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Altered Cortical Oscillations: Investigations into a Putative Neural Correlate of Tinnitus

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Abstract

Abnormal cortical oscillations have been implicated in tinnitus generation. To gain further insight into this relationship, we performed two Experimental Series, both employing behavioural, pharmacological, and *in vivo* electrophysiological techniques in an animal model. To that end, we revealed three novel findings: (1) While exposure to 250 mg/kg sodium salicylate or transient loud noise induced behavioural evidence of tinnitus, these insults caused dissimilar effects on spontaneous cortical oscillations; (2) Despite these dissimilar effects, sodium salicylate and loud noise exposure caused similar deficits in the evoked oscillatory activity elicited by the auditory steady state response; and (3) Manipulation of medial geniculate body GABAergic inhibition is sufficient to alter spontaneous cortical oscillations, but does not induce tinnitus-like behaviour. Collectively, these findings suggest that there is no clear link between altered cortical oscillations and tinnitus, and the 40 Hz ASSR might be a useful tool for assessing the presence of tinnitus in animals.

Keywords

Tinnitus; Cortical Oscillations; Auditory Cortex; Thalamocortical Dysrhythmia; Medial Geniculate Body; Thalamic Tonic Inhibition; *in vivo* Electrophysiology; Auditory Steady State Response; Operant Conditioning; Rat Model

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2AFC, Two-Alternative Forced-Choice

AC, Auditory Cortex

aCSF, Artificial Cerebrospinal Fluid

AM, Amplitude-Modulated

ASSR, Auditory Steady-State Response

CN, Cochlear Nucleus

DCN, Dorsal Cochlear Nucleus

EC_{50}, Half Maximal Effective Concentration

EEG, Electroencephalography

EP, Evoked Power

FC, Frontal Cortex

FFT, Fast Fourier Transform

fMRI, Functional Magnetic Resonance Imaging

GABA, Gamma-Aminobutyric Acid

GPIAS, Gap Pre-Pulse Inhibition of the Acoustic Startle Reflex

IC, Inferior Colliculus

IP, Intraperitoneal

IR, Infrared

ITC, Inter-Trial Coherence

LED, Light-Emitting Diode

LFP, Local Field Potential

MEG, Magnetoencephalography

MGB, Medial Geniculate Body

mRNA, Messenger Ribonucleic Acid

NBN, Narrow-Band Noise

NE, Noise Exposure

PB, Phosphate Buffer

PET, Positron Emission Tomography

RM-ANOVA, Repeated Measures Analysis of Variance

SEM, Standard Error of the Mean

SPL, Sound Pressure Level

SQ, Subcutaneous

SS, Sodium Salicylate

TC, Thalamocortical

TCD, Thalamocortical Dysrhythmia

TDT, Tucker-Davis Technologies

THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol

vMGB, Ventral Division of the Medial Geniculate Body

Chapter 1

1 Historical Review

1.1 Tinnitus Overview

Tinnitus is a condition characterized by the perception of a sound with no corresponding external acoustic source. There are two different types of tinnitus that someone may experience: objective tinnitus and subjective tinnitus (Henry, Dennis, and Schechter 2005). Objective tinnitus refers to the perception of a sound that is generated from within the body (i.e., an internal acoustic source), such as those resulting from various vascular system blockages (Hofmann et al. 2013). Alternatively, subjective tinnitus – the more common type of tinnitus – has a far more enigmatic origin, and refers to the condition in which the subject experiences a phantom sound perception (Henry et al. 2014; Eggermont and Roberts 2015). This thesis focuses exclusively on the subjective type of tinnitus, which will henceforth be referred to simply as "tinnitus."

Nearly all adults will experience tinnitus at some point in their life, likely due to short-term exposure to loud noise (e.g., music concert or sports stadium). In contrast to this transient form of tinnitus, it has been estimated that 10 – 15 % of the general adult population experiences persistent tinnitus, with 0.5 – 3 % of the population being plagued by a debilitating tinnitus at all times (Axelsson and Ringdahl 1989; Davis and El Rafaie 2000; Baguley, McFerran, and Hall 2013; Kim et al. 2015). Making matters worse are the numerous comorbidities that often accompany the chronic form of tinnitus, such as difficulty concentrating, insomnia, anxiety, and/or depression (Tyler and Baker 1983; Axelsson and Ringdahl 1989; Erlandsson and Hallberg 2000; Folmer et al. 1999; Folmer and Griest 2000; Shargorodsky, Curhan, and Farwell 2010). Despite decades of investigation into the neuropathological mechanisms underlying tinnitus, there has yet to be an effective treatment developed that has been widely accepted to suppress the phantom percept. There are, however, reports that some treatments (e.g., sound therapy, cognitive behavioural therapy) may improve the quality of life for some sufferers (Kochkin and Tyler 2008; Eggermont and Tass 2015; Allman et al. 2016). Given its high

prevalence rate in combination with its negative impact on quality of life, further research is needed into the underlying mechanisms that give rise to tinnitus, as these findings will likely underscore the development and refinement of future therapies.

1.2 Tinnitus Etiology

Historically, tinnitus was viewed as the consequence of a peripheral phenomenon; i.e., it was a condition localized to the ear (Møller 2011). This intuitive line of thinking was reinforced by the observations that (1) tinnitus is a subjective auditory percept and (2) it often occurs following trauma to peripheral auditory structures (e.g., the cochlea) (Kiang, Moxon, and Levine 1970; Jastreboff 1990). Over time, this "peripheral origin" view of tinnitus faced increasing scrutiny as contradictory findings emerged. For example, if tinnitus was in fact localized to the auditory periphery, then surgical transection of the auditory nerve (the anatomical connection that delivers acoustic signals from the cochlea to the central auditory system) should eradicate the phantom perception. In contrast to this prediction, studies found that transection of the auditory nerve did not abolish tinnitus in all participants (Fisch 1970; Pulec 1984; Gardner 1984), and in some instances, the tinnitus severity actually worsened following surgery (House and Brackmann 1981). Ultimately, over the past few decades, technological advances in neuroimaging and neural recordings in humans as well as numerous studies using electrophysiological recordings in animal models has resulted in a gradual shift toward considering tinnitus as emerging from aberrant neural mechanisms within the central nervous system (Henry et al. 2014; Eggermont and Roberts 2015).

In addition to age-related hearing loss (Podoshin, Ben-David, and Teszler 1997; Nicolas-Puel et al. 2002), tinnitus can also result from excessive exposure to high doses of ototoxic drugs [e.g., sodium salicylate (Mongan et al. 1973; Cazals 2000)] or loud noise (Loeb and Smith 1967; Atherley, Hempstock, and Noble 1968). It is generally accepted that these tinnitus-inducing insults cause a functional deafferentation of peripheral auditory structures (i.e., reduced output from the cochlea entering the central auditory pathway), which then initiates the neuropathological mechanisms that are essential for emergence and persistence of tinnitus (Henry et al. 2014). Aside from the role that functional deafferentation plays in the onset of tinnitus, the actual mechanisms

themselves – and more specifically, the changes that are critical for tinnitus generation – are poorly understood. Indeed, it has proven challenging to uncover the relationship between a tinnitus-inducing insult and the central changes required for tinnitus generation, because the changes accompanying these insults do not localize to one specific region, but instead trigger widespread aberrant activity along the entire central auditory pathway (Eggermont and Roberts 2004; Henry et al. 2014; Eggermont and Roberts 2015). Identifying the changes that are essential for tinnitus are further complicated by the fact that tinnitus onset is often accompanied by hearing loss (i.e., failure to hear relatively quiet sounds) and hyperacusis (i.e., increased sensitivity to moderately loud sounds) (Nelson and Chen 2004; Schecklmann et al. 2014), both of which could bring about confounding changes in neural activity that may be falsely interpreted as being important in tinnitus generation.

Although we have an incomplete understanding of the neural basis of tinnitus, numerous studies over the past 25+ years in both humans and animals have ultimately led to the development of a variety of putative models of tinnitus. In addition to briefly summarizing the experimental techniques used to study the aberrant neural activity associated with tinnitus, the remainder of this chapter will outline our current understanding of the possible pathophysiological mechanisms that have been proposed to generate tinnitus.

1.3 Human Studies of Tinnitus

Over the past few decades, findings from human studies have provided support to the suggestion that tinnitus arises from aberrant central mechanisms that take place following damage to the auditory periphery (i.e., peripheral deafferentation). Investigations employing neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), were instrumental in early studies as these techniques suggested that aberrant neural activity was indeed present in several auditory and non-auditory regions of the brains of tinnitus sufferers compared to healthy controls (Lanting, de Kleine, and van Dijk 2009; Adjamian, Sereda, and Hall 2009; Roberts, Husain, and Eggermont 2013). Although these neuroimaging studies were crucial in supporting the central theory of tinnitus, it has been noted that features inherent

in their associated methodology have limited the ability of these techniques to fully elucidate the neural basis of tinnitus. For example, fMRI and PET assess changes in blood flow or metabolism, respectively, and thus, these changes provide only an indirect assessment of neural activity differences between subjects (Lanting, de Kleine, and van Dijk 2009; Adjamian 2014). Furthermore, neuroimaging cannot be used to assess changes in neural activity that are temporally coordinated with changes in a subject's tinnitus severity because these techniques are restricted by low temporal resolution (Adjamian 2014). Lastly, magnetic resonance scanners used in fMRI studies generate a considerable amount of acoustic noise, which may interfere with neural activity that is important for tinnitus (Adjamian 2014).

As a complement to the numerous neuroimaging studies on tinnitus sufferers, additional studies have used neural recording techniques, such as electroencephalography (EEG) and magnetoencephalography (MEG), to compare the brain activity in subjects with or without tinnitus (Adjamian, Sereda, and Hall 2009; Eggermont and Tass 2015). The primary benefit of these neural recording techniques is that, unlike neuroimaging, it is possible to directly record ongoing cortical activity with extremely high temporal precision, thereby allowing for further exploration into the relationship between fluctuations in neural activity that correspond to fluctuations in a subject's tinnitus severity (Kahlbrock and Weisz 2008; Sedley et al. 2012). Additionally, because of the high temporal resolution and direct neural activity recordings afforded by EEG/MEG, these techniques can uncover changes in communication between different brain regions (Adjamian 2014). Further benefits of neural recordings are their minimal invasiveness (neural activity recorded from an array of sensors placed onto the scalp), minimal time commitment required from participants (typical recording times are five to ten minutes), and the fact that the equipment does not generate acoustic noise (Adjamian 2014).

The fundamental principles underlying EEG and MEG is that these experimental techniques record the ongoing electrical (EEG) or magnetic (MEG) activity that results from temporally coordinated changes in the transmembrane potentials of large neuronal populations due to synchronous synaptic input (Buzsáki, Anastassiou, and Koch 2012). This synchronous synaptic input causes the neuronal population's collective

transmembrane potentials to oscillate at several frequencies, which are widely believed to be crucial for normal brain processing (Uhlhaas et al. 2008; Buzsáki, Anastassiou, and Koch 2012). Such physiologically relevant frequencies (commonly grouped into frequency ranges or "bins") include the delta (0 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 12 Hz), and gamma (> 30 Hz) bins. Neural oscillations within the delta bin are believed to reflect a functional uncoupling between cortical regions and their thalamocortical afferents (Steriade 2006), such as during episodes of deep sleep (Gath & Bar-On 1983; Benoit et al. 2000). Theta oscillations are often investigated in the context of hippocampal activity, but are also observed in sensory cortices, and are believed to be crucial for communication between distant brain regions (Uhlhaas et al. 2008). Alpha oscillations are the human brain's most prominent resting oscillatory activity, and are widely believed to reflect the balance between excitation and inhibition within a neural region – with a high degree of alpha activity representing increased inhibition and a low degree of alpha activity representing decreased inhibition (i.e., increased excitatory activity) (Klimesch, Sauseng, and Hanslmayr 2007; Klimesch 2012; Weisz et al. 2011). Finally, gamma activity has been suggested to be crucial for short-range neural communication (e.g., intra-cortical communication) and coordinating multiple sensory stimuli into a single cognitively relevant percept (i.e., conscious awareness of stimuli) (Joliot, Ribary, and Llinás 1994; Tallon-Baudry and Bertrand 1999; Sohal 2016; Cardin 2016). It is worth noting that investigations into gamma activity have increased over the past two decades, with animal studies attempting to elucidate the neuronal mechanisms responsible for generating gamma activity (Cardin et al. 2009; Sohal et al. 2009; Carlén et al. 2011), and studies on patient populations which have shown abnormal gamma activity in various neuropathological states [e.g., schizophrenia (Uhlhaas and Singer 2010) as well as autism spectrum disorder (Simon and Wallace 2016)]. Importantly, oscillatory activity within the various frequency bins do not operate independently of one another, but instead are believed to modulate aspects of one another's activity (e.g., the phase of theta oscillations can modulate the amplitude of gamma oscillations; also known as phase-amplitude coupling), which has been suggested to underpin cognitively relevant functions (Le Van Quyen and Bragin 2007; Simon and Wallace 2016). Lastly, it is worth noting that abnormalities within each of these frequency bins have been proposed to

underlie the pathological neural mechanisms that ultimately give rise to tinnitus (Llinás et al. 1999; Llinás et al. 2005; De Ridder et al. 2015; Weisz et al. 2011), which is discussed further in section 1.6.

Cortical oscillatory activity can be investigated during periods of no external acoustic stimuli (i.e., during the "resting state", often termed *spontaneous oscillations*), or during periods of stimulus-evoked activity (often termed *evoked oscillations*). The majority of human studies investigating the relationship between aberrant cortical oscillations and tinnitus have focused on spontaneous oscillations, largely motivated by the fact that individuals afflicted with tinnitus experience the phantom percept at all times. To a lesser extent, evoked cortical oscillations have been explored in human tinnitus research, and these studies have revealed some deficits in evoked oscillations localized to the auditory cortex (Adjamian 2014). Methodologically, the auditory steady state response (ASSR) can be used to elicit cortically-evoked oscillations, whereby an acoustic stimulus (typically either many repeated clicks or sinusoidally amplitude-modulated sounds) is presented, and the extent to which the evoked response maintains its consistency over several trials can be assessed (Picton et al. 2003; Brenner et al. 2009; Uhlhaas and Singer 2010). The ASSR can be an extremely useful tool for investigating the functional abnormalities of a certain neural population (Brenner et al. 2009); however, this approach has rarely been used in tinnitus subjects. In fact, studies on tinnitus patients utilized the ASSR largely in the context of investigating cortical reorganization of the tonotopic map in tinnitus subjects (Wienbruch et al. 2006), probing for functional connectivity abnormalities between brain regions (Schlee et al. 2007; Schlee et al. 2008), or assessing changes in the evoked response amplitude when the acoustic stimulus was presented at various frequencies (Diesch et al. 2004; Diesch et al. 2010).

