network towards the tinnitus and thereby reducing attention towards external stimuli (Hubbard et al., 2020b).

Reduced attention to external stimuli may facilitate the persistence of tinnitus.

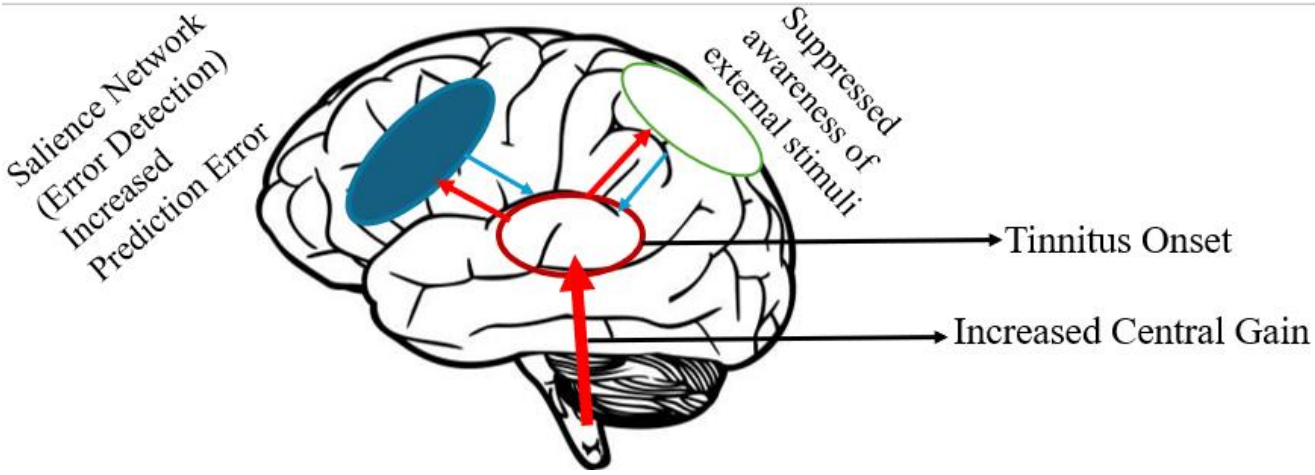
In the current research, we concentrated solely on the cortical region with the greatest t statistic as our region of interest, potentially restricting the evaluation of other brain regions that may demonstrate independent activity or possess pertinent functional connections. However, evaluating the current investigation at face value, it is intriguing to observe the comparisons between Acute and Chronic Tinnitus both cross-sectionally and longitudinally, the longitudinal comparisons yielded significantly more robust results, demonstrating non-significant trend to significant reductions in relative amplitude across conditions from Acute to Post Acute stages. The inferior parietal lobe which was the other region of interest in this study apart from the anterior cingulate cortex has been known to contribute to both novelty processing (Kiehl et al., 2001) and in P3b generations too (Volpe et al.,2007). The role of the inferior parietal cortex can be interpreted in two manners: either as a heightened awareness of the tinnitus at its inception or as a reduced attentiveness to external stimuli throughout the chronic stages of tinnitus. We would favour the latter based on the evidence from the current

study. In addition to the alterations in the inferior parietal lobe, we observed a decrease in P300 amplitudes in both target deviant circumstances at sensor space, indicating attention relocation between frequencies (Volpe et al., 2007).

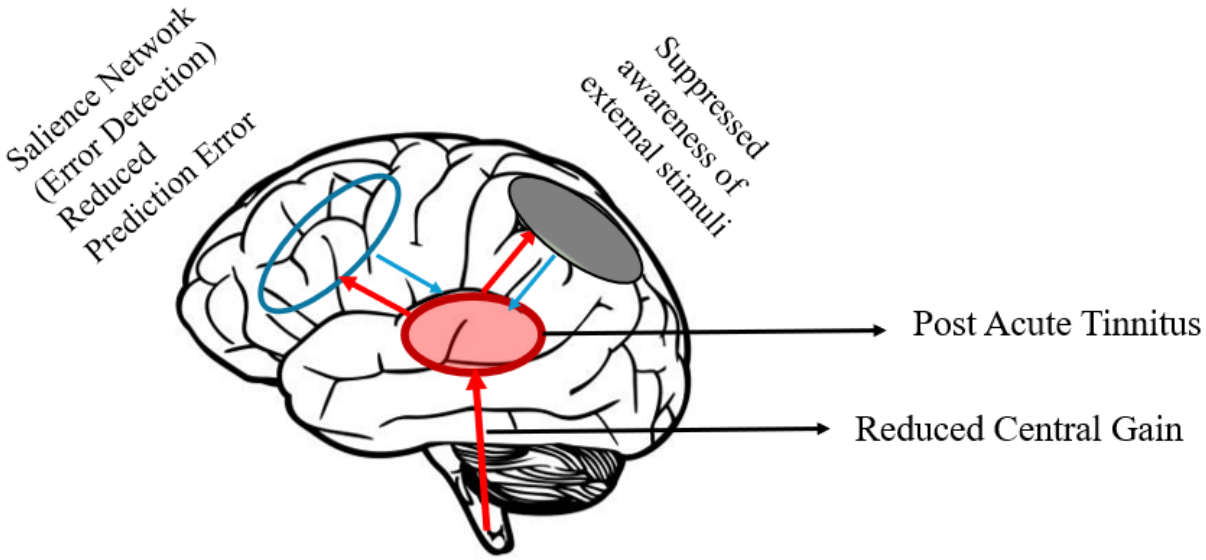
It is imperative to note that the increased activity in the salient processing of the tinnitus signal increases attention orientation to any externally incoming salience/novel stimulus. But it is the contrast for frequency deviance detection with reduced activity in the inferior parietal lobe during the later stages or Post Acute stages of tinnitus, thereby reducing the attention to the external stimuli, due to the increase in attention towards the tinnitus. We further propose that there is a possibility of the anterior cingulate cortex to play a role in tinnitus formation through error detection in the *precursor stages*, leading to tinnitus as a mechanism to mitigate prediction error. The other possibility is that the prominence or novelty of tinnitus is affected by the anterior cingulate cortex, which functions as an error detector at the onset of tinnitus, subsequently engaging additional attentional networks that reduce focus on external stimuli, thereby amplifying concentration on tinnitus and ensuring its persistence. These findings align with the initial resting state EEG results, which emphasise the anterior cingulate cortex for cross-sectional differences between Acute and Chronic Tinnitus, and the inferior parietal cortex for longitudinal differences between Acute and Post-Acute Tinnitus, further underscoring the collaborative role of the anterior cingulate cortex and inferior parietal cortex in the onset and persistence of tinnitus.

Figure 6.7 depicts a newly proposed account of tinnitus persistence based on the attentional orientation towards the tinnitus further explaining its onset and persistence. A significant drawback of this study is the Chronic Tinnitus cohort, which could not be matched for high-frequency hearing, hence preventing the current study from interpreting the cross-sectional comparisons of Chronic Tinnitus with the other two groups.

a.


Salience Network
(Error Detection)
Increased
Prediction Error
Suppressed
awareness of
external stimuli
Tinnitus Onset
Increased Central Gain

b.


Salience Network
(Error Detection)
Reduced
Prediction Error
Suppressed
awareness of
external stimuli
Post Acute Tinnitus
Reduced Central Gain

c.

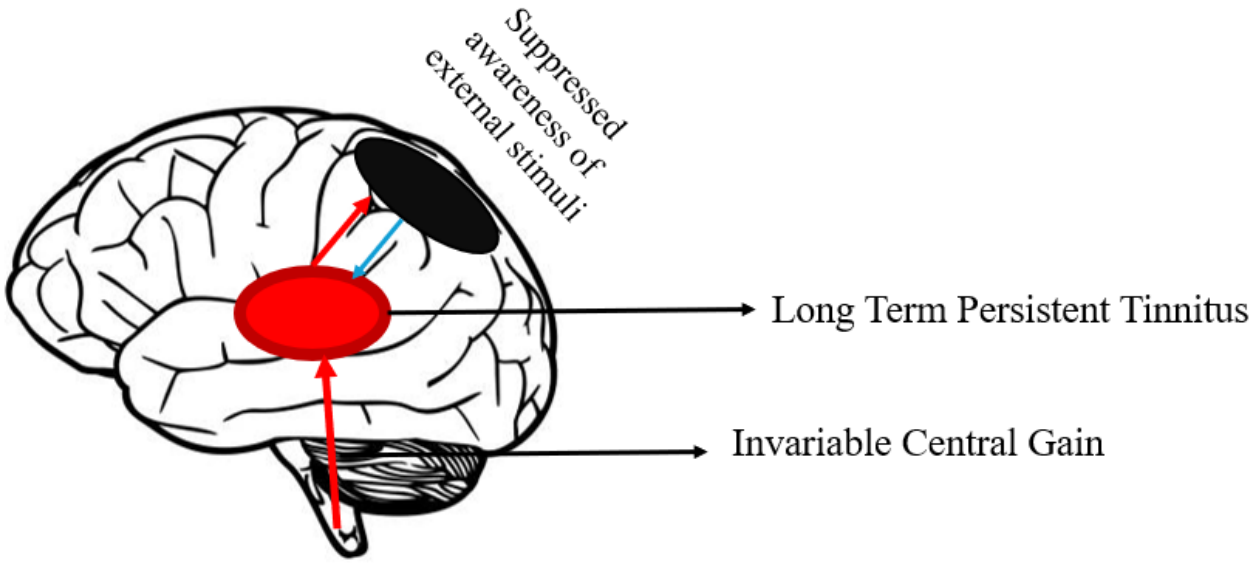


Figure 6.7: Illustration of tinnitus mechanism based on stimulus novelty. *The three figures illustrate changes in tinnitus mechanism post onset of tinnitus. There are three major elements included in the figure which include anterior cingulate cortex (blue) highlighting error detection, inferior parietal lobe (black) highlighting increased attention orientation towards the tinnitus, auditory cortex (red) highlighting the tinnitus activity, and increased central gain (red arrow) which increases during the onset and regresses to the mean during the Post Acute and Chronic stages based on our results from Chapters 3, 4, and 5.*
a. Acute Tinnitus: There is increased activation of salience network around the time of the onset of tinnitus which either may cause tinnitus or be a consequence of tinnitus. There is increased prediction error around this time concurrently activating the attentional networks which includes the inferior parietal cortex that starts to reorganize, diminishing focus on external stimuli and redirecting it towards the tinnitus. The central gain increases during this stage that results in tinnitus onset.

b. Post Acute Tinnitus: The propagation of tinnitus diminishes prediction error by altering the mental model, shifting the default prediction from quiet to tinnitus, so causing the salience network to reduce the error detection that was present around the onset of tinnitus. Conversely, the attentional networks (through fronto-parietal attentional networks) enhance the focus on the tinnitus while diminishing the perception of exterior stimuli. Central gain reduces as a function of regression to the mean

c. Chronic Tinnitus: The mental model has transitioned from default silence to tinnitus, thereby minimizing prediction error. Concurrently, the attentional networks (specifically sustained attention) experience a permanent alteration in connectivity, which enhances focus on tinnitus while diminishing attention to external stimuli, thus facilitating a persistent perception of tinnitus. Central gain remains constant indicating the tinnitus activity remaining constant over time after initial reduction.

6.5 Conclusion

Previous research has proposed potential processes for the generation and persistence of tinnitus, either independently or through cross-sectional comparisons between acute and chronic tinnitus. The primary areas of focus have been the prefrontal, parietal, and insular regions in the generation and maintenance of tinnitus. Alongside the previously discussed findings, including our preliminary EEG results emphasising the anterior cingulate cortex and inferior parietal lobe, we opted to examine the hypothesis using a novelty based P300 paradigm, which is typically elicited from both prefrontal and parietal regions, to investigate the distinction between acute and chronic tinnitus and to corroborate earlier findings. This research likely represents the first study employing a longitudinal design that includes a follow-up on Acute Tinnitus six months post-onset of Chronic Tinnitus. Our data indicate a significant drop in P300 amplitude during the chronic stage, highlighting the substantial influence of the anterior cingulate cortex/salience network in possible generation of tinnitus and inferior parietal lobe in the persistence of tinnitus.

Chapter 7 (Overall discussion). An integrative model for Tinnitus based on Tinnitus as an invariance to central gain.

7.1 Summary of results.

This research conducted four primary experiments, three of which indirectly assessed hyperexcitability and addressed the central gain process, while one experiment concentrated on the attentional mechanisms related to tinnitus. Furthermore, tinnitus was assessed according to its psychophysical characteristics (pitch and loudness) and the degree of distress experienced, and preliminary resting-state EEG results are also presented. These data provide a comprehensive examination of tinnitus as it progresses from acute to chronic stages. This research represents the inaugural neuroscientific study on Acute Tinnitus employing objective measures tracked from onset to the Chronic stage. To summarise our findings, we will place primary emphasis on the longitudinal changes from the Acute to Post-Acute stages, as these offer the most robust insights. Cross-sectional comparisons between the Acute, Chronic, and Control groups will serve a complementary role—helping to interpret the direction of change relative to controls and to integrate our results with existing literature on Chronic Tinnitus. However, we interpret the cross-sectional differences with caution, as they may be confounded by unmatched high-frequency hearing and other variances such as the level of hyperacusis. The comparisons between Acute and Control groups, which were well matched, provide a framework for interpreting the data when comparing Acute and Post-Acute Tinnitus. It is noteworthy that the presentation levels for the evoked potentials (IDAEP, ASSR, and P300) were customised to the subject's dynamic range, allowing for comfortable presentation levels, recruitment control, control for hyperacusis, and partial control of hearing asymmetries. However, cortical reorganisation resulting from hearing loss abnormalities and other consequences may not be fully accounted for and could potentially confound the results which is also a potential weakness in all cross-sectional observational studies of tinnitus and in the current study, the Chronic Tinnitus group specifically.

7.2 Neurobiological Explanation

7.2.1 Tinnitometry and Distress.

This section will summarise the differences in tinnitus-related characteristics across groups, including psychophysical correlates such as tinnitus frequency, loudness, and overall distress

associated with tinnitus.

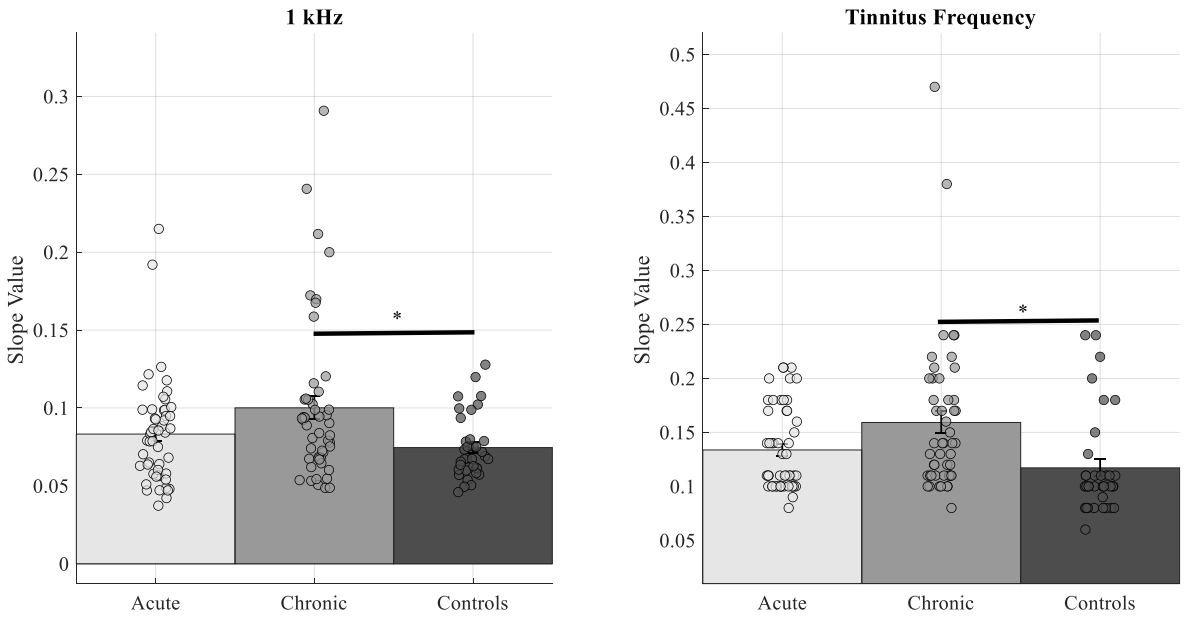
Firstly, the tinnitus frequencies were the same across groups for Acute and Chronic (and identical stimulus frequencies used for Controls), and even did not change from Acute to Post Acute Tinnitus longitudinally ensuring the obtained results were not confounded by differences in tinnitus frequency. Secondly, there was a drop in tinnitus intensity from Acute to Post Acute Tinnitus longitudinally and even cross sectionally between Acute and Chronic Tinnitus in some instances. This is in line with our primary hypothesis regarding central gain, where the tinnitus loudness (an indirect metric of auditory sensitivity/central gain) is maximal around the onset and reduces over time. As to the reduction in loudness from Acute to Post Acute Tinnitus, we can attribute habituation to the perception of tinnitus over time. Our Chronic Tinnitus group was similar to the Acute Tinnitus group in terms of distress but given that the Acute group's distress reduced by the Post-Acute stage, it can be inferred that the Chronic group maintained a higher long-term level of tinnitus distress. The previous chapters have discussed in detail the selection bias often present in Chronic Tinnitus studies, where increased distress tends to be overrepresented due to the inclusion of individuals with long-term, persistent tinnitus. In contrast, our Post-Acute Tinnitus group was followed longitudinally, allowing for a more representative understanding of tinnitus progression independent of distress-related sampling bias.