The ASSR is a method of evoking cortical oscillations from various regions of the brain; primarily from the auditory cortex (Spencer et al. 2009; Vohs et al. 2010; Vohs et al. 2012; Sivarao et al. 2013), but ASSRs have also been evoked from the hippocampus and parietal cortex (Sullivan et al. 2015). ASSRs do this by entraining the neural region of interest into a state of oscillatory activity that is phase-locked with the repetitive acoustic stimulus frequency (Brenner et al. 2009). For example, investigations into the functional

capacity of a neuronal circuit to support gamma activity can be explored through evoking a 40 Hz ASSR, and then assessing the ASSR through two measures: namely, the inter-trial coherence (ITC) and the evoked power (EP). The ITC is a measure of the entrained neural region's ability to maintain phase with the repetitive acoustic stimulus over multiple trials (i.e., the degree to which the neural region can become "phase-locked"), whereas the EP is the magnitude of the evoked steady-state response (Brenner et al. 2009; Roach and Mathalon 2008). Importantly, ITC and EP are complementary measurements, as ITC relies exclusively on the phase angles of the driven oscillatory response, and therefore does not take magnitude information into account (thereby allowing ITC to detect subtle, magnitude-irrelevant changes in evoked oscillatory activity), whereas EP takes both phase and magnitude information into account (Roach and Mathalon 2008). As mentioned previously, abnormal gamma activity has been observed in several different neuropathologies (Uhlhaas and Singer 2010; Simon and Wallace 2016), and has been implicated in the generation of the tinnitus percept (though this remains a contentious topic; discussed in section 1.6). Therefore, investigations into the 40 Hz ASSR abnormalities that are potentially present in tinnitus subjects could yield further insight into the neuropathological activity that underlies tinnitus generation.

Overall, the aforementioned neuroimaging and neural recording studies have revealed aberrant neural activity in the brains of tinnitus subjects; however, these human studies can do little more than establish that tinnitus subjects do indeed exhibit altered neural activity compared to their non-tinnitus counterparts. The principal shortcoming of human studies investigating tinnitus pathophysiology is their inherent need for between-subject comparisons; the subjects involved in tinnitus studies already have tinnitus and, evidently, the neuropathological changes necessary for tinnitus have already taken place. Due to the ethical conflicts of inducing tinnitus in humans, the longitudinal studies (i.e., assessing neural activity within the same subject before versus after tinnitus induction) that are needed for identifying causal relationships between altered neural activity following a tinnitus-inducing insult and the generation of tinnitus are problematic for human participants.

1.4 Uncovering the Neural Basis of Tinnitus Using Animal Models

The need to carry out longitudinal studies in order to uncover the neural changes responsible for tinnitus generation has led to the widespread use of animal models in tinnitus research. Animal models offer several advantages over human studies, such as the ability to compare a given animal's neural activity before versus after tinnitus induction. Additionally, animal models provide the experimenter with control over several variables, such as the method of tinnitus induction (e.g., exposure to high doses of ototoxic drugs or loud noise), which cannot be managed in human studies. Furthermore, animal models permit highly invasive neural recordings that allow for high spatial resolution (e.g., single neuron recordings from a specific central auditory structure) that would only be attainable in human studies under extreme circumstances (e.g., recordings from the thalamus during neurosurgery by Jeanmonod, Magnin, and Morel 1996).

Typically, the experimental techniques used to investigate neural activity in animal models are *in vivo* electrophysiological recordings using microelectrodes that can record single unit (i.e., single neuron) or multi-unit (i.e., a neuronal cluster) activity while the animal is awake (Kalappa et al. 2014; Zhang et al. 2011) or anesthetized (Noreña and Eggermont 2003; Seki and Eggermont 2003; Stolzberg et al. 2011). Additionally, these microelectrodes can be used to record local field potentials (LFP) (Stolzberg et al. 2013; Noreña et al. 2010; Berger et al. 2017), which are the changes in transmembrane potential of the local neuronal region (i.e., region surrounding the electrode) that receive synchronous, temporally coordinated synaptic input (Buzsáki, Anastassiou, and Koch 2012). Similar to EEG/MEG recordings in humans, LFP recordings allow for investigations into synchronous neural activity (i.e., spontaneous and evoked cortical oscillations) that might be altered following tinnitus induction, but with the advantage of greater spatial resolution compared to the human neural recording techniques (Buzsáki, Anastassiou, and Koch 2012).

The considerable downside to using animal models of tinnitus, however, is that they do not allow for easily attainable confirmation of tinnitus, in contrast to human studies, where psychoacoustic characterization of a subject's tinnitus (e.g., pitch and loudness of

percept) can be verbally communicated (Norena et al. 2002; Pan et al. 2009; Sereda et al. 2011; Sedley et al. 2012). This need to confirm that animals are in fact experiencing tinnitus has led to the development of numerous behavioural paradigms. Within the last quarter century, three overarching categories of behavioural paradigms for the assessment of tinnitus in animals have emerged: (1) conditioned avoidance paradigms, (2) paradigms based on inhibition of the acoustic startle reflex, and (3) two-choice operant conditioning paradigms, which are presented here in chronological order of development. While each of these behavioural paradigms are essentially indirect measures of confirming whether or not an animal is experiencing tinnitus, each successive paradigm attempts to circumvent the shortcomings of the previous paradigms, with the most robust paradigm emerging within the last five years.

1.4.1 Conditioned Avoidance Paradigms

Conditioned avoidance paradigms were the first behavioural tasks developed for assessing the presence of tinnitus in animals (Jastreboff et al. 1988). These paradigms operate on the basis that animals can be trained to associate an unpleasant stimulus (e.g., a foot shock) with a certain behaviour (e.g., licking a waterspout) and then they will stop performing that behaviour. Animals in these paradigms are trained to perform one task either more or less frequently depending on which acoustic stimulus they perceive. For example, water-deprived animals were trained to lick a waterspout in the presence of an acoustic stimulus and stop licking the waterspout in the absence of an acoustic stimulus (i.e., during quiet) (Jastreboff et al. 1988). Following a tinnitus inducing insult, such as exposure to a high doses of sodium salicylate or loud noise, animals were screened for exhibiting evidence of tinnitus based on the rate at which their conditioned behaviour would extinguish (Jastreboff et al. 1988) or based on how frequently they licked the waterspout during the different acoustic conditions (Lobarinas et al. 2004). For paradigms using the former assessment (Jastreboff et al. 1988), it was predicted that animals experiencing tinnitus would exhibit faster rates of extinction because they were experiencing the phantom percept during periods of quiet, and thus would continue licking as they were allowed to do for periods when an acoustic stimulus was present. For paradigms using the latter assessment (Heffner and Harrington 2002; Rüttiger et al. 2003;

Lobarinas et al. 2004), it was predicted that animals experiencing tinnitus would lick more or less frequently during the different acoustic conditions (e.g., acoustic stimulus present or absent) compared to animals not experiencing tinnitus.

Despite several attempts to improve upon the challenges associated with the earlier tasks (Bauer et al. 1999; Bauer and Brozoski 2001; Heffner and Harrington 2002; Rüttiger et al. 2003; Lobarinas et al. 2004), all conditioned avoidance paradigms exhibit shortcomings in being able to accurately assess evidence of tinnitus in animals. For example, some of these paradigms are unable to test for chronic tinnitus, since they assess for behavioural evidence of tinnitus based on the rate of conditioned behaviour extinction (Hayes et al. 2014; Heffner and Heffner 2012). For tests that are based on tracking increased/decreased licks during quiet periods (Heffner and Harrington 2002; Lobarinas et al. 2004; Rüttiger et al. 2003), these are susceptible to behavioural extinction since the foot shock is turned off (Hayes et al. 2014). Furthermore, these paradigms are easily affected by confounding factors that often arise from the methods of tinnitus induction, such as decreased motivation or hearing loss (Hayes et al. 2014).

1.4.2 Paradigms Based on Inhibition of the Acoustic Startle Reflex

In an attempt to overcome the challenges of the conditioned avoidance paradigms, Turner and colleagues (2006) developed a novel behavioural paradigm termed the "gap pre-pulse inhibition of the acoustic startle reflex," or GPIAS. The rationale for the GPIAS paradigm comes from observations that an animal's acoustic startle reflex – a reflexive contraction of muscles elicited by a loud noise burst – can be inhibited when the noise burst is preceded by a gap in the otherwise constant background tone (i.e., gap pre-pulse) (Koch and Schnitzler 1997). The premise for the ability of the GPIAS paradigm to detect tinnitus is that an animal that has a tinnitus percept with a pitch similar to that of the background tone will exhibit less inhibition of their acoustic startle reflex because the animal's tinnitus percept will "fill in" the pre-pulse gap (Turner et al. 2006).

While originally thought to be an improvement over the previous conditioned avoidance paradigms, proposing such advantages as high-throughput due to no training requirements and the ability to characterize an animal's tinnitus pitch (Turner et al.

2006), there has been an increase in evidence over time suggesting that the GPIAS paradigm is prone to many confounding factors. Firstly, there is evidence suggesting that hearing loss can affect an animal's ability to detect the pre-pulse gap embedded in the background tone (Deng, Lu, and Sun 2010), which could cause an animal experiencing hearing loss without tinnitus to be wrongly screened as experiencing tinnitus. Secondly, there have been inconsistencies reported between studies using GPIAS with respect to where the pitch of an animal's tinnitus lies following loud noise exposure: some studies have found the tinnitus pitch to lie above the noise exposure frequency (Wang et al. 2009; Holt et al. 2010; Turner et al. 2012), while others have found it to lie below the noise exposure frequency (Turner et al. 2006; Engineer et al. 2011). Lastly, there is evidence from both human (Campolo, Lobarinas, and Salvi 2013; Fournier and Hébert 2013) and animal (Hickox and Liberman 2014) studies suggesting that the notion of tinnitus "filling in" the pre-pulse gap is inaccurate. While the GPIAS model remains the most commonly used behavioural assessment for tinnitus employed in animal studies, largely due to its high throughput capabilities, it has been recommended that researchers be careful when interpreting the results of the GPIAS paradigm due to the aforementioned confounding factors (Allman et al. 2016).

1.4.3 Two-Choice Operant Conditioning Paradigms

The third category of behavioural paradigms for the assessment of tinnitus in animals is two-choice operant conditioning paradigms. These paradigms (Heffner and Koay 2005; Guitton and Dudai 2007; Sederholm and Swedberg 2013; Stolzberg et al. 2013) were developed to circumvent the challenges associated with the previous paradigms. Recall that in conditioned avoidance tasks, the animals are performing only one specific task either more or less frequently (e.g., lick waterspout or press lever more/less times when experiencing tinnitus), whereas in the GPIAS model, the animals are not required to do anything but startle to a noise burst. In contrast, the methodological approach that underscores the two-choice operant conditioning paradigms is to train an animal to be able to make a conscious choice when reporting what they are hearing; e.g., press the left lever for sound or the right lever for quiet. This distinction allows two-choice operant conditioning paradigms to be far more resilient to confounding factors that often

accompany tinnitus induction, such as loss of motivation, hearing loss, or hyperacusis (Hayes et al. 2014), and also allows these paradigms to obtain the most direct measure of what an animal perceives.

In 2013, Stolzberg and colleagues developed a novel two-alternative forced-choice (2AFC) operant conditioning task that was intended to provide additional resilience against the potential confounding factors associated with tinnitus induction. In contrast to the previous two-choice operant conditioning paradigms (Heffner and Koay 2005; Guitton and Dudai 2007; Sederholm and Swedberg 2013), which trained rats to consciously discriminate between two acoustic conditions – namely, the presence or absence of a continuous sound – Stolzberg et al. (2013) incorporated a third acoustic stimulus into their behavioural training regimen. This third acoustic stimulus was a sinusoidally amplitude-modulated broadband noise (referred to as *AM*), a sound that is readily distinguishable from quiet and continuous sounds. Animals were trained to go to the left feeder trough when they perceived a continuous sound and to go to the right feeder trough when they perceived either quiet or the AM stimulus (Stolzberg et al. 2013). The strength of this paradigm was that animals experiencing tinnitus would exhibit an increased proportion of quiet trials perceived as a continuous sound (as indicated by increased incorrect feeder trough selections during quiet trials), but would still select the correct feeder troughs during AM stimulus and continuous sound trials, indicating that the animal's ability to perform the task was not affected by potential confounding factors, such as hearing loss or guessing on feeder trough selection due to decreased motivation or an impaired ability to remember the task (Stolzberg et al. 2013). This two-choice operant conditioning paradigm has particular relevance for this thesis as the behavioural experiments conducted herein used a two-choice operant conditioning paradigm based on the work of Stolzberg et al. (2013).

In addition to increasing the resilience of their paradigm to potential confounds by incorporating a third acoustic stimulus into the task, Stolzberg et al. (2013) were the first to successfully couple *in vivo* electrophysiological recordings with a behavioural paradigm. This novel coupling of electrophysiological and behavioural approaches in an animal model allows for the direct investigation of neural activity within the same

animals before versus after induction of tinnitus (i.e., a longitudinal investigation) while obtaining behavioural confirmation that the animals are experiencing tinnitus. Also of importance is that the auditory cortex LFPs recorded by Stolzberg et al. (2013) was one of the very few animal model investigations into aberrant cortical oscillatory activity observed following a tinnitus-inducing insult (in this case, high dose sodium salicylate), which has implications in the role of altered cortical oscillations (and also for the thalamocortical dysrhythmia (TCD) model of tinnitus) in the generation of tinnitus, which is discussed further in the following sections.

1.5 Putative Models of Tinnitus

Over time, the collective findings from human and animal studies have resulted in the proposal of several putative models that seek to explain the underlying pathological mechanisms that are responsible for tinnitus. The organizational hierarchy that these models span is staggering; from the putative generator of tinnitus being localized to a single central auditory structure (e.g., dorsal cochlear nucleus hyperactivity; tonotopic reorganization or enhanced neural micro-synchrony within the auditory cortex), to aberrant communication *between* central auditory structures (e.g., TCD), to dysfunctional communication within entire brain regions involved in complex functions, such as attention, emotion, and memory (network models of tinnitus). However, as others have stressed (Henry et al. 2014; Eggermont and Roberts 2015), it is entirely possible that these disparate models may not be mutually exclusive, but may instead act in concert to generate tinnitus. For example, increased spontaneous firing rate and neural synchrony at the single neuron level could alter large scale neural oscillations, which may be involved in recruiting several brain regions (e.g., those involved in attention, emotion, and memory), possibly giving rise to the tinnitus percept and the negative attention and emotional components that often accompany it (Henry et al. 2014; Allman et al. 2016).

While several of these putative models have received extensive investigation (e.g., dorsal cochlear nucleus hyperactivity; auditory cortex tonotopic reorganization; central gain enhancement), largely through neural recordings performed in animal studies, others have received little investigation at the mechanistic level (e.g., TCD model of tinnitus). The following sections briefly summarize each putative model of tinnitus, with an emphasis

on the experimental findings that either support or refute each model. The final section to be discussed is the TCD model of tinnitus, as it represents the theoretical framework for the experiments conducted in this thesis.

1.5.1 Dorsal Cochlear Nucleus (DCN) Hyperactivity

The DCN hyperactivity model postulates that sensory deafferentation induces several mechanisms of aberrant plasticity that result in hyperactivity (i.e., increased spontaneous firing of neurons) within the DCN (Kaltenbach, Zhang, and Finlayson 2005). Importantly, it has been proposed that this local hyperactivity in the DCN is subsequently relayed to each successive structure in the central auditory system, ultimately giving rise to tinnitus (Kaltenbach, Zhang, and Finlayson 2005). Support for this model comes exclusively from animal studies that have found increased spontaneous firing rates of DCN neurons following ototoxic drug administration (Kaltenbach et al. 2002) or loud noise exposure (Kaltenbach et al. 1998; Kaltenbach, Zhang, and Afman 2000), as well as in animals exhibiting behavioural evidence of tinnitus (Brozoski and Bauer 2005). Despite these supportive results, there have been several findings that challenge this model, such as the observation that DCN hyperactivity typically does not appear until several days following loud noise exposure (Kaltenbach et al. 1998; Kaltenbach, Zhang, and Afman 2000), whereas the onset of tinnitus occurs almost immediately after loud noise exposure in humans (Atherley, Hempstock, and Noble 1968) and animals (Beh et al. 2016). Furthermore, lesioning the DCN does not abolish behavioural evidence of tinnitus in animals (Brozoski and Bauer 2005). Interestingly, lesioning the DCN in animals prior to loud noise exposure has been shown to prevent the onset of tinnitus (Brozoski et al. 2012), which suggests that aberrant DCN activity may be important for setting in motion the neuropathological mechanisms that underlie tinnitus percept generation, but is likely not the neural generator of tinnitus itself (Henry et al. 2014; Eggermont and Roberts 2015).

1.5.2 Tonotopic Reorganization of the Auditory Cortex

One of the consequences of restricted cochlear damage (e.g., following loud noise exposure) is a reorganization of the frequency-place map that is normally present in each

successive relay nucleus within the central auditory system (Kaltenbach, Czaja, and Kaplan 1992; Noreña, Tomita, and Eggermont 2003; Kamke, Brown, and Irvine 2003; Izquierdo et al. 2008). At the level of the auditory cortex, this tonotopic reorganization has been proposed to underlie tinnitus (Rauschecker 1999). Essentially, it has been suggested that because a portion of the auditory cortex is deprived its normal input, there is an increase in spontaneous activity in an unaffected neighboring region, and this hyperactivity ultimately manifests as tinnitus (Rauschecker 1999; Eggermont and Roberts 2004). While there have been findings of tonotopic reorganization in the auditory cortices of tinnitus subjects, (Mühlnickel et al. 1998; Wienbruch et al. 2006), there is a major shortcoming of this model: if cortical tonotopic reorganization were in fact the neural generator of tinnitus, then it would be expected that the tinnitus pitch would correspond to the preferred frequency represented in the *spared region* of the cortex. In stark contrast, the majority of psychoacoustic studies in humans have revealed that their tinnitus pitch is often located within the frequency region of their hearing loss, and not at the spared frequencies (Norena et al. 2002; Pan et al. 2009; Sereda et al. 2011; Schecklmann et al. 2012). Furthermore, this model fails to explain how people can experience a broadband tinnitus pitch or how tinnitus can emerge in the absence of hearing loss. Lastly, as was an issue with the DCN hyperactivity model, tonotopic reorganization following noise-induced hearing loss takes a minimum of a few hours to occur (Noreña, Tomita, and Eggermont 2003), whereas the tinnitus percept is present almost immediately following exposure to loud noise (Atherley, Hempstock, and Noble 1968; Beh et al. 2016).

1.5.3 Central Gain Enhancement

The central gain enhancement model proposes that aberrant homeostatic mechanisms following sensory deafferentation act to preserve the sensitivity of the central auditory pathway for relaying auditory signals from the periphery to the cortex (Noreña 2011; Auerbach, Rodrigues, and Salvi 2014). These aberrant mechanisms ultimately increase the “gain” (i.e., neural sensitivity) along the central auditory pathway, which is maximized at the level of the cortex. The proposed consequences of this enhanced gain are (1) the amplification of auditory stimuli, which results in hyperacusis, and (2) the

amplification of the "neural noise" level (i.e., spontaneous neuronal activity), which manifests as tinnitus (Noreña 2011; Auerbach, Rodrigues, and Salvi 2014). Indeed, neural recordings in animal models of tinnitus have revealed a progression of enhanced evoked responses along the central auditory pathway following a tinnitus-inducing insult: e.g., decreased auditory nerve fiber (Stypulkowski 1990) and CN (Jiang et al. 2016) evoked responses, normal or enhanced IC evoked responses (Salvi et al. 1990; Jiang et al. 2016), and increased auditory cortex evoked responses (Lu et al. 2011; Zhang et al. 2011; Laundrie and Sun 2014; Jiang et al. 2016). While the aforementioned findings do lend support for hyperacusis emerging as predicted by the central gain enhancement model, there is far less consistency in neural recordings in animals that assess the changes in spontaneous activity along the central auditory pathway following a tinnitus-inducing insult. In fact, it seems that the methods of tinnitus induction – that is, exposure to sodium salicylate or loud noise – have disparate effects on spontaneous activity along the central auditory pathway (Eggermont and Roberts 2015). For example, exposure to loud noise is generally associated with decreased spontaneous activity in the auditory nerve fibers (Liberman and Kiang 1978) and increased spontaneous activity in all other central auditory structures (Kaltenbach, Zhang, and Afman 2000; Noreña and Eggermont 2003; Ma, Hidaka, and May 2006; Mulders and Robertson 2013; Kalappa et al. 2014). Conversely, exposure to sodium salicylate causes variable effects on spontaneous activity of auditory nerve fibers [increased if high dose (Evans, Wilson, and Borerwe 1981), insignificant effect if moderate dose (Stypulkowski 1990)] and a general decrease in the spontaneous activity of the other central auditory structures (Ma, Hidaka, and May 2006; Yang et al. 2007; Wei et al. 2010; Lu et al. 2011; Zhang et al. 2011). Thus, the findings that two tinnitus-inducing methods cause directly opposing effects on the level of "neural noise" that supposedly underlies tinnitus generation is a shortcoming of this model. Additionally, as was an issue with the previously discussed putative models, changes in spontaneous activity within central auditory structures is typically not observed until hours or even days following a tinnitus-inducing insult (Kaltenbach, Zhang, and Afman 2000; Noreña and Eggermont 2003; Mulders and Robertson 2013), whereas the phantom percept emerges almost immediately after the insult.

1.5.4 Altered Neural Synchrony

A structural hierarchy of neural synchrony exists within the central nervous system (Buzsáki, Anastassiou, and Koch 2012; Eggermont and Tass 2015). For example, synchronous activity can be observed at the level of single neurons (i.e., micro-synchrony; the temporally-coordinated firing of action potentials from multiple neurons) or on a global scale (i.e., macro-synchrony; the oscillatory activity exhibited by large neural assemblies recorded with EEG/MEG). Importantly, in the context of tinnitus, alterations in neural synchrony at either of these structural levels have been implicated in generation of the tinnitus percept (Eggermont and Tass 2015). For example, support for enhanced micro-synchrony comes from electrophysiological animal studies revealing an increased cross-correlation coefficient for single unit firing in regions of the auditory cortex that are affected by exposure to loud noise (Seki and Eggermont 2003; Noreña and Eggermont 2003). The aforementioned findings have been regarded as important for tinnitus because there are findings suggesting that changes in neural synchrony are a better predictor of the presence or absence of a stimulus more so than firing rate (DeCharms and Merzenich 1996; Eggermont 1997). Based on this logic, it has been proposed that increased neural micro-synchrony may be indicative of the presence of the tinnitus percept (Seki and Eggermont 2003; Eggermont and Roberts 2004).

Support for the role of altered macro-synchrony in the underlying pathology of tinnitus comes from the multitude of EEG/MEG investigations into aberrant neural oscillations exhibited by those afflicted with tinnitus (Weisz et al. 2005; Weisz et al. 2007; Ashton et al. 2007; Kahlbrock and Weisz 2008; Lorenz et al. 2009; van der Loo et al. 2009; Ortmann et al. 2011; Adjamian et al. 2012; Sedley et al. 2012), which were motivated by the initial MEG findings of Llinás et al. (1999) and their proposal of the TCD model of tinnitus. Additional support for altered macro-synchrony in tinnitus (or specifically applying to the TCD model) comes from recent *in vitro* studies (Richardson et al. 2011; Sametsky et al. 2015) and *in vivo* neural recordings in animal models (Stolzberg et al. 2013; Kalappa et al. 2014; Berger et al. 2017), all of which are discussed further in section 1.6.

1.5.5 Network Models

Network models of tinnitus emerged from human neuroimaging studies, which revealed aberrant neural activity in several auditory and non-auditory regions of the brain of tinnitus subjects (Lanting, de Kleine, and van Dijk 2009; Adjamian, Sereda, and Hall 2009; Husain and Schmidt 2014). Generally, the network models postulate that generation of the tinnitus percept arises from neuropathological mechanisms within central auditory structures (as is common to other models), but that the conscious awareness of the tinnitus percept is a result of aberrant activity involving several brain regions (i.e., aberrant neural networks), which are also responsible for the negative attention and emotional components that often accompany tinnitus (Leaver et al. 2011; De Ridder et al. 2011; De Ridder et al. 2014; Rauschecker et al. 2015).

1.6 Thalamocortical Dysrhythmia (TCD)

The thalamocortical dysrhythmia (TCD) model was proposed to explain the underlying mechanisms that give rise to several different neuropathologies, including Parkinson's, depression, neurogenic pain, and tinnitus (Llinás et al. 1999). Focusing on this model's relevance for tinnitus (i.e., the TCD model of tinnitus), Llinás et al. (1999) proposed that peripheral deafferentation leads to increased tonic inhibition in the medial geniculate body (MGB; the putative auditory gating center of the thalamus). Tonic inhibition in the MGB causes prolonged hyperpolarization of the resting membrane potentials of the thalamocortical (TC) relay cells – the sensory relay neurons of the MGB that project to the auditory cortex. This prolonged hyperpolarization of TC relay cells causes deinactivation of voltage-gated T-Type Ca^{2+} channels, which triggers a shift in TC relay cell firing type from the regular tonic mode of firing to a burst-like mode of firing. Importantly, this TC relay cell burst firing is proposed to occur within the delta-theta frequency range (i.e., < 8 Hz) (Jeanmonod, Magnin, and Morel 1996; Llinás et al. 1999; Llinás et al. 2005), which is detected as increased delta-theta activity in the spontaneous oscillatory profiles of tinnitus subjects (Llinás et al. 1999). The functional consequence of TC relay cell burst firing at the level of the auditory cortex is increased inhibition in the focal region that receives the aberrant TC relay cell input and decreased lateral inhibition of the area surrounding the inhibited cortical region (detected as increased gamma

activity in the spontaneous oscillatory profile). Ultimately, the model proposes that these disinhibited regions of the auditory cortex, resulting from dysrhythmic thalamocortical activity, give rise to the tinnitus percept (Llinás et al. 1999; Llinás et al. 2005).

There were two seminal findings that led to the proposal of this model: The first finding was from a study performing single unit recordings during medial thalamotomies of neuropathology-afflicted patients (Jeanmonod, Magnin, and Morel 1996). The researchers found that 99 % of the single units did not respond to sensory stimuli (indicating peripheral deafferentation), and approximately 45 % of these single units were firing in bursts at a frequency of ~ 4 Hz (i.e., within the delta-theta range) (Jeanmonod, Magnin, and Morel 1996). The second finding was the MEG results obtained by Llinás et al. (1999), which showed that neuropathology-afflicted patients exhibited a shift in profile of their oscillatory spectrum: the peak of the spectrum had shifted from the normal alpha range that was observed in control subjects to the theta range, which the authors interpreted as evidence for increased thalamic burst firing onto cortical regions.

Since its conception in the late 1990s, the TCD model of tinnitus has received support from human studies employing EEG/MEG recordings. Indeed, there have been several studies that provide evidence of the neural oscillatory correlates of tinnitus proposed by the TCD model; i.e., increased delta-theta activity (Llinás et al. 1999; Weisz et al. 2005; Adjamian et al. 2012), decreased alpha activity (Weisz et al. 2005; Lorenz et al. 2009), and increased gamma activity (Weisz, Dohrmann, and Elbert 2007; Ashton et al. 2007; Lorenz et al. 2009; Ortmann et al. 2011). There has also been support for the positive correlation between gamma activity and tinnitus severity (van der Loo et al. 2009). Additionally, an MEG study found that patients exhibiting residual inhibition (a method that involves playing an external tone that matches a tinnitus sufferer's pitch and then turning off the tone, which results in a transient decrease in tinnitus loudness) demonstrated a correlation between decreased delta activity and decreased tinnitus severity (Kahlbrock and Weisz 2008). Another MEG study using residual inhibition found that tinnitus sufferers demonstrated a positive correlation between increased activity in the delta-theta and gamma frequencies and tinnitus severity (Sedley et al. 2012). Interestingly, the same study (Sedley et al. 2012) found, contrary to what was

predicted, that tinnitus sufferers exhibiting residual excitation (opposite to residual inhibition, such that their tinnitus severity *increased* once the external tone was shut off) showed a *decrease* in gamma activity while reporting that their tinnitus severity had increased. This important finding calls into question the direct correlation between changes in gamma activity and tinnitus (Sedley et al. 2012). Nevertheless, approximately two decades of EEG/MEG recordings have provided some support for the TCD model of tinnitus as well as the broader relationship between the presence of aberrant neural oscillations and tinnitus.