Tinnitus handicap and hyperacusis questionnaires generally improve over time from Acute to Post Acute Tinnitus, reflecting adaptation and habituation. In Chapter 2, we suggested that heightened focused attention on tinnitus, stemming from the novelty of the condition and hypervigilance, may lead to higher distress upon the beginning of tinnitus. Cross-sectionally, we further identified across chapters that the sample for Chronic Tinnitus may reflect a selection bias towards those experiencing distress, whose distress-related scores resemble those of Acute Tinnitus rather than Post-Acute Tinnitus. Controls however showed normalized reduced levels in the hyperacusis scores which was reflected in the CLS test with a reduced slope when compared to Chronic Tinnitus. In summary the loudness and distress of Tinnitus are maximal around its onset and reduces over time which is in line with our primary hypothesis that neural changes underlying tinnitus are maximal around the time of onset but later subside as a regression to the mean, and also compatible with adaptation or habituation-based explanations.

7.2.2 Central gain and tinnitus

There were 3 tests that were used which could tap into the central gain mechanisms of Tinnitus. The CLS which is a subjective loudness scaling test measuring sound intolerance and with Tinnitus and Hyperacusis both originating from a shared basis of abnormal central gain enhancement; the subjective loudness assessment of loudness intolerance would effectively quantify central gain in both conditions (Hébert et al., 2013). Our results of CLS indicated that Chronic Tinnitus exhibited a heightened slope and loudness category compared to Acute Tinnitus and Controls at the Tinnitus frequency, which we attributed to the influence of hyperacusis (which may have related to more impaired hearing and/or greater long-term tinnitus distress in the Chronic Tinnitus group). Interestingly, there were no differences in the CLS slope and loudness categories between Acute Tinnitus and Controls and between Acute Tinnitus and Post Acute Tinnitus, indicating that if the slope of subjective loudness is a measure of central gain, there is no alterations in central gain indicating that central gain increase does not seem to be necessary for either the onset or maintenance of tinnitus (though can nonetheless be increased in people with chronically bothersome tinnitus) as we had a cohort of Chronic Tinnitus individuals that may have replicated increased auditory sensitivity due to long term tinnitus distress (Chapter 3). Figure 7.1 summarizes the slope across loudness levels between the groups cross sectionally and longitudinally.

A)



B)

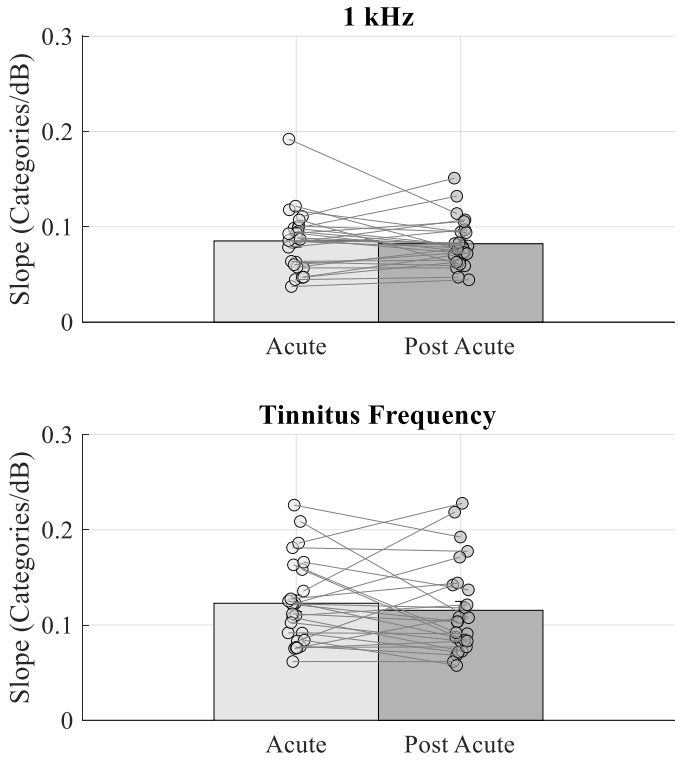


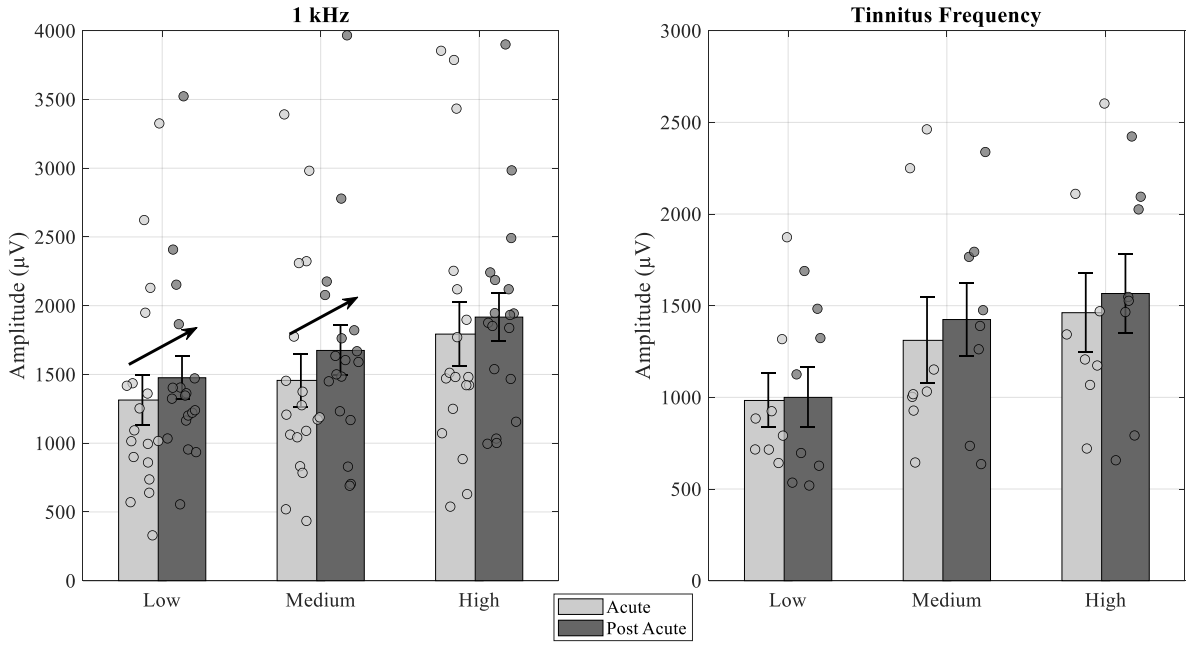
Figure 7.1: The illustration is a reproduction of the slope referenced in chapter 3. *The slope across loudness levels is an essential metric of central gain (auditory sensitivity) and shows no variations between Acute and Controls (A), Acute and Post Acute Tinnitus (B) indicating that auditory sensitivity does not influence the tinnitus activity in isolation. The changes observed in Chronic Tinnitus (A) is due to the level of hyperacusis in the tinnitus groups causing increased sensitivity.*

Surprisingly, this observation of no clear difference between Acute Tinnitus and Controls in the CLS slope was replicated partially in both ASSR and IDAEP, where cross sectionally there was no significant difference between the groups after controlling for hearing and recruitment, indicating that once level of hyperacusis (which may have contributed to the cross-sectional changes in CLS) is factored in, there is no mean difference in the level of central gain. Conversely, in longitudinal comparisons, the slope of IDAEP at 1 kHz decreases over time following the onset of tinnitus, with an increase in N1-P2 amplitude at low intensity levels becoming more control like. Likewise, the ASSR amplitudes exhibit a trend

in increase at low to mid intensity levels over time between Acute and Post Acute Tinnitus, becoming like controls.