While the investigation of aberrant cortical oscillations and tinnitus (with implications for the TCD model of tinnitus) have almost exclusively been carried out in human neural recording studies, more recent *in vitro* and *in vivo* electrophysiological recordings in animal models have provided new insight into this long-standing putative model. Recently, patch-clamp recordings have shown that TC relay cells from the rat MGB can be triggered to shift their primary mode of firing from tonic to bursting when they are tonically inhibited (thereby causing a hyperpolarization of the resting membrane potential) through selective activation of extrasynaptic, δ-subunit containing $GABA_A$ receptors (Sametsky et al. 2015). $GABA_A$ receptors that contain the δ-subunit in place of the γ-subunit are preferentially expressed at extrasynaptic locations within the TC relay cell membrane (Farrant and Nusser 2005; Belelli et al. 2009). The presence of the δ-subunit causes these extrasynaptic $GABA_A$ receptors to exhibit enhanced GABA affinity as well as increased duration of channel opening, both of which contribute to tonic Cl^- influx and, consequently, prolonged hyperpolarization of the TC relay cell resting membrane potential (Cope, Hughes, and Crunelli 2005; Belelli et al. 2009). In the same study (Sametsky et al. 2015), researchers found elevated levels of mRNA for δ -subunit containing $GABA_A$ receptors in the MGB of animals that were exhibiting behavioural evidence of tinnitus following exposure to loud noise, suggesting that inhibition within the MGB might be increased in tinnitus. Furthermore, microelectrode recordings from the MGBs of noise-exposed rats revealed that rats exhibiting behavioural evidence of tinnitus had an increased rate of thalamic burst firing compared to control rats (Kalappa et al. 2014). While the utility of animal models provides the ability for a mechanistic approach

of investigating the putative models of tinnitus, such a direct investigation has yet to be taken for the TCD model of tinnitus. Therefore, as described in detail below, the over-arching focus of this thesis was to conduct a mechanistic approach to investigating the TCD model of tinnitus, as well as to gain further insight into the relationship between altered cortical oscillations and tinnitus in general.

1.7 Thesis Objectives & Hypotheses

Over the last few decades, human studies employing neuroimaging and neural recording techniques have revealed that there is widespread aberrant neural activity exhibited by those who are afflicted with tinnitus. Several of these studies have investigated the tinnitus-related abnormalities in large-scale neural synchrony (i.e., cortical oscillatory activity) through EEG and MEG recordings (Adjamian 2014; Eggermont and Tass 2015). Unfortunately, experiments using human subjects can do little to uncover the causal relationship between altered cortical oscillations and tinnitus, since these studies rarely allow for longitudinal investigations (i.e., comparing a subject's oscillatory activity before versus after tinnitus induction). Consequently, animal models of tinnitus have been widely used over the last two decades, as they allow for both longitudinal and invasive investigations. Additionally, given the high spatial resolution permitted by electrophysiological recordings in animal models, the ability to control for the method of tinnitus induction, and the capacity to manipulate excitatory/inhibitory activity within specific central auditory structures, animal models permit a suitable approach for investigating the putative mechanisms that have been proposed to underlie tinnitus generation.

Despite the experimental conditions afforded by animal models, there have been very few studies that have investigated the relationship between altered cortical oscillations and tinnitus (Noreña et al. 2010; Stolzberg et al. 2013; Berger et al. 2017) and, to our knowledge, there have been no direct investigations of the TCD model of tinnitus. Collectively, the aforementioned studies have found altered spontaneous oscillatory activity from the auditory cortex following induction of tinnitus through exposure to loud noise (Noreña et al. 2010) or high doses of sodium salicylate (Noreña et al. 2010; Stolzberg et al. 2013; Berger et al. 2017). However, the findings between studies are not

consistent with one another. For example, compared to the relevant controls, Stolzberg et al. (2013) found decreased theta, decreased alpha, and increased gamma activity; Berger et al. (2017) found decreased alpha activity; and Noreña et al. (2010) found a general decrease in ~ 10 – 30 Hz activity following either exposure to loud noise or a high dose of sodium salicylate. The low number of studies combined with the inconsistent findings among them provides a strong rationale for further investigation into the relationship between aberrant cortical oscillations and tinnitus using animal models.

Beyond the possible alterations in spontaneous oscillatory activity associated with tinnitus, the use of animal models for exploring potential evoked oscillation deficits following tinnitus induction has yet to be performed. As discussed above, employing the 40 Hz ASSR could uncover abnormalities in a cortical region's ability to maintain stimulus-driven oscillatory activity, which could have implications for understanding the neural basis of tinnitus.

The overarching hypothesis of this thesis was that altered cortical oscillations underlie the presence of tinnitus. Thus, the overall objective of this work was to gain further insight into the relationship between altered cortical oscillations and tinnitus. To achieve this objective, two Experimental Series – both capitalizing on the behavioural and *in vivo* electrophysiological techniques permitted by the use of animal models – were performed.

1.7.1 Experimental Series 1

The objective of Experimental Series 1 was to investigate the changes in cortical oscillations following exposure to either a high dose (250 mg/kg, i.p.) of sodium salicylate (SS) or a single bout of loud noise (acute NE). Importantly, both of these treatments are known to induce tinnitus in humans and animals. Support for altered cortical oscillations underlying tinnitus comes from the neural recording studies carried out in tinnitus subjects (Llinás et al. 1999; Weisz et al. 2005; Weisz et al. 2007; Ashton et al. 2007; Lorenz et al. 2009; Ortmann et al. 2011), as well as the findings in previous studies that exposed animals to tinnitus-inducing insults (Noreña et al. 2010; Stolzberg et al. 2013; Berger et al. 2017). To carry out the objective of this Experimental Series,

behavioural and *in vivo* electrophysiological techniques were performed in awake, freely-moving rats.

Experimental Series 1 addressed three Specific Aims:

1. **Confirm that the SS (250 mg/kg, i.p.) and acute NE treatments cause behavioural evidence of tinnitus in rats and that the control (sham) conditions do not affect behavioural performance using a two-choice operant conditioning paradigm.** Based on the overwhelming support from both human and animal studies, it was predicted that administration of either the SS (250 mg/kg, i.p.) or acute NE treatments would induce behavioural evidence of tinnitus. Furthermore, it was predicted that the behavioural paradigm would be resistant to false-positives, and as such, animals would not show tinnitus-like behaviour during the control conditions for the SS (250 mg/kg, i.p.) and acute NE treatments (i.e., saline and sham NE, respectively).

2. **Investigate the alterations in spontaneous oscillatory activity from the auditory and frontal cortices (AC and FC, respectively) following either SS (250 mg/kg, i.p.) or acute NE treatments.** Based on the evidence from human and animal studies showing altered oscillatory activity associated with tinnitus, it was predicted that both SS (250 mg/kg, i.p.) and acute NE treatments would result in increased delta-theta, decreased alpha, and increased gamma activity of the spontaneous AC oscillations, compared to the control conditions (i.e., saline vs SS; sham NE vs acute NE). Additionally, because the SS (250 mg/kg, i.p.) and acute NE treatments are systemic treatments (i.e., effects not localized to a specific neural region), it was predicted that alterations in FC spontaneous oscillatory activity would be similar to that of the AC.

3. **Investigate the alterations in evoked oscillatory activity from AC and FC, through implementation of the 40 Hz ASSR, following either SS (250 mg/kg, i.p.) or acute NE treatments.** Based on the support for the relationship between enhanced neural synchrony at the micro/macro-synchronous scale and tinnitus, as

well as the proposal that enhanced gamma activity might underlie generation of the tinnitus percept, it was predicted that both ITC and EP of the AC 40 Hz ASSR would be increased following treatment with either SS (250 mg/kg, i.p.) or acute NE, compared to their relative control conditions. In line with the predictions made for Specific Aim 2, differences in the ITC and EP of the FC 40 Hz ASSR following treatment with either SS (250 mg/kg, i.p.) or acute NE were predicted to be similar to that of the AC.

1.7.2 Experimental Series 2

The objective of Experimental Series 2 was to perform the first mechanistic investigation of the TCD model of tinnitus: a long-standing theory that posits dysrhythmic thalamocortical activity, resulting from enhanced tonic inhibition of the MGB, to be the neural basis of tinnitus (Llinás et al. 1999; Llinás et al. 2005). In line with this model, the hypothesis of this Experimental Series was that increased thalamic tonic inhibition results in thalamocortical dysrhythmia, which is responsible for the tinnitus percept. Over the past two decades, support for the TCD model of tinnitus has mounted from human EEG/MEG studies revealing aberrant neural oscillations exhibited by tinnitus subjects that are in line with the proposed oscillatory correlates of TCD; i.e., increased delta-theta (Llinás et al. 1999; Weisz et al. 2005; Adjamian et al. 2012), decreased alpha (Weisz et al. 2005; Lorenz et al. 2009), and increased gamma activity (Weisz et al. 2007; Ashton et al. 2007; Lorenz et al. 2009; Ortmann et al. 2011). Importantly, recent investigations into the relationship between altered cortical oscillations and tinnitus using electrophysiological recording techniques in animals have given some support for the TCD model of tinnitus. For example, animals exposed to high-doses of sodium salicylate, a known tinnitus-inducing agent, have exhibited decreased alpha activity (Berger et al. 2017; Stolzberg et al. 2013) and increased gamma activity (Stolzberg et al. 2013). Additionally, enhanced MGB burst firing activity has been reported in animals exhibiting behavioural evidence of tinnitus following exposure to loud noise (Kalappa et al. 2014). Furthermore, recent *in vitro* experiments have revealed a high density of δ-subunit-containing $GABA_A$ receptors (Richardson et al. 2011) within the rat MGB, which, when selectively targeted by the drug THIP, triggers a shift from the regular tonic mode of

firing to an aberrant burst mode of firing (Sametsky et al. 2015), similar to that described in the TCD model of tinnitus. Collectively, the aforementioned findings support different aspects of the TCD model; however, the mechanisms proposed by this model have yet to be explicitly tested. Therefore, the objective of Experimental Series 2 was to perform the first direct investigation of the TCD model of tinnitus using behavioural, *in vivo* electrophysiological, and local thalamic drug infusion approaches in awake, freely-moving rats.

Experimental Series 2 addressed three Specific Aims:

1. **Investigate the alterations in spontaneous oscillatory activity from AC and FC following the increase or decrease in local MGB inhibition.** Based on the TCD model of tinnitus, enhanced tonic inhibition of the MGB via local infusion of THIP, a preferential δ-subunit-containing $GABA_A$ receptor agonist, was predicted to cause TCD-like spontaneous oscillations (i.e., increased delta-theta, decreased alpha, increased gamma activity) in the AC in a dose-dependent manner (i.e., 100 μM THIP effects > 50 μM THIP effects) compared to the aCSF control condition. In contrast, decreased inhibition of the MGB via local infusion of 50 μM gabazine, a potent $GABA_A$ receptor antagonist, was predicted to cause the inverse of the TCD-like oscillations (i.e., decreased delta-theta, increased alpha, decreased gamma activity) in the AC compared to the aCSF control condition. Importantly, since the local drug infusions target the ventral division of the MGB (vMGB), which projects almost exclusively to the primary auditory cortex (Winer et al. 1999; Smith et al. 2012), the FC spontaneous oscillations were predicted to remain unchanged between the various thalamic infusions.

2. **Investigate the alterations in evoked oscillatory activity from AC and FC, through implementation of the 40 Hz ASSR, following the increase or decrease in local MGB inhibition.** In line with the TCD model's prediction that increased thalamic tonic inhibition induces tinnitus, and the belief that increased gamma activity and increased neural synchrony are closely associated with tinnitus, it was predicted that local MGB infusion of THIP would increase the ITC

and EP of the AC 40 Hz ASSR in a dose-dependent manner, as compared to the aCSF control condition. In contrast, local MGB infusion of 50 μM gabazine was predicted to decrease the ITC and EP of the AC 40 Hz ASSR, as compared to aCSF. In line with the predictions from Specific Aim 1, it was predicted that ITC and EP of the FC 40 Hz ASSR would not differ between the various thalamic infusions, since the AC, and not the FC, receives the majority of output from the vMGB.

3. **Determine whether the local increase or decrease in MGB inhibition is sufficient to cause behavioural evidence of tinnitus in rats using a two-choice operant conditioning paradigm.** Consistent with the TCD model of tinnitus, local MGB infusion of THIP was predicted to induce behavioural evidence of tinnitus. In contrast, local MGB infusion of either 50 μM gabazine or aCSF was not expected to cause behavioural evidence of tinnitus (i.e., rats would exhibit a normal behavioural profile following infusion).

Chapter 2

2 Materials & Methods

The studies performed in this thesis project included two Experimental Series, both of which contained behavioural and *in vivo* electrophysiological experiments using adult male Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA, USA). Experimental Series 1A-Behaviour used 8 rats; Experimental Series 1B-Electrophysiology used 11 rats; Experimental Series 2A-Electrophysiology used 13 rats; and Experimental Series 2B-Behaviour used the same 8 rats from Experimental Series 1A. All rats were housed on a 12-h light-dark cycle and were given food and water *ad libitum* unless otherwise stated. All experimental procedures were approved by the University of Western Ontario Animal Care and Use Committee, and were in accordance with the guidelines established by the Canadian Council of Animal Care. All necessary measures were taken to reduce animal number and to minimize the pain and suffering of the animals.

2.1 Experimental Series 1

The first Experimental Series was an investigation into the behavioural (Series 1A) and electrophysiological (Series 1B) consequences of exposure to two known tinnitus-inducing insults: (1) a high dose (250 mg/kg, i.p.) of sodium salicylate (SS) and (2) a single bout of loud noise (acute NE). In Experimental Series 1, the behavioural experiments (n = 8 rats) preceded the electrophysiological experiments that were conducted on a separate group of rats (n = 11) so that we could be certain that the specific treatment parameters (e.g., dose and timing of SS; loudness and duration of acute NE) were indeed capable of inducing behavioural evidence of tinnitus prior to comparing the associated electrophysiological changes caused by these treatments.

2.1.1 Experimental Series 1A – Behaviour

Behavioural Apparatus

Behavioural training and test sessions (described in detail, below) were conducted using a

standard modular operant conditioning chamber (ENV-008CT; Med Associates, Inc., St. Albans, VT, USA), located inside of a sound-attenuating box (29" L x 23.5" D x 23.5" H; ENV-017M; Med Associates, Inc.), which was further housed inside of a single-walled, walk-in sound-attenuating booth (SE 2000 Series; WhisperRoom Inc., Knoxville, TN, USA). At the front of the behavioural test chamber, there was a center nose poke and a feeder trough positioned on either side; each containing infrared (IR) detectors. There were two lights present in the test chamber: an LED (ENV-229M; Med Associates, Inc.) located on the front wall above the nose poke, which served as a "go" cue (see Behavioural Training Sessions below), and a house light located at the top of the rear wall, which remained on at all times. Acoustic stimuli were delivered via a ceiling-mounted speaker (FT28D Dome Tweeter; Fostex, Tokyo, Japan) located near the front of the test chamber. The intensity of the acoustic stimuli was calibrated with custom Matlab software (MathWorks Inc., Natick, MA, USA) using a 1/4" microphone (2530; Larson Davis, Depew, NY, USA Larson Davis) and a preamplifier (2221; Larson Davis). A webcam (LifeCam Cinema HD; Microsoft, Redmond, WA, USA) was mounted on the rear wall to permit constant monitoring of the rat's behaviour during training and test sessions. Stimulus delivery, nose poke responses, "go" cue light triggering, feeder trough selection, and positive/negative reinforcement were all controlled and monitored using custom Matlab behavioural protocols running in Matlab (EPsych Toolbox; dstolz.github.io/epsych/), which was interfaced with real-time processing hardware (RZ6; Tucker-Davis Technologies, Alachua, FL, USA).