This suggests that certain neural responses associated with tinnitus may decrease over time, as longitudinal comparisons are considerably more reliable than cross-sectional ones. However, the question arises regarding the nature of this neural response: is it an additional metric of central gain or merely the effect of tinnitus on the processing of incoming sound? This was demonstrated in the Auditory Steady-State Response (ASSR), where we suggested that tinnitus, in isolation, may affect synchronous firing to AM sounds. Furthermore, as tinnitus reduces over time—evidenced by reductions in both distress and tinnitus loudness—the synchrony with AM sounds improves. The same applies to IDAEP, where the onset of tinnitus is marked by heightened tinnitus activity, resulting in reduced inhibitory functions within the auditory pathway, as indicated by an increased N1-P2 slope of the IDAEP. Over time, this slope decreases, thereby enhancing inhibitory functions. Figure 7.2 highlights the changes in ASSR amplitude and IDAEP slope between Acute and Post Acute Tinnitus.

A)



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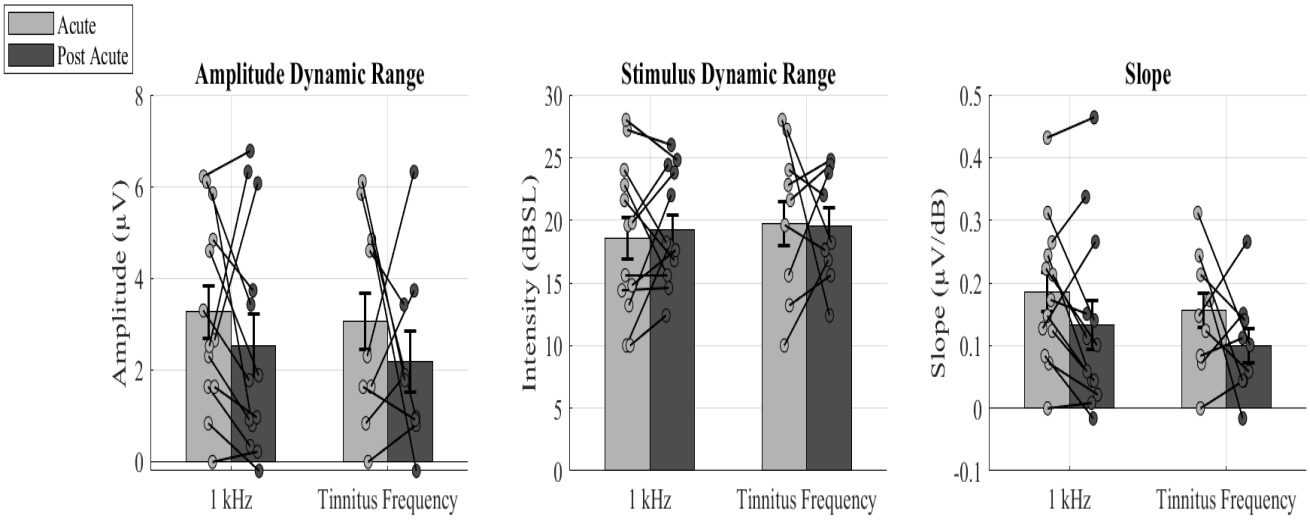


Figure 7.2: The illustration is replicated from Chapters 3 and 4 with depiction of ASSR amplitude and IDAEP slope variations between Acute and Post Acute Tinnitus. *The figure illustrates another metric of auditory sensitivity/central gain that shows variations between Acute and Post Acute Tinnitus with a non significant trend of increase in the ASSR amplitude and reduction of IDAEP slope over time indicating neural response linked to tinnitus habituates over time.*

The question remains as to how the results are to be interpreted. Reiterating the fact that we presented the stimulus that was tailored to the dynamic range to each subject in ASSR and IDAEP, the hyperacusis was controlled further indicating that the central gain too was largely compensated, if going by the definition of Zheng et al.,(2013) where central gain is associated to hyperacusis, and central noise was associated with Tinnitus. In our case we are in agreement with Zeng (2013) that to a large extent central gain was controlled for the objective measures of ASSR and IDAEP by providing the stimulus tailored to the dynamic range of each subject, hence what is remaining is the central noise which can be tapped into. However, the central noise according to Zeng (2013) indicates a baseline shift where the output of the slope function is an additive increase from its pre-tinnitus configuration, but in our case, we did not see an entire baseline shift from Acute to Post Acute Tinnitus but rather a positive shift at low to mid-levels but not (or to a lesser extent) at high levels. This in

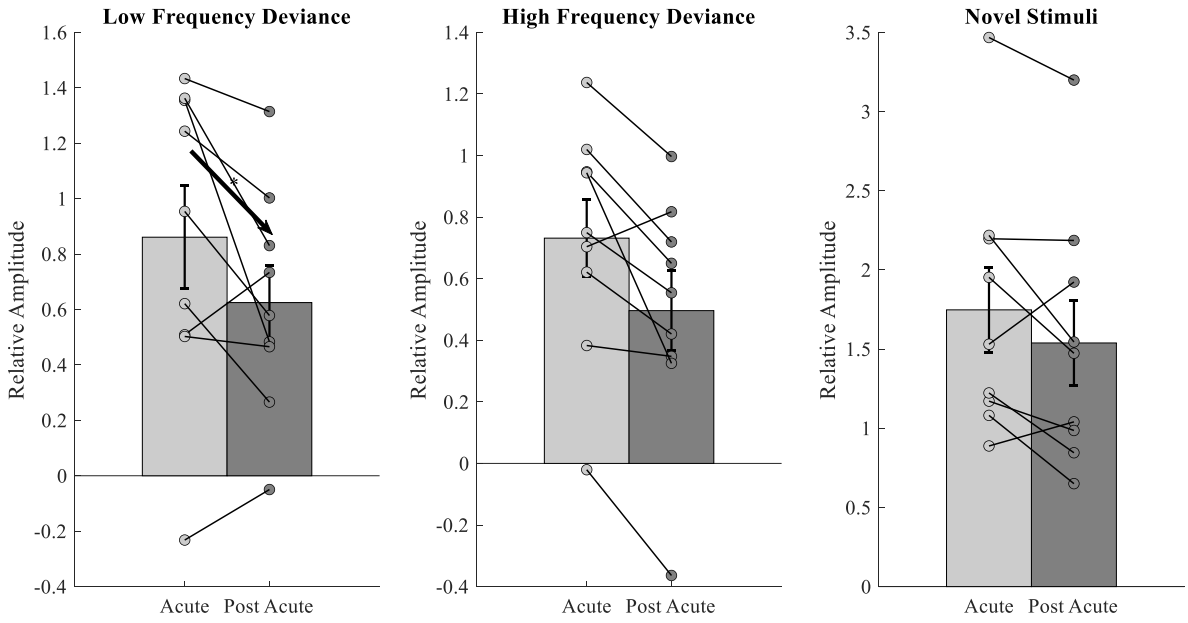
combination with exploratory regression analysis in Chapter 5 between SDR and IDAEP slope, where a trend of negative relationship was seen for the Acute Tinnitus, which indicates that responses associated with acute tinnitus are more indicative of dynamic range adaptation rather than simply increased central gain or central noise. Diesch & Hassel-Adwan (2017) explored dynamic range adaptation in their study, where they used a varied amplitude modulation tones in both ascending and descending run to compare tinnitus with controls. They suggested that tinnitus individuals had stronger undershoot (strong decrease in amplitude with decrease in stimulus) and weaker overshoot (small increase in amplitude with increase in stimulus). The cumulative results lead to our assertion that Tinnitus is a byproduct (or cause) of a particular process that attenuates excessive gain. When integrating these findings, it becomes evident that gain in tinnitus in general remains relatively stable in comparison to controls, whilst in certain distressing scenarios, it may be associated with hyperacusis. In the absence of tinnitus, there would likely be an excess of central gain; however, tinnitus mitigates this excess by attenuating it. Furthermore, we argue that tinnitus regulates gain by enhancing dynamic range adaptation (Chapter 5), ensuring that soft to medium sounds are amplified while preventing further amplification or even reducing the amplitude of loud sounds. Tinnitus is hence an invariance to the central gain by keeping the central gain relatively constant, or constraining its limits, despite the changes in the incoming environmental sounds. This would explain the reason for the generation and persistence of tinnitus as the activity of tinnitus ensures central gain doesn't increase substantially further. Also, the increase in amplitude values at low to mid-levels, and the reduction of tinnitus loudness over time, signifies the tendency of tinnitus to reduce as a consequence of adaptation and habituation keeping the central gain constant.