Acoustic Stimuli

During both training and testing sessions, rats were exposed to three different types of acoustic conditions within the behavioural test chamber: (1) quiet (speaker off), (2) amplitude modulated noise (AM; 1 – 32 kHz broadband noise with a 100% modulation depth occurring at 5 Hz), and (3) narrow-band noise (NBN; $1/8^{th}$ octave band centered at 8, 12, 16, 20 or 24 kHz; five NBN stimuli total). The speaker was calibrated to deliver AM and NBN stimuli at 75 dB SPL. One of these acoustic conditions was always present in the test chamber, and the type of stimulus being presented would only change following completion of a given trial (i.e., after the rat had selected either the left or right

feeder trough following a nose poke into the center port). The order of presentation for the different acoustic stimuli was randomized to achieve an approximate proportion of 20 % quiet trials, 30 % AM trials, and 50 % NBN trials for all training and testing sessions.

Behavioural Training Sessions

Between one and two weeks following their arrival, male Sprague-Dawley rats (n = 8; received at 50 - 60 days old) were placed on a food-restricted diet to maintain approximately 85 % of their free feeding body mass. Two days following food restriction onset, rats began habituating to the operant chamber for 30 min/day. During this habituation period, spontaneous nose pokes into the center port (as detected with the IR beam) triggered (1) the "go" cue (i.e., transient illumination of the LED light above the nose poke), (2) a change in acoustic stimulus being presented (for the habituation period, only quiet or 16 kHz NBN stimuli were possible), and (3) dispensing of a 45 mg food pellet (Bio-Serv, Frenchtown, NJ, USA) into the associated feeder trough (i.e., left feeder trough for NBN trials; right feeder trough for quiet trials). If the rat selected the correct feeder trough (as detected with the IR beam), a second food pellet was dispensed into that trough to reinforce the association between the particular feeder trough and a specific acoustic stimulus. Once the rats had associated nose pokes with selecting a feeder trough for a food pellet reinforcement (typically within two to three days), the initial dispensing of a food pellet for spontaneous nose pokes ceased, and the rats were now only rewarded with a single food pellet for selecting the correct feeder trough.

During each 30 min/day training session, behaviour continued to be reinforced for selecting the correct feeder trough and punished for selecting the incorrect feeder trough. Punishment consisted of a 15 s timeout period in which the center port would not register any nose poke attempts. Once rats achieved ≥ 90 % correct trials for at least two consecutive days, additional acoustic stimuli were added to the task, starting with the addition of the four other NBN stimuli (centered at 8, 12, 20 and 24 kHz). Following ≥ 90 % correct trials for at least five consecutive days, the AM stimulus was added to the task, and the rats now had to discriminate quiet and AM stimuli (right trough) from the five NBN stimuli (left trough). During successive training sessions, there was also a gradual

increase (~ 100 ms/day) in nose poke holding time required to trigger the "go" cue, until a max of 2000 – 3000 ms was reached, as well as a gradual reduction in reward rate for correct trials from 100 % to 70 %. From our observations and those of our colleagues (Stolzberg et al. 2013), reducing the reward rate seemed to enhance performance on the task. Rats underwent behavioural training for 30 min/day, 6 days/week, for approximately four months until they were able to achieve ≥ 90 % correct trials for all acoustic stimuli, at which time they were ready to commence the interspersed testing sessions.

Behavioural Testing Sessions

Behavioural testing sessions differed from training sessions in three ways: (1) there were no rewards or punishments for feeder trough selection on quiet trials so as to limit the likelihood of tinnitus-like behaviour being confounded by food-motivated biases; (2) the reward rate for correct AM and NBN trials was increased to 90% to compensate for the lack of food pellet rewards that would be given for correct quiet trials during training sessions, and; (3) rats performed a maximum of 130 trials (typically 20 – 25 min) to reduce the possibility of extinguishing the originally-learned quiet trial behaviour. The time course for the interspersed testing sessions was consistent for all treatments: rats were administered a given treatment, followed by a waiting period (which differed according to the actual treatment; see below), and then they performed the testing session. The following day, rats performed another testing session in the absence of a treatment. Subsequently, there was a minimum of two regular training sessions before rats performed the next testing session associated with a different treatment.

All rats (n = 8) received the same treatments – each treatment separated by a minimum of 4 days – in the following order: (1) saline (equivalent volume to the SS treatment, i.p.), (2) SS dissolved in saline (250 mg/kg, i.p.; Sigma-Aldrich, St. Louis, MO, USA), (3) sham NE, and (4) acute NE. For the saline and SS (250 mg/kg, i.p.) treatments, rats began their testing sessions two hours post-injection. The experimental parameters chosen for these treatments were based on findings of peak electrophysiological and/or tinnitus-related behavioural effects resulting from SS administration in rodents at similar dosages

and times post-injection (Stolzberg et al. 2013; Jiang et al. 2016; Yang et al. 2007). For the sham NE and acute NE treatments, rats were placed inside of a noise exposure box, which consisted of a standard rat home cage equipped with a ceiling-mounted speaker (T90A Horn Tweeter; Fostex), that was housed inside of a sound-attenuating box (ENV-022MD; Med Associates, Inc.). For the sham NE, rats remained inside of the noise exposure box for 15 min in the absence of acoustic stimuli. For the acute NE, rats were subjected to a 12 kHz tone presented at 112 dB SPL for 15 min. The intensity of the acute NE tone was calibrated as described above. These acute NE parameters were selected based on previous work from our lab demonstrating that they were sufficient for inducing behavioural evidence of tinnitus in rats (Beh et al. 2016). Rats that received either the sham NE or acute NE treatments began their testing sessions 10 min after removal from the noise exposure box in order to remain consistent with the electrophysiological recordings (see section 2.1.2).

Data Analysis

For each rat, its performance on the quiet, AM, and NBN trials was calculated as the proportion of trials identified as NBN (i.e., the proportion of left feeder trough selections) for (1) the day before a given treatment (*Pre*), (2) the day of a given treatment (*Test*), and (3) the day after a given treatment (*Post*). Next, the results of all rats in a given treatment group were plotted as group mean ± SEM for all treatments. Consistent with previous studies (Stolzberg et al. 2013), behavioural evidence of tinnitus was assessed on the basis of a treatment's ability to increase the proportion of quiet trials incorrectly identified as NBN (i.e., the rat mistakenly perceived the quiet condition as though a steady sound was present; findings consistent with the presence of tinnitus). Statistical comparisons were decided *a priori* and were conducted as follows: For each treatment and its respective control condition (e.g., SS (250 mg/kg, i.p.) vs saline; acute NE vs sham NE), a two-way repeated measures analysis of variance (RM-ANOVA) was conducted for time x treatment. If a significant main effect or a significant interaction of time x treatment ($\alpha = 0.05$) was found, a *post hoc* paired-samples, two-tailed t test was used to further determine if there were significant differences between treatments on a given day (i.e., *Pre*, *Test*, *Post*) or between *Pre* and *Test* of a given treatment. Since there were five *post*

hoc comparisons being made, the Bonferroni-corrected significance level was set at $\alpha = 0.01$. All statistical analyses were performed using SPSS Statistics 24 (IBM; Armonk, NY, USA) and figures were generated in Matlab 2012 (MathWorks Inc., Natick, MA, USA) or GraphPad Prism 6 (GraphPad; La Jolla, CA, USA).

2.1.2 Experimental Series 1B – Electrophysiology

Surgical Procedure

A separate group of naïve, adult male Sprague-Dawley rats (350 – 470 g, n = 11) than those tested in Experimental Series 1A were placed into a gas anesthesia induction chamber, and anesthetized with isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane; 4 % mixed with pure O_2, 2 L/min; Forane; Baxter Corporation, Mississauga, ON, Canada). Following loss of consciousness, the rat was removed from the induction chamber, and fixed into a stereotaxic frame with blunt ear bars. A surgical level of anesthesia, as assessed by the absence of a pedal withdrawal reflex, was maintained throughout the surgery with isoflurane (1.5 – 2 % mixed with pure O_2, 2 L/min) administered through a nose cone that was fixed to the stereotaxic frame. An electronic heating pad (Homeothermic Monitor; Harvard Apparatus, Holliston, MA, USA) was used to maintain the rat's body temperature within 36.5 °C – 37.5 °C. The rat's head was shaved, and then cleaned with antimicrobial soap, followed by 70% isopropyl alcohol, and then betadine solution (Purdue Products L.P., Stamford, CT, USA). Eye lubricant was applied, and the rat was given the analgesic agent Metacam (1 mg/kg, s.c.; Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany). A midline incision was made along the dorsal aspect of the skull, cutting through the skin of the scalp. Fascia was removed from the dorsal surface of the skull, revealing the skull's sutures, and the left temporalis muscle was resected from the lateral aspect of the skull. Three epidural screw electrodes (E363-20; PlasticsOne Inc., Roanoke, VA, USA) were used to record local field potentials (LFP), and were implanted at (1) 2.0 mm rostral and 2.0 mm left of bregma (over left frontal cortex), (2) -4.3 mm caudal to bregma and -4.5 mm ventral to the dorsal skull surface on the left lateral aspect of the skull (over auditory cortex), and (3) -2.0 mm caudal to lambda (over cerebellum; to serve as the reference and ground electrode) (Paxinos and Watson 2007). The gold pins from the three electrode

wires were fed into a 6-pin pedestal (MS363; PlasticsOne Inc.), and dental cement was placed around the exposed regions of the skull to secure the implants and pedestal. Skin at the rostral and caudal aspects of the incision were sutured using 3-0 silk suture material (Perma-Hand Silk; Ethicon, Inc., Somerville, NJ, USA), and the rat was administered the antibacterial agent Baytril (10 mg/kg, s.c.; Bayer AG, Leverkusen, Germany) to decrease the likelihood of infection following surgery. Upon completion of the surgery, the rat was placed into an empty home cage for recovery and monitored until it became ambulatory. Rats were administered Metacam (1 mg/kg, s.c.) for the next three mornings following surgery, and their body weight, appearance, and activity were monitored closely for seven days. Rats were given a minimum of five days to recover from surgery prior to performance of the electrophysiological recording experiments.

Electrophysiological Recording Apparatus

Electrophysiological recordings were performed in a standard 9” L x 17” D x 9” H rat home cage (hereafter referred to as the *recording cage*) housed inside of a 24” L x 13” D x 17.5” H sound-attenuating box. The sound-attenuating box was equipped with a house light that remained on at all times, as well as a rear wall-mounted webcam (LifeCam Cinema HD; Microsoft). The recording cage was equipped with a ceiling-mounted speaker (FT17H Horn Super Tweeter; Fostex) centered over the front of the cage, which delivered the acoustic stimulus during recording sessions. The intensity of the acoustic stimulus was calibrated with custom Matlab software using a 1/4” microphone (2530; Larson Davis) and a preamplifier (2221; Larson Davis). During recording sessions, rats were placed inside the recording cage, their electrode pedestals were connected to a commutator (SL6C-SB; PlasticsOne Inc.) located above the center of the cage lid. The headstage cable (363-363; PlasticsOne Inc.) used to connect the pedestal to the commutator was long enough to allow for uninhibited movements throughout the cage. A separate cable (363-441-6; PlasticsOne Inc.) connected the commutator to a RA4LI low-impedance headstage (TDT), which amplified the LFP signal by 20-fold. The LFP signal was then digitized at 1017.25 Hz and bandpass filtered at 0.5 – 300 Hz using a RA4SD Medusa preamp (TDT), which was connected to an RZ6 processor (TDT) via fiber optic cable. Both acoustic stimulus delivery and LFP signal acquisition were controlled and

monitored through custom Matlab protocols (EPsych Toolbox; dstolz.github.io/epsych/) running in Matlab, which was interfaced with an RZ6 processor.

Stimulus Paradigm

The stimulus paradigm used in the present experiment was based on previous studies that investigated the auditory steady state responses (ASSRs) of various brain regions (Vohs et al. 2010; Vohs et al. 2012; Sullivan et al. 2015; Sivarao et al. 2013); however, it was modified to also permit the recording of spontaneous cortical activity in addition to the evoked-responses associated with the ASSR. Each trial of the ASSR stimulus paradigm (150 total trials) was 5.5 s in duration, and included three consecutive epochs: (1) a quiet period lasting 4 s, (2) a 500 ms duration 40 Hz click train (composed of 20 clicks; each click was a 1 – 45 kHz noise burst presented at 87 dB SPL lasting 10 ms with 0.1 ms rise/fall time), and (3) a quiet period lasting 1 s (see **Fig. 1A** for schematic). A new trial began immediately following the end of the third epoch. A single recording session lasted approximately 13 min 50 s.

Electrophysiological Recordings

The experimental protocol for all recording sessions was similar, regardless of treatment (see **Fig. 1A** for schematized overview). First, rats were placed inside the recording cage, tethered with the headstage cable, and allowed a 10 min acclimation period. The purpose of the acclimation period was to maintain a consistent arousal level between rats across recording sessions. The acclimation period was immediately followed by the onset of the stimulus paradigm. Electrophysiological recordings obtained during this time are referred to henceforth as *pre-treatment recordings*. Once the pre-treatment recording had ended, rats were untethered, removed from the recording cage, and the given treatment was administered (saline, SS, sham NE or acute NE). After waiting a certain amount of time (which differed according to treatments; see below), rats were placed inside the recording cage, tethered, and allowed a 10 min acclimation period, which was immediately followed by the stimulus paradigm. Electrophysiological recordings obtained during this time are referred to henceforth as *post-treatment recordings*. During both the pre- and post-treatment recordings, rats were monitored closely via the wall-mounted webcam and

the real-time LFP traces on screen to ensure that they were not asleep. Rats were given a minimum of 48 hours between recording sessions to allow sufficient time for any transient treatment effects to resolve.

Upon completion of pre-treatment recordings, rats were untethered and removed from the recording cage. All rats received the same treatments in the following order, on separate days: (1) saline (equivalent volume to the SS treatment, i.p.), (2) SS dissolved in saline (250 mg/kg, i.p.), (3) sham NE, and (4) acute NE. For the saline and SS (250 mg/kg, i.p.) treatments, rats were placed inside of the recording cage and tethered 1 hour 50 min post-injection, and allowed a 10 min acclimation period, which was followed by the stimulus paradigm. The sham NE and acute NE treatments were carried out as described in Behavioural Test Sessions in section 2.1.1. Immediately following removal from the noise exposure box, rats were placed inside of the recording cage, tethered, and allowed a 10 min acclimation period, which was followed by the stimulus paradigm.

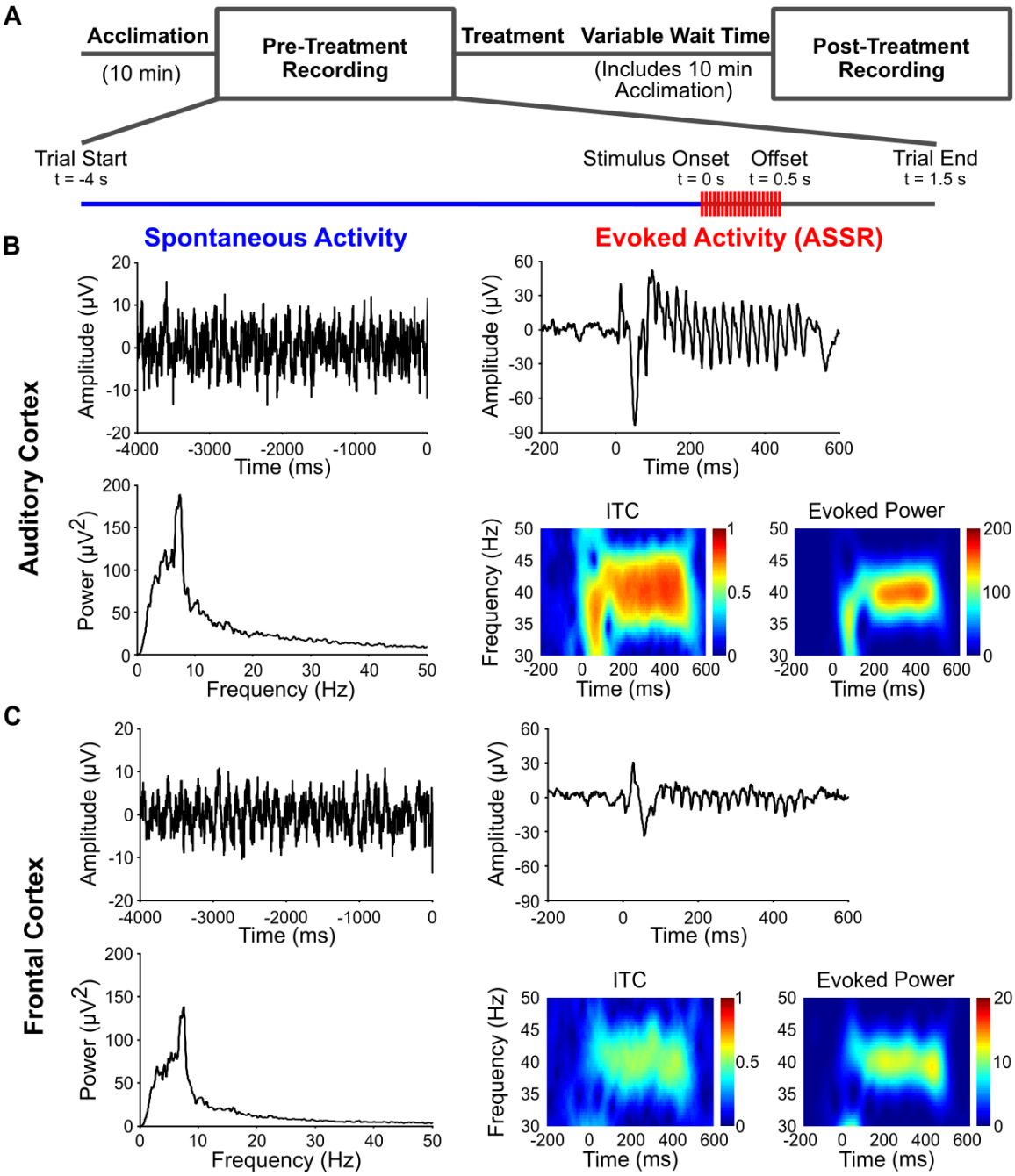

Figure 1. Electrophysiology experimental overview. (A) Schematics of the overall experimental protocol (Acclimation; Pre-Treatment Recording period; Treatment administration; Wait Time; Post-Treatment Recording period), as well as a zoomed in example of one of the 150 trials included in the Pre-Treatment Recording period. Within the single trial, note that the timing of the 4 s of spontaneous LFP activity is shown in

blue, and the 500 ms of evoked LFP activity (elicited by 20 clicks presented at 40 Hz) is shown in red. Representative examples of the (**B**) auditory cortex and **(C)** frontal cortex spontaneous LFP traces and corresponding power spectra (left panel), as well as the evoked LFP traces and corresponding ITC and evoked power (right panel). Sample data were derived from the 150 trials of the stimulation paradigm for an individual rat's Pre-Treatment Recoding period (see Materials and Methods for details). Note that all x-axis time values are shown with respect to stimulus onset (occurs at 0 ms). Scale bars denote magnitudes of ITC (units normalized on a 0 – 1 scale) and evoked power (in μV^2) with warmer colours indicating larger magnitudes.

Data Analysis

All data analysis for Experimental Series 1B was performed in Matlab using custom scripts and functions from the FieldTrip toolbox (Oostenveld et al. 2011). LFP values within the 150 raw trials from a single pre- or post-treatment recording were converted from units of Volts into μV by multiplying all values by 10^6 and dividing these values by 20 to account for the 20-fold increase from the RA4LI low-impedance headstage. Each raw trial was subjected to a range-based artifact rejection process (Spencer et al. 2009; Spencer 2012) in which the entire trial would be removed from further analyses if its LFP amplitude range exceeded two-thirds of the LFP amplitude range of the entire recording. For all electrophysiological recordings across all treatments, the number of accepted trials for the auditory cortex was 123 ± 24 and for the frontal cortex was 126 ± 20 (mean ± SD). The accepted trials were then used for further analyses related to spontaneous oscillations and ASSR activity as described below.

Spontaneous Oscillations Analysis

For spontaneous oscillations analysis pertaining to each cortical area, LFP values between -4 s to 0 s relative to stimulus onset from each accepted trial were subjected to time-frequency decomposition via Fast-Fourier Transformation (FFT) that utilized a Hanning window taper. The magnitudes of the resulting complex values were squared to yield power values, and then were averaged across trials to create a single power spectrum representing average spontaneous oscillatory activity (see left panel of **Fig. 1B, C** for an example). To account for variability in LFP signal strength between rats, each rat's 0.5 – 50 Hz post-treatment power spectrum was normalized by the mean power of their same-day 0.5 – 50 Hz pre-treatment power spectrum, thereby converting a rat's individual power spectrum from units of μV^2 to units of scaled power; a normalization method based on the work of Weisz and colleagues for oscillations analyses of tinnitus patients and healthy controls (Weisz et al. 2005; Weisz et al. 2007). For each treatment, scaled power spectra were averaged across rats and plotted as group mean ± SEM for both auditory and frontal cortices as a 0.5 – 30 Hz power spectrum, with a 30 – 50 Hz power spectrum inset to highlight potential changes in gamma power. Further

quantification was performed by calculating each rat's mean scaled power within four frequency bins of interest: delta, 2 – 4 Hz; theta, 4 – 8 Hz; alpha, 8 – 12 Hz; gamma, 30 – 50 Hz, which were then averaged across rats for each treatment and plotted as group mean ± SEM. Statistical comparisons were decided *a priori* and were conducted as follows: A two-way RM-ANOVA was performed for treatment x frequency to compare frequency bin power between a treatment and its control condition (e.g., SS (250 mg/kg, i.p.) vs saline; acute NE vs sham NE). If a significant main effect or a significant interaction of treatment x frequency ($\alpha = 0.05$) was found, a *post hoc* paired-samples, two-tailed t test was used to further determine if there were significant differences between treatments within a specific frequency bin. Since there were four *post hoc* comparisons being made, the Bonferroni-corrected significance level was set at $\alpha = 0.0125$.

ASSR Analysis

For ASSR analysis pertaining to each cortical area, each accepted trial was subjected to time-frequency decomposition via the '*ft_freqanalysis'* function in the FieldTrip toolbox. Using this function, the *'mtmconvol'* method was used, which performed a time-frequency analysis on the time series data (i.e., the LFP values comprising the accepted trial) using a conventional Hanning window taper. A complex value (containing magnitude and phase information from the LFP values) was created for each frequency of interest (i.e., 0 Hz to 50 Hz in 0.5 Hz steps) from the beginning to the end of the trial (i.e., from 0 s to 5.5 s) using a 200 ms window centered on 1 ms steps. The resulting complex values for each trial were then used to calculate the inter-trial coherence (ITC) and evoked power (EP) of the ASSR; two commonly reported ASSR metrics. ITC depends exclusively on the phase angles of complex values; it does not take magnitude information into account. ITC is a measure of the neural region's ability to become entrained (i.e., become "phase-locked") with the acoustic stimulus over multiple trials, and is suggested to represent the ability of that region's neuronal circuitry to support a certain type of oscillatory activity (e.g., ability to support gamma activity when the ASSR is driven by a 40 Hz stimulus) (Brenner et al. 2009; Roach and Mathalon 2008). ITC was calculated by dividing each complex value by its magnitude (i.e., the absolute of the

complex value), thereby preserving the complex value's phase angle while transforming its magnitude to one (Roach and Mathalon 2008). All of these magnitude-normalized complex values were then averaged across trials, yielding a value between zero and one, which represents one minus the circular variance of phases for each point corresponding to a specific time and frequency (Roach and Mathalon 2008). An ITC value of zero signifies complete random distribution of phase angles between trials (i.e., no inter-trial phase synchrony), whereas a value of one signifies completely synchronized phase angles across trials (i.e., perfect inter-trial phase synchrony). EP represents frequency-specific changes in power that are phase-locked with the stimulus (Roach and Mathalon 2008); i.e., EP is the magnitude of the evoked response. EP was calculated by averaging the complex values across trials, thereby preserving values that are phase-locked with the stimulus, and then squaring the magnitudes of these averaged complex values to derive the power (see right panel of **Fig. 1B, C** for examples of ITC and EP).

Consistent with previous studies (Spencer et al. 2008; Spencer et al. 2009; Vohs et al. 2010; Vohs et al. 2012), the calculated ITC and EP values were then baseline corrected; a process that is important for revealing changes in these measurements that may not be evident from the raw values (Roach and Mathalon 2008). For ITC baseline correction, a mean ITC baseline value was calculated within a -400 ms to -100 ms time window with respect to stimulus onset at each frequency of interest (i.e., from 0 Hz to 50 Hz in 0.5 Hz steps). These mean ITC baseline values were then subtracted from all ITC values from 0 s to 5.5 s of corresponding frequencies to yield the baseline-corrected ITC values. For EP baseline correction, a mean EP baseline value was calculated within a -400 ms to -100 ms time window with respect to stimulus onset for each frequency of interest. All EP values from 0 s to 5.5 s were then divided by their EP baseline values of corresponding frequency. The $\log_{10}$ values of the resulting quotients were calculated and multiplied by 20, yielding values that represent the ratio of EP change from baseline in units of dB, a common way of expressing this measure (Roach and Mathalon 2008). It is important to note that ITC values are already normalized on a scale of 0 to 1, but EP values are in units of power (μV^2), which can differ between animals due to variability in LFP signal strength. Therefore, the conversion of EP values from units of μV^2 to units of dB permits the calculation of group averages. For both cortical regions (auditory and frontal),

baseline corrected ITC and EP values for each treatment are shown as group averaged spectrograms plotted as frequency (30 Hz – 50 Hz) x time (-500 ms to 1000 ms with respect to stimulus onset) x magnitude of response (values ranging from 0 – 1 for ITC or values ranging from 0 to 50 dB for EP). These baseline-corrected values were further quantified by calculating each rat's mean ITC or EP between 100 ms – 400 ms post-stimulus onset and 35 – 45 Hz, thereby incorporating the maximum region of the evoked response, and then averaged across rats to yield group averaged ITC and EP values (plotted as group mean ± SEM). Statistical comparisons were decided *a priori* and were conducted as follows: For both cortical regions, paired-samples, two-tailed t tests were used to determine if ITC or EP values were significantly different ($\alpha = 0.05$) between a treatment and its control condition (e.g., SS (250 mg/kg, i.p.) vs saline; acute NE vs sham NE). All statistics were performed using SPSS Statistics 24 and figures were generated in Matlab 2012 or GraphPad Prism 6.

2.2 Experimental Series 2

The second Experimental Series included an investigation into the electrophysiological (Series 2A) and behavioural (Series 2B) consequences of manipulating the level of inhibition in the medial geniculate body (MGB) through local drug delivery via micro-infusion cannulae. In Experimental Series 2, the electrophysiological experiments (n = 13 rats) preceded the behavioural experiments that were conducted on a separate group of rats (n = 8) to ensure that the chosen doses of the various drugs micro-infused into the MGB did indeed cause disruption of cortical activity before investigating whether or not these treatments also induced behavioural evidence of tinnitus. Importantly, this Experimental Series represents the first direct investigation of the TCD model of tinnitus (Llinás et al. 1999; Llinás et al. 2005) using an animal model.

2.2.1 Experimental Series 2A – Electrophysiology

Experimental Series 2A used the same materials and methods as described above in section 2.1.2, with any notable deviations outlined below.

Surgical Procedure

Naïve, adult male Sprague-Dawley rats (350 – 470 g, n = 13) underwent the same surgical procedure and epidural screw electrode implantation as described in section 2.1.2. Additionally, these rats were implanted with a 26-gauge, 4.5 mm-long guide cannula (C315GA-SPC; PlasticsOne Inc.) at -5.2 mm caudal and 3.4 mm to the left of bregma (unilateral), which projected to approximately 2.0 mm dorsal to the ventral division of the MGB (vMGB) according to Paxinos and Watson (2007). This region of the MGB was targeted for drug infusion because, in contrast to the dorsal and medial divisions of the MGB, the vMGB thalamocortical relay cells project almost exclusively to the primary auditory cortex (Winer et al. 1999; Smith et al. 2012), and thus, this thalamic region has relevance for the TCD model of tinnitus. To maintain guide cannula patency, a dummy stylet (C315DC-SPC; PlasticsOne Inc.) was inserted into the guide cannula (projecting to the guide cannula tip), except for when rats were receiving drug infusion treatments (see below).