7.2.3 Attention and Tinnitus

In the previous section, we addressed the question of *why* tinnitus occurs (increased dynamic range adaptation to modulate gain). From the current section onwards, we would discuss *how* does the tinnitus and persist (mechanisms linked to propagation of tinnitus). From our previous chapter (Chapter 6), we have discussed different attentional mechanisms that might play a role in the generation and persistence of Tinnitus. We used a novelty based P300 paradigm to explore the potential roles of the salience and attention network in the generation and persistence of tinnitus, as novelty response of the P300 taps into the salience network

with strong contributions from the anterior cingulate cortex which is essential for the salience network (Kim et al., 2014). We found that novelty-based P300 responses were elevated at tinnitus onset but gradually declined from the acute to the chronic stage. Source analysis identified the anterior cingulate cortex (ACC) as the primary region involved, with ACC activity decreasing over time in a pattern that progressively aligned with that of the control group. This might potentially explain at least part of the mechanism of onset of tinnitus, as anterior cingulate cortex also plays a role of error detection or conflict monitoring as reported by Dali et al. (2023). The increased contribution of the anterior cingulate cortex to the onset of Tinnitus may imply the presence of error detection; however, it remains uncertain whether this error detection exists to the novelty of the tinnitus onset or exists prior to the onset of Tinnitus, given that tinnitus has been identified as a regulator of central gain (from our findings) or be present as both cause and effect of tinnitus. With respect to the stages prior to the onset of tinnitus, the novelty response from the anterior cingulate cortex might results from the heightened central gain induced by continuous error detection. Even though there is hypervigilance in response to the onset of tinnitus as discussed in Chapter 1, the error detection precedes even the onset of the tinnitus. This mechanism may explain tinnitus onset, potentially driven either by the anterior cingulate cortex (ACC) initiating a “switch-on” process or by the ACC responding to tinnitus after its onset—via heightened engagement of the salience network and increased error detection which. This is illustrated in figure 7.3

A)



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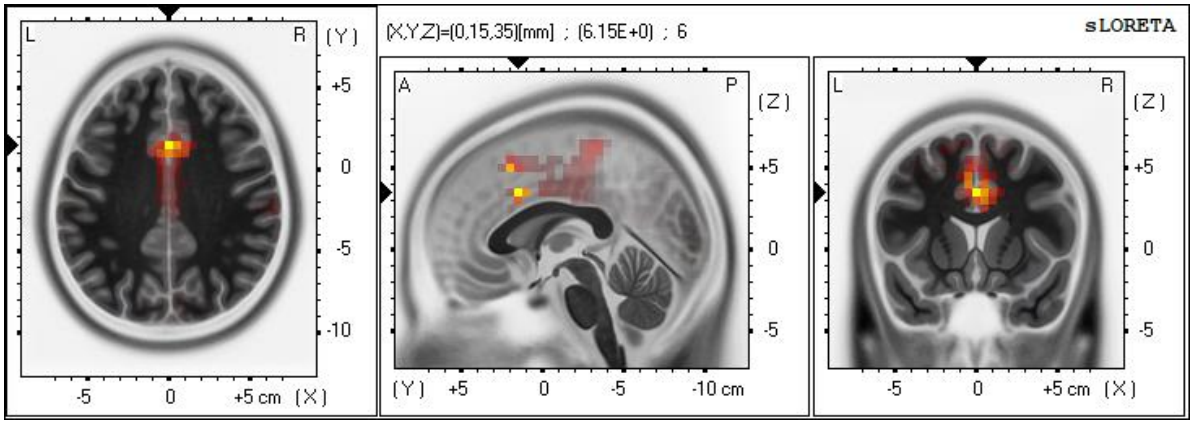
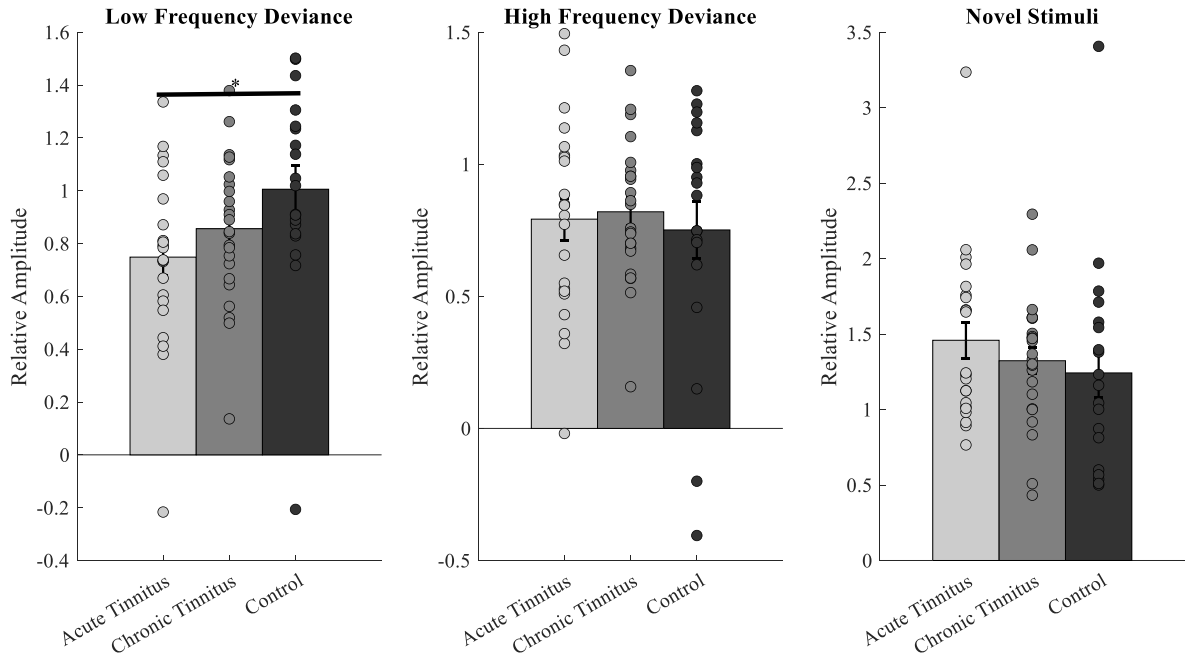


Figure 7.3: A) Relative P3 amplitude differences between Acute and Post Acute Tinnitus across conditions. *Significant difference noted for LFD and non-significant trend noted for HFD and NS.* ***B) Source localized to the Anterior Cingulate Cortex when comparing Acute and Post Acute Tinnitus for novelty-based stimulus***

Now with respect to the mechanism for its persistence, can be explained by the reduction of the frequency deviance response over time, which is away from the direction of controls (i.e. becoming more abnormal, unlike other observed changes in acute tinnitus, which tend to

normalise). The inferior parietal cortex was implicated, in the source space analysis, in deviance detection, which exhibited diminished activity over time, and is linked to the fronto-parietal attentional network. This region corresponds to our preliminary resting-state EEG findings (Chapter 6, section 6.1.1), where longitudinal analysis indicates heightened oscillatory activity of delta oscillations in the inferior parietal lobe. Recent studies highlight the intensified focused attention on tinnitus, thereby augmenting the fronto-parietal attentional network's engagement with tinnitus and concurrently diminishing attention to external stimuli (Burton et al., 2012). Decreasing focus on exterior stimuli may result in attention being directed towards Tinnitus, hence ensuring its persistence. This is depicted in figure 7.4. Tinnitus might be generated by the novelty or salience-based mechanisms even during its precursor stages and the persistence by diverting attention towards the tinnitus over time. Tinnitus, as a consequence of an excess input-output function, may initially be detected during the precursor stage through error detection mechanisms. The anterior cingulate cortex (ACC) likely contributes to the onset of tinnitus by initiating an adaptive response—interpreted as an increased dynamic range adaptation—to stabilize the excessive input-output function and reduce prediction error. Once the brain recognizes tinnitus as a compensatory stabilizing mechanism, it may recruit networks involving the inferior parietal lobe to maintain and persist this perception over time.

A)



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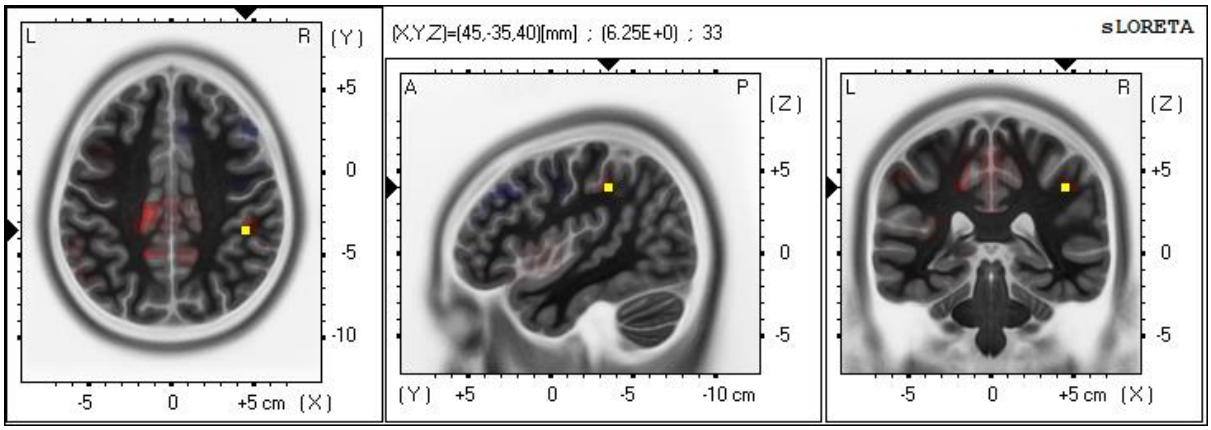

Figure 7.4: Figure 7.3: A) Relative P3 amplitude differences between Acute, Chronic, and Controls across conditions. *Significant difference noted for LFD and no significant differences noted for HFD and NS. Also to be noted that controls have increased LFD relative amplitude which is the direction considered when interpreting results for LFD* ***B) Source localized to the Inferior Parietal Lobe when comparing Acute and Post Acute Tinnitus for Low Frequency Deviance***

7.2.4 Potential role of GABA and Serotonin in tinnitus onset and persistence

Chapter 1 provided a comprehensive analysis of the potential functions of several neurotransmitters and neuromodulators in the processes of tinnitus. The current PhD does not directly quantify neurotransmitters, but rather utilizes neurophysiological measures as indirect indicators of neurotransmitters and neuromodulators: ASSR amplitude, which has been demonstrated as an indirect correlate to the alterations in GABA, and IDAEP slope, which has proved to be a measure and indirect measure of serotonergic functions. With respect to GABA and ASSR, Onitsuka et al. (2022) reports that by boosting GABAergic transmission via lorazepam, a benzodiazepine, increased the 40 Hz ASSR in the early auditory cortex. Toso et al. (2024) further discusses how boosting GABAergic transmission increases the amplitude of the 40 Hz auditory steady-state response in the early auditory cortex. Specifically, the administration of lorazepam, a GABAergic modulator, led to a significant increase in both the early transient and later sustained components of the 40 Hz ASSR compared to placebo and memantine. On the other hand, with respect to serotonin, the loudness dependence of the auditory evoked potential slope has been proposed as a non-invasive measure of central serotonergic function, with a steeper slope theorized to reflect lower serotonin activity and hypervigilance to sensory stimulation, and a shallow IDAEP slope indicates the opposite (Carrillo-de-la-Peña et al., 2006). In our case, we found reduced ASSR amplitude at low to mid-levels, and increased IDAEP slope, at the onset indicating a trend of possible reduced GABA and serotonin at the time of onset of tinnitus, and their normalisation over time. This reduction in GABA and Serotonin found in the acute stages of tinnitus, may also be a potential cause of tinnitus.

Increase central gain is a normal phenomenon post hearing loss to achieve homeostasis but at times becomes excessive. In our results some potential markers of gain such as categorical loudness scaling or cross-sectional variations in measures like ASSR and IDAEP did not show any changes. Other measures such as measures of distress, attention, tinnitus loudness, and longitudinal variations of ASSR and IDAEP tend to show changes linking to possible excess hypersensitivity such as gain. As to why the central gain becomes excessive, it could be modulated by the abnormality in neurotransmitters like GABA and serotonin.

With respect to serotonin and Tinnitus, results have been mixed. Several studies explore the relationship between serotonin and tinnitus. One perspective suggests that tinnitus may be a symptom of serotonin depletion, alongside other conditions like hypersensitivity to noise, sleep disturbances, and depression (Rauschecker et al., 2010). This hypothesis stems from the observation that these conditions frequently co-occur with tinnitus. However, the efficacy of serotonin-modulating drugs, such as selective serotonin reuptake inhibitors, in treating tinnitus has yielded mixed results, requiring further investigation with rigorous clinical trials. One study comparing melatonin and sertraline (an SSRI) found melatonin to be more effective in reducing tinnitus symptoms (Abtahi et al., 2017). Another case report documented the development of tinnitus in a patient taking a low dose of sertraline (Miller, 2016). While the exact mechanisms are unclear, it's hypothesized that serotonin may influence tinnitus through its modulation of neural plasticity in the auditory system (Simpson & Davies, 2000). Changes in serotonin levels could affect auditory filtering and habituation to tinnitus (Baguley, 2002). The decrease in slope in the IDAEP corresponds with the increase in ASSR at low to medium frequencies over time, indicating the reduction of Tinnitus, suggesting that serotonin modulation has a direct relationship with alterations in Tinnitus activity. The reason for highlighting serotonin in the generation of tinnitus is that there is a high possibility of factors like serotonergic deficiency to be present even in the precursor stages of tinnitus. Pattyn et al. (2016) emphasized that tinnitus is frequently a comorbid or simultaneously exists with anxiety and other psychological disorders, leading to significant distress and impacting quality of life. They further explore that it is this comorbid presence that enables a causal link between these conditions and acknowledging the difficulty in establishing a definitive direction of causality of whether it is tinnitus that influences anxiety or distress or whether it is the other way around. It suggests that while anxiety disorders may not directly cause tinnitus, they could influence the perception and distress associated with it. They further added that some studies potentially discuss the possibility of distress prior to the onset of tinnitus. Increased distress, closely linked to reduced serotonin, may facilitate tinnitus, and could contribute to its onset by excessively elevating central gain, as serotonin may serve as the neuromodulator that could potentially trigger the tinnitus. Although we did not directly measure serotonin or GABA levels, our electrophysiological assessments likely reflect indirect correlates of these neurotransmitter systems. The observed patterns—particularly around the time of tinnitus onset—suggest a potential reduction in both

GABAergic and serotonergic activity. These findings provide a basis for future research, where direct measurement of serotonin and GABA could help validate and expand upon this hypothesis, deepening our understanding of their roles in the onset and progression of tinnitus.

7.3 Limitations

We conducted a comprehensive investigation over the past three years to understand the neurological foundations of tinnitus onset and persistence. Despite implementing a comprehensive process to achieve beneficial results, there have been potential limitations. Firstly, in our participant sampling, we aimed to collect data both cross-sectionally—across Acute Tinnitus, Chronic Tinnitus, and Control groups—and longitudinally, following individuals from Acute to Post-Acute Tinnitus stages. However, this design may have introduced a selection bias in the Chronic Tinnitus group. Specifically, these participants tended to represent individuals with persistently distressing tinnitus and showed more pronounced asymmetries in high-frequency hearing loss compared to the Acute Tinnitus group. This suggests that chronic tinnitus in these individuals may be a consequence of progressive hearing loss and sustained tinnitus distress over time. There also appears to be a potential selection bias in the Post-Acute Tinnitus group. All participants who returned for follow-up six months after tinnitus onset showed a pattern of reduced tinnitus distress. However, only about 50% of the original Acute Tinnitus participants completed the follow-up. The majority of those who did not return failed to respond to reminder emails and phone calls, making it difficult to determine whether their tinnitus improved, worsened, or remained the same. This loss to follow-up limits our ability to draw comprehensive conclusions about the broader trajectory of their tinnitus over time. Another potential limitation of our study is the use of only two time points for assessing Acute Tinnitus. Including additional follow-up points—such as at one month and one year post-onset—could have provided a more detailed understanding of the timeline and mechanisms underlying the transition from acute to chronic tinnitus. Furthermore, within the Acute Tinnitus group, a more balanced distribution of participants across different stages of onset—such as early onset (e.g., less than 2 weeks) versus late onset (e.g., more than 2 weeks but less than 6 weeks)—would have allowed for a more nuanced analysis of when tinnitus begins to chronify. Secondly, with respect to the methodology of tests conducted, we could have adopted more broader set of questionnaires

assessing distress including questionnaires linked to general anxiety and stress and our CLS test was done in a single trial ascending run which could have been done in a more randomized trial to yield even more consistent and valid results. Thirdly, we have carried out additional tests of resting state EEG and residual inhibition which is not included as a part of this PhD due to incomplete data analysis. Lastly, a more comprehensive measurement of dynamic range adaptation (IDAEP at various stimulus dynamic range) and neurotransmitter variations could be potentially measured in future studies based on the initial analysis from this PhD. Lastly, future studies could benefit from a more comprehensive assessment of dynamic range adaptation—such as measuring the intensity-dependent auditory evoked potentials (ID-AEP) across a wider range of stimulus intensities. In addition, incorporating direct measurements of neurotransmitter variations (e.g., GABA and serotonin) would build on the initial findings of this PhD and offer deeper insight into the neurophysiological mechanisms underlying tinnitus onset and progression.