Electrophysiological Recordings

Upon completion of the pre-treatment recording, rats were untethered and removed from the recording cage. The dummy stylet was removed from the guide cannula and a 33-gauge, 6.5 mm-long infusion cannula (C315LI-SPC; PlasticsOne Inc.) was inserted into the guide cannula, projecting into the vMGB. All rats (n = 13) received each of the following four drug infusions – with each infusion separated by a minimum of 48 hours – in a randomized order: (1) aCSF (Harvard Apparatus), (2) 50 μM gabazine (SR-95531; Sigma-Aldrich), (3) 50 μM THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; Sigma-Aldrich), and (4) 100 μM THIP (Sigma-Aldrich). THIP was selected as the drug to increase tonic inhibition in the MGB through its preferential agonism of δ-subunit-containing $GABA_A$ receptors (Brown et al. 2002; Jia et al. 2005; Stórustovu and Ebert 2006; Herd et al. 2009; Mortensen et al. 2010; Meera, Wallner, and Otis 2011). The selection of THIP concentrations used in this Experimental Series was based on significant behavioural (Paydar et al. 2014) and electrophysiological (Mesbah-Oskui, Orser, and Horner 2014) effects observed in rodents when infused into other thalamic

nuclei at similar concentrations. Additionally, care was taken when choosing the upper THIP concentration based on evidence that when THIP is infused into certain thalamic nuclei at ~ 100 μM concentrations, there is an increased risk of eliciting electrophysiological and behavioural correlates of absence seizure activity (Cope et al. 2009). Indeed, pilot testing using 200 μM THIP induced absence seizures in a subset of rats; however, we observed no evidence of this at 100 μM THIP in the present study.

The potent $GABA_A$ receptor antagonist, gabazine, was also selected for intra-vMGB infusion as it is a well-known agent capable of decreasing the levels of local inhibition (Brown et al. 2002; Cope, Hughes, and Crunelli 2005; Bright, Aller, and Brickley 2007). The concentration of gabazine used in this Experimental Series was based on significant behavioural effects observed in rodents following intra-thalamic infusion (Paydar et al. 2014).

Stock solutions of gabazine and THIP were prepared in aCSF, which were subsequently aliquoted and frozen. As needed, an aliquot was thawed to room temperature and diluted with aCSF to the proper concentration. Drug infusions were performed at a rate of 0.1 μL/min for 3 min (total volume of 0.3 μL) using a micro-infusion pump (Model 22 Syringe Pump Series; Harvard Apparatus). The infusion cannula was removed two minutes after the infusion pump was shut off to allow for drug diffusion from the infusion cannula tip, and then the dummy stylet was reinserted into the guide cannula. Rats were then placed inside of the recording cage, tethered, and allowed a 10 min acclimation period, which was followed by the stimulus paradigm.

Data Analysis

Statistical comparisons were decided *a priori* and were conducted as follows: For spontaneous oscillations, the statistical comparisons being performed are the same as those discussed in section 2.1.2 (i.e., two-way RM-ANOVAs and *post hoc* paired-samples, two-tailed t tests with Bonferroni-corrected $\alpha = 0.0125$), but in this Experimental Series, a comparison was made between the control condition (aCSF) and the other treatments (50 μM gabazine, 50 μM THIP, 100 μM THIP). For ITC and EP statistical comparisons, a one-way RM-ANOVA was conducted for treatment (i.e., aCSF,

50 µM gabazine, 50 µM THIP, 100 µM THIP). If there was a significant main effect ($\alpha = 0.05$), *post hoc* paired-samples, two-tailed t tests were used to further investigate the possible significant differences between the control condition (aCSF) and any of the other treatments (i.e., three total *post hoc* comparisons). Since there were three *post hoc* comparisons being made, the Bonferroni-corrected significance level was set at $\alpha = 0.0167$.

Histology

Once the final recording session was completed (i.e., all treatments had been administered to a given rat), rats were injected with either sodium pentobarbital (100 mg/kg, i.p.) or ketamine-xylazine (80 mg/kg ketamine, 5 mg/kg xylazine, i.p.). Upon reaching a surgical plane of anesthesia, as assessed with the absence of a pedal withdrawal reflex, rats were transcardially perfused (Gage, Kipke, and Shain 2012; Schormans et al. 2017) with 300 mL of 0.9 % saline, and then 400 mL of 4 % paraformaldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer (PB, pH 7.2). Brains were extracted and kept in 4 % paraformaldehyde in 0.1 M PB for 24 hours post-perfusion, followed by a 30 % sucrose solution in 0.1 M PB for 48 hours. Brains were then cut into 50 µm coronal sections using a freezing microtome (HM 430/34; Thermo Scientific, Walktham, MA, USA). Following thionin staining, coronal sections were imaged using an Axio Vert A1 inverted microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Single images were stitched together to produce a full coronal section image for each rat using Zen Blue imaging software (Carl Zeiss Microscopy GmbH). One of the 13 rats showed an infusion cannula track that was located outside of the targeted region, so data pertaining to that rat were removed from all electrophysiological analyses.

2.2.2 Experimental Series 2B – Behaviour

Experimental Series 2B used the same materials and methods as described above in section 2.1.1, with any notable deviations described below. The rats (n = 8) used in this Experimental Series were the same as those used for Experimental Series 1A. Once rats were able to consistently achieve ≥ 90% correct trials for a minimum of three weeks, they were selected to undergo surgery.

Surgical Procedure

Adult male Sprague-Dawley rats (350 – 450 g, n = 8) were placed into a gas anesthesia induction chamber and anesthetized with isoflurane (4 % mixed with pure O_2, 2 L/min). Following loss of consciousness, the rat was removed from the induction chamber and fixed into a stereotaxic frame with blunt ear bars. A surgical level of anesthesia, as assessed by the absence of a pedal withdrawal reflex, was maintained throughout the surgery with isoflurane (1.5 – 2 % mixed with pure O_2, 2 L/min) administered through a nose cone that was fixed to the stereotaxic frame. An electronic heating pad (Homeothermic Monitor; Harvard Apparatus, Holliston, MA, USA) was used to maintain the rat's body temperature within 36.5 °C – 37.5 °C. The rat's head was shaved and then cleaned with antimicrobial soap, followed by 70% isopropyl alcohol, and then betadine solution. Eye lubricant was applied and the rat was given the analgesic agent Metacam (1 mg/kg, s.c.). A midline incision was made along the dorsal aspect of the skull, cutting through the skin of the scalp, and fascia was removed from the dorsal surface of the skull, revealing the skull's sutures. Two small holes were drilled into the skull at -5.2 mm caudal and 3.4 mm to the left and right of bregma. A stereotaxic arm was used to insert a 26-gauge, 4.5 mm-long guide cannula into each of these holes (bilateral). These guide cannulae projected to approximately 2.0 mm dorsal to the ventral division of the MGB (vMGB) (Paxinos and Watson 2007). Stainless steel screws were secured into the skull, and dental cement was placed around the guide cannulae and screws. Skin at the rostral and caudal aspects of the incision were sutured using 3-0 silk suture material, and the antibacterial agent Baytril (10 mg/kg, s.c) was administered to decrease the likelihood of infection following surgery. At the conclusion of the surgery, the rat was removed from the stereotaxic frame, and placed into an empty home cage for recovery. The rat was administered Metacam (1 mg/kg, s.c.) the following morning and its body weight, appearance, and activity were monitored closely for seven days following surgery.

Behavioural Test Sessions

Approximately two to three days following surgery, rats resumed the 30 min/day behavioural training sessions. Once they achieved ≥ 90 % correct trials for at least three

consecutive days, rats began the same testing time course, as described in section 2.1.1, with the following drug infusion treatments (in randomized order): (1) aCSF, (2) 50 μM gabazine, (3) 50 μM THIP, and (4) 100 μM THIP. All rats received the same treatments, and each treatment was separated by a minimum of 4 days.

Histology

The histological procedures performed in Experimental Series 2B were the same as those described in section 2.2.1.

Chapter 3

3 Results

3.1 Experimental Series 1

The objective of the first Experimental Series was to investigate the behavioural (Series 1A) and electrophysiological (Series 1B) consequences of exposure to either of two known tinnitus inducers; namely, (1) 250 mg/kg (i.p.) sodium salicylate (SS) or (2) a single bout of loud noise (acute NE).

3.1.1 Experimental Series 1A – Behaviour

The behavioural experiments performed in Experimental Series 1A served to confirm that our two-choice operant conditioning task was sensitive to detecting behavioural evidence of tinnitus following a putative tinnitus-inducing insult (e.g., treatment with SS (250 mg/kg, i.p.) or acute NE), while being able to detect a normal behavioural profile following a control treatment (e.g., administration of saline or sham NE). There were 8 rats used for this Experimental Series.

SS vs Saline. For quiet stimulus trials (left panel of **Fig. 2A**), a two-way RM-ANOVA revealed a significant treatment x time interaction ($F_{(2,14)} = 30.53$; $p < 0.001$), and *post hoc* testing (paired-samples, two-tailed t tests) revealed further significant differences. Specifically, the proportion of quiet trials identified as NBN was significantly increased for the SS (250 mg/kg, i.p.) treatment (SS Test: 32.73 ± 5.71 %) compared to both the saline treatment (saline Test: 1.92 ± 0.73 %; $p < 0.01$) and the day prior to the SS (250 mg/kg, i.p.) treatment (SS Pre: 2.04 ± 1.09 %; $p < 0.01$). Additionally, *post-hoc* paired samples, two-tailed t tests failed to reveal any significant differences for the proportion of quiet trials identified as NBN between the saline treatment (saline Test) and the day prior to the saline treatment (saline Pre: 2.07 ± 1.08 %; $p = 0.92$). Taken together, these findings confirm that the rats exhibited behavioural evidence of tinnitus following treatment with SS (250 mg/kg, i.p.), but not following treatment with saline.

For the AM stimulus trials (middle panel of **Fig. 2A**), a two-way RM-ANOVA revealed a significant treatment x time interaction ($F_{(2,14)} = 29.59$; $p < 0.001$). Additionally, *post hoc* paired-samples, two-tailed t tests revealed that the proportion of AM trials identified as NBN was significantly increased for the SS (250 mg/kg, i.p.) treatment (SS Test: 24.53 ± 4.54 %) compared to both the saline treatment (saline Test: 3.853 ± 0.968 %; $p < 0.01$) and the day prior to SS (250 mg/kg, i.p.) treatment (SS Pre 1.93 ± 1.10 %; $p < 0.001$), suggesting that the SS (250 mg/kg, i.p.) treatment might cause a difficulty with discriminating between AM noises and steady sounds (i.e., NBNs).

Finally, similar to the quiet trials and the AM noises, a two-way RM-ANOVA revealed a significant treatment x time interaction ($F_{(2,14)} = 14.93$; $p < 0.001$) for the NBN stimulus trials (right panel of **Fig. 2A**). Furthermore, *post hoc* paired-samples, two-tailed t tests revealed that the proportion of correctly identified NBN trials was significantly decreased for the SS (250 mg/kg, i.p.) treatment (SS Test: 84.75 ± 2.26 %) compared to both the saline treatment (saline Test: 96.55 ± 0.80 %; $p < 0.01$) and the day prior to SS (250 mg/kg, i.p.) treatment (SS Pre: 95.99 ± 1.08 %; $p < 0.01$). These findings were not overly surprising given that SS (250 mg/kg, i.p.) treatment is known to cause a mild hearing loss, which could lead to difficulty accurately discriminating the NBN trials from the quiet trials.

Acute NE vs Sham NE. For quiet stimulus trials (left panel of **Fig. 2B**), a two-way RM-ANOVA revealed a significant treatment x time interaction ($F_{(2,14)} = 91.71$; $p < 0.001$), and further significant differences were revealed by *post hoc* paired-samples, two-tailed t tests. Specifically, the proportion of quiet trials identified as NBN was significantly increased for the acute NE treatment (acute NE Test: 46.49 ± 3.44 %) compared to both the sham NE treatment (sham NE Test: 2.89 ± 1.58 %; $p < 0.001$) and the day prior to acute NE treatment (acute NE Pre: 0.45 ± 0.45 %; $p < 0.001$). Furthermore, there were no significant differences revealed by *post hoc* paired-samples, two-tailed t tests between the sham NE treatment (sham NE Test) and the day prior to sham NE treatment (sham NE Pre: 3.42 ± 1.39 %; $p = 0.61$). Collectively, these findings confirm that rats treated with acute NE exhibited behavioural evidence of tinnitus, whereas the sham NE-treated rats did not. Additionally, there was a significant increase in the proportion of quiet trials

identified as NBN on the day following acute NE treatment (acute NE Post: 13.57 ± 2.35 %) compared to the day following sham NE treatment (sham NE Post: 3.26 ± 1.07 %; $p < 0.01$); findings which could be explained by the acute NE-treated rats continuing to experience noise-induced tinnitus the next day, whereas the sham NE-treated rats did not.

For the AM stimulus trials (middle panel of **Fig. 2B**), a two-way RM-ANOVA revealed a significant treatment x time interaction ($F_{(2,14)} = 14.23$; $p < 0.001$), and *post hoc* paired-samples, two-tailed t tests revealed that the proportion of AM trials identified as NBN was significantly increased for the acute NE treatment (acute NE Test: 24.71 ± 3.66 %) compared to both the sham NE treatment (sham NE Test: 6.37 ± 1.77 %; $p < 0.01$) and the day prior to acute NE treatment (acute NE Pre: 5.48 ± 2.29 %; $p < 0.001$). Thus, similar to the SS treatment described above, the acute NE treatment appeared to increase the difficulty with discriminating AM noises from steady sounds. There was also an increase in the proportion of AM trials identified as NBN on the day following acute NE treatment (acute NE Post: 8.26 ± 1.96 %) compared to the day following sham NE treatment (sham NE Post: 1.91 ± 0.94 %; $p = 0.012$).

For the NBN stimulus trials (right panel of **Fig. 2B**), a two-way RM-ANOVA revealed a significant treatment x time interaction ($F_{(2,14)} = 11.00$; $p < 0.01$), and *post hoc* paired-samples, two-tailed t tests revealed that the proportion of correctly identified NBN trials was significantly decreased for the acute NE treatment (acute NE Test: 82.29 ± 3.13 %) compared to both the sham NE treatment (sham NE Test: 98.86 ± 0.80 %; $p < 0.01$) and the day prior to acute NE treatment (acute NE Pre: 96.95 ± 0.91 %; $p < 0.01$). The aforementioned finding suggests that, similar to the SS treatment, the acute NE treatment may have induced a mild hearing loss. Lastly, there was also a decrease in the proportion of correctly identified NBN trials on the day following acute NE treatment (acute NE Post: 92.72 ± 2.08 %) compared to the day following sham NE treatment (sham NE Post: 98.53 ± 0.63 %; $p = 0.026$), as well as an increase in this measure for the sham NE treatment (sham NE Test) compared to the day prior to sham NE treatment (sham NE Pre: 97.69 ± 0.68 %; $p = 0.026$).