7.4 Conclusion

Our integrated tinnitus model for its generation and persistence centres on the simultaneous engagement of attentional networks, dynamic range adaptation, and central gain, facilitated by mechanisms such as increased auditory hypersensitivity and hypervigilance. These mechanisms clearly overlap. Initially, the abnormally heightened central gain resulting from hearing loss enhances attentional networks, particularly the salience network, to identify discrepancies between the aberrant central gain and the homeostatically elevated central gain. The salience network's error detection promotes the emergence of tinnitus (enhanced dynamic range adaptation) to enhance stability by serving as an invariance to central gain. Once the brain recognises that tinnitus stabilises aberrant hyperactivity, additional mechanisms involving the inferior parietal lobe, which suppresses attention to external stimuli, contributes to its persistence by enhancing focus on it over time. The physiological basis of tinnitus may differ according to individual responses, thereby influencing the level of distress experienced. Section 7.4.1 details on the progression of tinnitus over time

7.4.1 Summary Model

In conclusion, there are several facets to our interpretation of the temporal progression of tinnitus from its predisposition, through onset, to its subsequent chronification.

1. Individuals with normal hearing have a mean input-output function (slope) that reflects their typical exposure levels, which undergo a normal dynamic range adaptation process in response to environmental changes.
2. Due to peripheral auditory insults (resulting in hearing loss), input to the central auditory system is reduced, thus altering the equilibrium of the input/output function. This prompts an error detection from the anterior cingulate cortex where the overall input-output function (slope) is altered (with reduced input at lower levels) than the required input-output function potentially resulting in increased variance further triggering increased prediction error between predicted outcome (normal input-output function with appropriate dynamic range) and actual outcome (altered input-output function with reduced dynamic range). The prediction error here can be defined as a negative prediction error.

   To maintain the input/output function, the brain typically changes the slope function by increasing the rate of excitatory to inhibitory firing to achieve homeostasis. This alteration of slope in the input/output function is increased neural gain and helps achieve homeostatic plasticity and reduce the prediction error. We attribute these changes in slope function at a very local level (within the auditory pathway itself) with further top-down feedback from the higher cortical regions such as the anterior cingulate cortex.
3. During certain circumstances, the increased central gain to achieve homeostasis has a synergistic effect due to the imbalance in the neuromodulatory systems potentially triggered by either physiological stress or psychological distress. This triggers further error detection from the anterior cingulate cortex where the overall input-output function (slope) increases resulting in higher central variance than the required input-output function potentially triggering increased prediction error between predicted outcome (normal input-output function) and actual outcome (increased input-output function). We call this a positive prediction error, and this further alters dynamic range with excess input-output function across intensity levels triggering the onset of tinnitus that manifests as increased dynamic range adaptation normalizing the excess input-output function. The anterior cingulate cortex not only initiates the onset of tinnitus but also increases the sensory precision of tinnitus to promptly respond to the surplus input-output function. This increased focus triggers increased loudness to the

tinnitus, increased attention, and increased distress during the onset of tinnitus (in cases where distress occurs).

4. The onset of tinnitus instantly attenuates the excess input-output function that begins to reduce the prediction error between the predicted and actual input and beginning to normalize central variance. Over time, there is adaptation and habituation to both the input-output function and the tinnitus signal where there is a learned behaviour that it is the tinnitus that stabilizes the input-output function, hence even though there is adaptation to the tinnitus signal with the brain reducing focus attention to the tinnitus signal with reduction in the anterior cingulate cortex, the brain still propagates the tinnitus activity with the brain still directing attention allocation (sustained attention) to the tinnitus signal through areas like the inferior parietal lobe enabling its persistence.
5. There still can be long term variations to the tinnitus signal with possible influence of long-term distress that can influence the characteristics of tinnitus. But regardless, the tinnitus will persist as it helps mitigate excess gain. Figure 7.5 illustrates the slope changes across the time course of tinnitus. Figure 7.6 illustrates a hypothetical spectrum of tinnitus based on input-gain function.

Slope

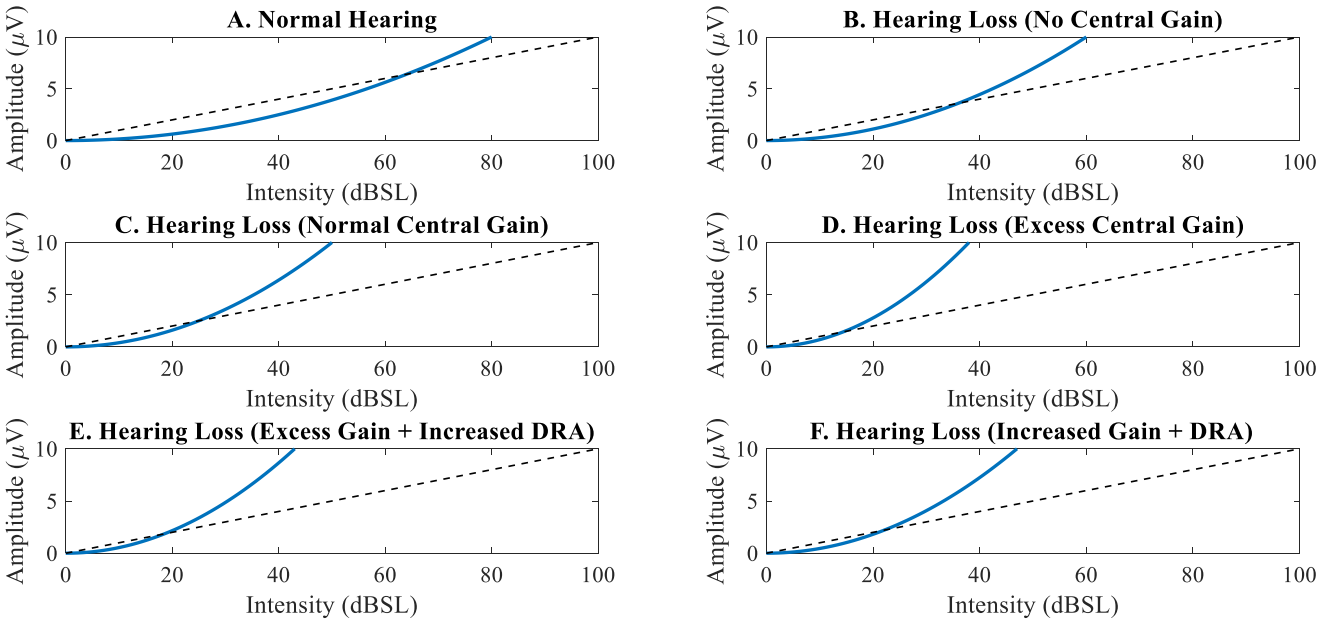

Figure 7.1: Input-output (slope) function explaining the onset and chronification of tinnitus. *The dotted line represents a standard growth function serving as a baseline reference; the x-axis denotes dBSL to account for degree of hearing loss, while the y-axis indicates neural response measured in microvolts. DRA indicates Dynamic Range Adaptation A. Normal hearing with normal slope closer to the baseline. B. Hearing loss with no central gain with altered slope due to reduced input where only the mid to high level intensities achieve absolute amplitude with no amplitude at low to mid-level intensities due to the degree of hearing loss and increased recruitment. C. Hearing loss with normal central gain function applied where input-output function is stabilized proportional to the rate of deafferentation. D. Hearing loss with excess central gain due to potential triggers caused by physiological stress or psychological distress results in abnormal increase in input-output function excess to the rate of deafferentation. E. Tinnitus is manifested as increased DRA that stabilizes the slope function from an excess increase. F. Chronic Tinnitus manifested as normal DRA persists to further stabilize the input-output function ensuring it doesn't go excess. Both Tinnitus and slope reduce over time because of adaptation and habituation.*

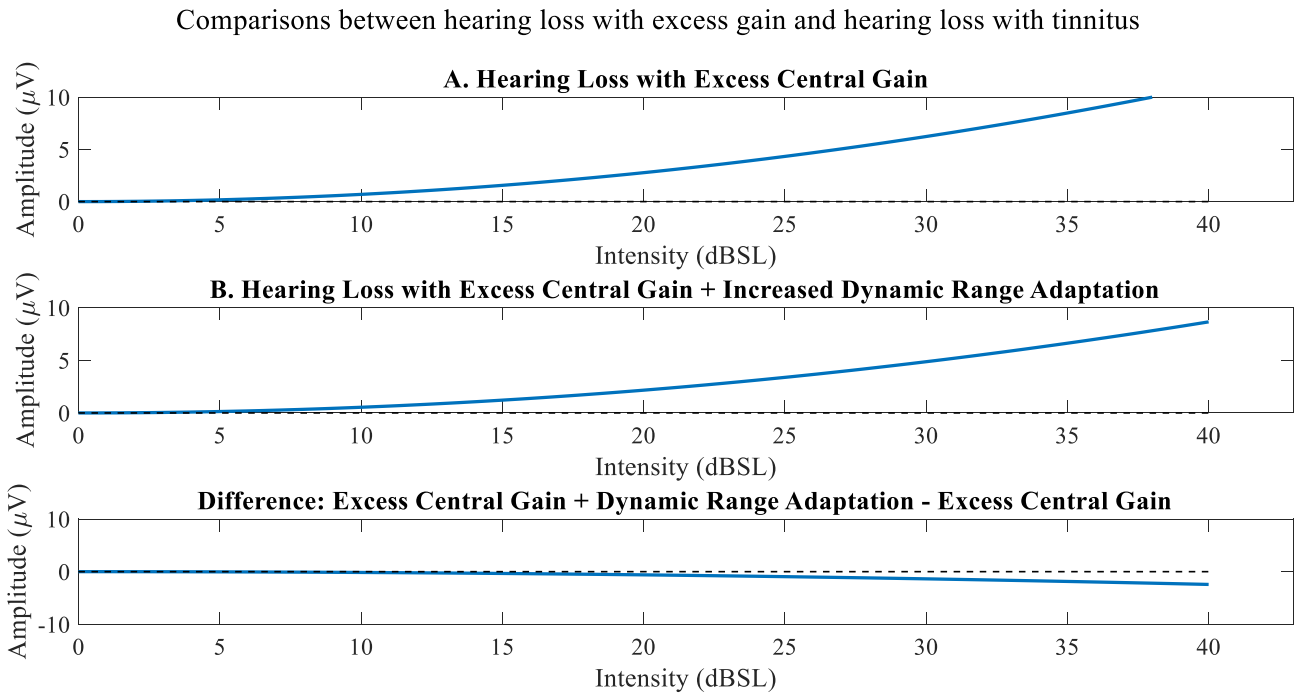

Figure 7.2: Comparisons between hearing loss with excess gain with hearing loss with tinnitus. *A. Hearing loss with excess central gain caused due to potential trigger mechanisms of physiological stress or psychological distress. B. Excess central gain and onset of tinnitus*

combined reduces the excess input-output function when compared to A. C. Differences between B and A which tentatively gives rise to the input-output function of the tinnitus alone which when visualized marginally decreases with increase in intensity highlighting the dynamic range adaptation properties of the tinnitus.

Tinnitus is not gain itself; rather, it is an factor promoting invariance to central gain that assists in regulating excessive growth of input-output function. It persists initially through various attentional networks that amplify emphasis on it, which accounts for its generation and likely be responsible for its short-to-medium term persistence. Regarding the mechanisms associated with the shift from acute to chronic tinnitus, there are no distinct acute stages of tinnitus (in cases of irreversible tinnitus resulting from excessive central gain), as the onset of tinnitus represents a chronification of tinnitus during the acute phases.

7.4.2 Responses to our research questions

Our research questions were framed based on our primary hypothesis which revolves around the fact that neural changes underlying tinnitus, principally central gain, and neural synchrony, are maximal around the time of tinnitus onset (to cross the threshold for tinnitus initiation), but later subside by way of regression to the mean. This hypothesis was framed from the sensory precision model by Sedley et al., (2016) where they state that tinnitus arises due to the changes in the predictive coding mechanisms where there is a mismatch between elevated spontaneous activity and default prediction of silence. The onset of the tinnitus is triggered by heightened sensory precision, with increased attention and gain to the aberrant signal, amplifying its perception. Over time, due to adaptation and habituation, the salience of the tinnitus signal reduces due to less weightage of sensory precision to the signal further resulting in reduction of the activity of the signal as a function of regression to the mean. Our results and interpretation are in line with the principal hypothesis through our subjective tinnitus loudness (Refer chapter 2), ASSR (Refer Chapter 4), and IDAEP (refer Chapter 5) findings where neural changes underlying tinnitus especially neural synchrony (refer Chapter 4) is maximal around the onset and subsides by way of regression to the mean. The diminishing salience of the tinnitus signal over time has been substantiated by our P300 findings (refer to Chapter 6), which indicate a decrease in novelty processing from Acute to Chronic Tinnitus, accompanied by a reduction in activity of the anterior cingulate cortex over

time. This has been further substantiated with the reduction of tinnitus distress over time too making tinnitus less intrusive during the chronic stages. The only addition to the sensory precision model (Sedley et al.,2016) is that tinnitus manifests as a mechanism to regulate/mitigate excess central gain through increased dynamic range adaptation. Further to our principal hypothesis, we posited research questions (refer chapter 1) that would help us understand the mechanisms of tinnitus and we aim to answer that through our results:

1. **What are the alterations in neural activity that are critical to initiating and driving tinnitus, and how can we set these apart from other consequences of hearing damage that are not directly relevant?** The alterations in neural activity that initiate tinnitus are excess central gain which that tends to be triggered following hearing loss, necessitating the onset of tinnitus to mitigate the excess central gain (rather than tinnitus being a direct result of increased gain). Neural reactivity following hearing loss generally tends to increase due to central gain proportional to the rate of deafferentation to achieve homeostasis. Excess neural activity such as gain that initiates tinnitus and normal increase in central gain can be set apart through variations in slope functions where excess central gain tend to have a steeper input-output function (slope). It is however essential to note that even though both excess gain and normal increase in central gain can be differentiated based on their slope, the onset of tinnitus does mitigate the excess gain and if both hyperacusis and hearing thresholds are matched it may not be yielding differences in neural activity between the two. But the increased dynamic range adaptation (stimulus dynamic range – slope function) visualized in tinnitus may not can be seen in hearing loss without tinnitus due to the property of the tinnitus to increasingly adapt to increased dynamic range and mitigate excess gain.
2. **For how long after tinnitus onset do these neural mechanisms of tinnitus remain excessively active?** Ideally neural mechanisms like central gain tend to not vary throughout the duration and course of tinnitus due to the nature of tinnitus to be an invariance to central gain where regardless of the level of input dynamic range, the gain remains unaltered. However, neural activity pertaining to tinnitus alone such as neural synchrony tend to be nonsynchronous around the time of onset due to the continuous spontaneous activity of tinnitus that reduces synchronous firing like phase

locking to the external stimulus and increases over time due to the reduction in tinnitus activity. We would state the time period of tinnitus onset to be less than 4 to 6 weeks.

3. **Conversely, are there neural processes related to tinnitus perpetuation that are absent in the acute stages but develop subsequently?** There are processes that aid in the propagation of tinnitus during the course of the chronic tinnitus. The onset of tinnitus triggers increases in salience or focussed attention due to the novelty of the stimulus. But during the chronic stages, there is likely an increased sustained attention through regions like inferior parietal lobe to the tinnitus signal to make it persist due to the regulatory nature of the tinnitus signal to excess gain. There is a learned behaviour from the onset linking tinnitus to stabilize gain and thereby warranting its presence to facilitate this over a period.
4. **Is there an early window, following onset, in which tinnitus might be more amenable to suppressive or curative treatment?** Although we did not run a treatment trial in this project, from our theoretical framework we could attribute the presence of an early window in which tinnitus might be more amenable to treatment. The Acute Tinnitus window (duration 1 – 6 months) can be aimed to provide curative or suppressive treatment however not aimed at tinnitus but aimed towards stabilizing excess gain. By directly focussing on stabilising excess neural gain, we circumvent the risk of developing a learnt response that associates tinnitus with the mitigation of gain (i.e., reducing the prediction error in relation to excess neural gain superseding the function of tinnitus).
5. **Are there Acute Tinnitus-related changes in phenomena linked to specific neurochemical processes, which might identify neurotransmitter or neuromodulator systems as potential targets for Acute or Chronic Tinnitus?** We have potentially explored GABA and serotonin to be the neurotransmitters/neuromodulators linked to the onset of tinnitus. Both were indicated to be reduced around the time of onset of tinnitus based on the slope and absolute amplitude measures of ASSR and IDAEP, indicating reduction of inhibitory neurochemicals that drive the onset of tinnitus. Both these neurochemicals could potentially play a role prior to the onset of tinnitus (precursor stages) by excessively

increasing central gain that further initiates tinnitus and both these neurochemicals can be targeted for Acute Tinnitus suppressive treatment.

These answers to the specific research questions help us propose a new integrative model for tinnitus mechanism based on tinnitus as an invariance to central gain and provides further research avenues focusing on the specific neurophysiological phenomena through which it manifests, and the likely neuromodulatory factors mechanistically underlying it.

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