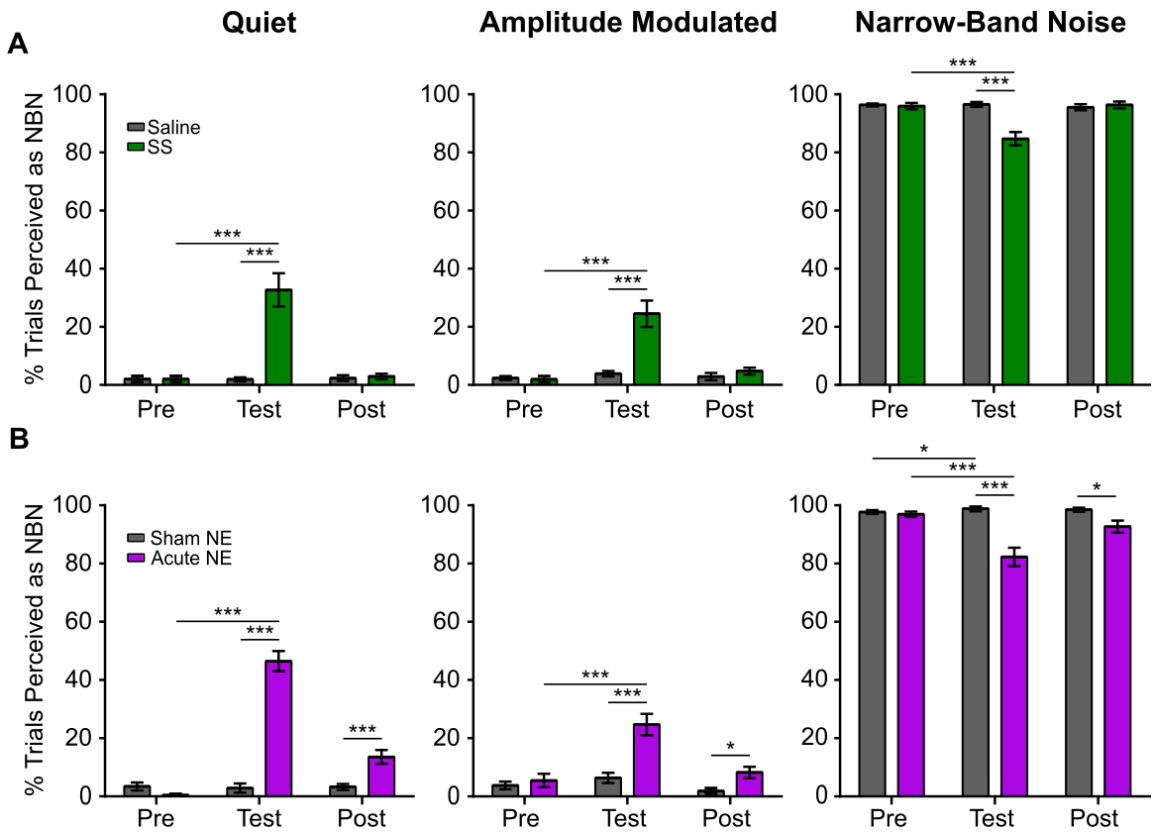

Figure 2. Rats trained on two-choice operant conditioning paradigm exhibit behavioural evidence of tinnitus following treatment with either SS (250 mg/kg, i.p.) or acute NE. Data are shown as group mean ± SEM for behavioural performance on the day before ("Pre"), the day of ("Test"), and the day after ("Post") treatment. Rats treated with either 250 mg/kg (i.p.) SS **(A)** or acute NE **(B)** exhibit behavioural evidence of tinnitus as evidenced by a significantly increased ($p < 0.01$) proportion of quiet trials perceived as NBN on Test day compared to the group's Test day performance for the respective control treatments and the group's Pre day performance. Both SS (250 mg/kg, i.p.) and acute NE treatments also resulted in a significantly increased ($p < 0.01$) proportion of AM trials perceived as NBN, suggesting that both of these treatments may enhance the difficulty with distinguishing AM noises from steady sounds (i.e., NBN), and a significant decrease ($p < 0.01$) in the proportion of correctly identified NBN trials, suggesting that both of these treatments may have induced a mild hearing loss. Refer to Results for further details on comparisons and statistical tests used. N = 8 for all treatments. ***$p < 0.01$ (Bonferroni-corrected α), *$p < 0.05$.

Summary of Experimental Series 1A – Behaviour. As predicted, treatment with SS (250 mg/kg, i.p.) or acute NE caused behavioural evidence of tinnitus in rats, as evidenced by the significantly increased proportion of quiet trials perceived as NBN following either of these treatments. Furthermore, the control conditions (i.e., saline and sham NE) did not result in behavioural evidence of tinnitus. Collectively, these results support the conclusions that (1) the experimental parameters for the SS (250 mg/kg, i.p.) and acute NE treatments (e.g., dosage of SS, duration and loudness of noise exposure) were appropriate for eliciting a tinnitus-like behavioural profile and (2) the behavioural paradigm was robust and did not falsely report the presence of tinnitus following a control treatment. Importantly, armed with these results, we were able to proceed to Experimental Series 1B with the confidence that our chosen treatments were effective for inducing tinnitus.

3.1.2 Experimental Series 1B – Electrophysiology

The aim of Experimental Series 1B was to investigate the potential effects that the tinnitus-inducing treatments used in Experimental Series 1A (i.e., SS (250 mg/kg, i.p.) and acute NE) had on oscillatory activity from the auditory (AC) and frontal (FC) cortices. There were 11 rats used for this Experimental Series.

SS vs Saline. For spontaneous AC oscillations (**Fig. 3A, B**), a two-way RM-ANOVA revealed a significant treatment x frequency interaction ($F_{(3,30)} = 6.80$; $p < 0.01$), and *post hoc* paired-samples, two-tailed t tests revealed further significant differences within individual frequency bins. Specifically, the SS (250 mg/kg, i.p.) treatment resulted in significantly decreased delta (saline: 2.53 ± 0.062 vs SS: 2.06 ± 0.11; $p < 0.01$), decreased alpha (saline: 2.15 ± 0.14 vs SS: 1.51 ± 0.11; $p < 0.01$), and increased gamma activity (saline: 0.36 ± 0.017 vs SS: 0.47 ± 0.029; $p < 0.01$), compared to the saline control condition. Similarly, for spontaneous FC oscillations (**Fig. 3C, D**), a two-way RM-ANOVA revealed a significant treatment x frequency interaction ($F_{(3,30)} = 6.54$; $p < 0.01$), and *post hoc* paired-samples two-tailed t tests revealed that SS (250 mg/kg, i.p.) treatment, compared to the saline control condition, resulted in significantly decreased alpha (saline: 2.27 ± 0.13 vs SS: 1.51 ± 0.16; $p < 0.001$) and increased gamma activity (saline: 0.32 ± 0.016 vs SS: 0.41 ± 0.022; $p < 0.01$). Additionally, treatment with SS (250

mg/kg, i.p.) resulted in decreased FC delta (saline: 3.46 ± 0.17 vs SS: 2.85 ± 0.21; p = 0.039) and FC theta activity (saline: 4.44 ± 0.27 vs SS: 3.53 ± 0.23; p = 0.024), compared to the saline treatment.

Acute NE vs Sham NE. For both spontaneous AC (**Fig. 3E, F**) and FC (**Fig. 3G, H**) oscillations, two-way RM-ANOVAs failed to reveal any significant treatment x frequency interactions. There were, however, significant effects of frequency for both AC ($F_{(3,30)} = 157.04$; $p < 0.001$) and FC ($F_{(3,30)} = 110.06$; $p < 0.001$), which is not surprising given the inherent differences in magnitude of the scaled power between the frequency bins (e.g., delta compared to gamma). Further analyses using *post hoc* paired-samples, two-tailed t tests revealed that, despite no significant differences between treatments within any of the frequency bins for either AC or FC, there was a slight decrease in AC gamma activity following acute NE treatment, compared to the sham NE control condition (sham NE: 0.38 ± 0.014 vs acute NE: 0.34 ± 0.016; p = 0.042).

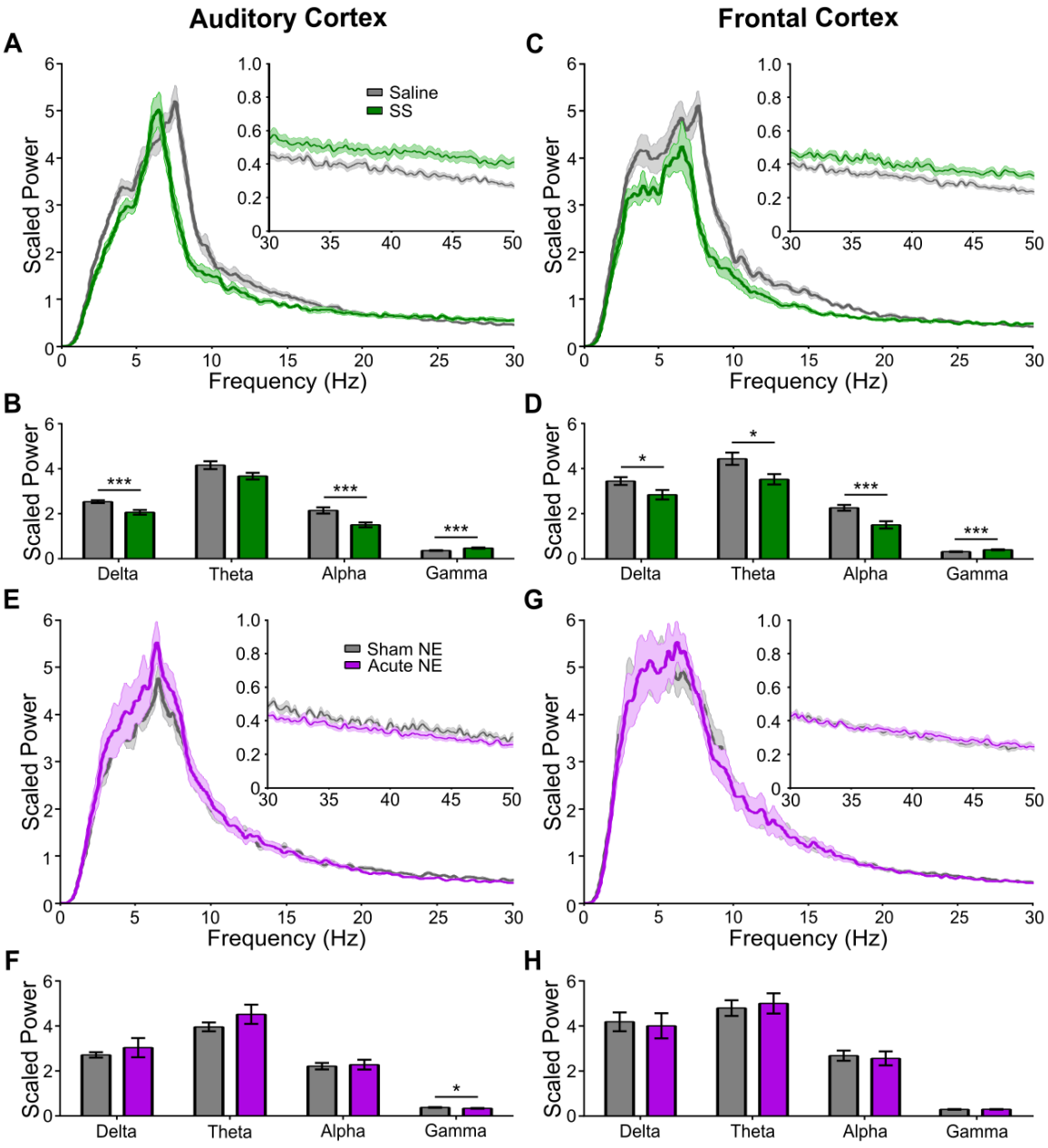

Figure 3. Spontaneous oscillatory profiles for both AC and FC are different between SS (250 mg/kg, i.p.) and saline treatments, but not between acute NE and sham NE treatments. (A) AC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for SS (250 mg/kg, i.p.) and saline treatments. **(B)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous AC oscillatory profile and presented as group mean ± SEM for the saline and SS (250 mg/kg, i.p.) treatments. Note that the delta, alpha, and gamma bins are all significantly different ($p < 0.0125$) between the SS (250 mg/kg, i.p.) and saline

treatments. **(C)** FC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for SS (250 mg/kg, i.p.) and saline treatments. **(D)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous FC oscillatory profile and presented as group mean ± SEM for the saline and SS (250 mg/kg, i.p.) treatments. Note that the alpha and gamma bins are significantly different ($p < 0.0125$) and the delta and theta bins are different ($p < 0.05$) between the SS (250 mg/kg, i.p.) and saline treatments. **(E)** AC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for acute NE and sham NE treatments. **(F)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous AC oscillatory profile and presented as group mean ± SEM for the sham NE and acute NE treatments. Note that the gamma bin is different ($p < 0.05$) between the acute NE and sham NE treatments. **(G)** FC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for acute NE and sham NE treatments. **(H)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous FC oscillatory profile and presented as group mean ± SEM for the sham NE and acute NE treatments. Note that the scaled power axes shown in A, C, E, and G are different between the 0 – 30 Hz and 30 – 50 Hz spontaneous oscillatory profiles. Refer to Materials and Methods for derivation of these measurements and Results for statistical tests used. $N = 11$ for all treatments. ***$p < 0.0125$ (Bonferroni-corrected α), *$p < 0.05$.

As a separate experiment to the aforementioned spontaneous oscillatory effects of tinnitus inducers, we also investigated the potential differences in the inter-trial coherence (ITC) and evoked power (EP) of the AC and FC 40 Hz ASSR following treatment with either SS (250 mg/kg, i.p.) or acute NE, and their respective control conditions. Contrary to what was predicted, paired-samples, two-tailed t tests failed to reveal any significant differences between SS (250 mg/kg, i.p.) and saline treatments for AC ITC or EP (**Fig. 4A, Fig. 5A**), but did reveal a significant decrease for FC ITC following the SS (250 mg/kg, i.p.) treatment compared to the saline treatment (SS: 0.20 ± 0.016 vs saline: 0.28 ± 0.020; $p < 0.01$) (**Fig. 4B, Fig. 5B**). Similarly, paired-samples, two-tailed t tests showed that neither AC ITC nor EP were significantly different between the acute NE and sham NE treatments (**Fig. 4A, Fig. 5A**), but the FC ITC and EP were significantly decreased following the acute NE treatment compared to the sham NE treatment (ITC: acute NE: 0.13 ± 0.012 vs sham NE: 0.27 ± 0.023; $p < 0.001$)(EP: acute NE: 16.49 ± 1.24 dB vs sham NE: 26.28 ± 2.11 dB; $p < 0.01$) (**Fig. 4B, Fig. 5B**).

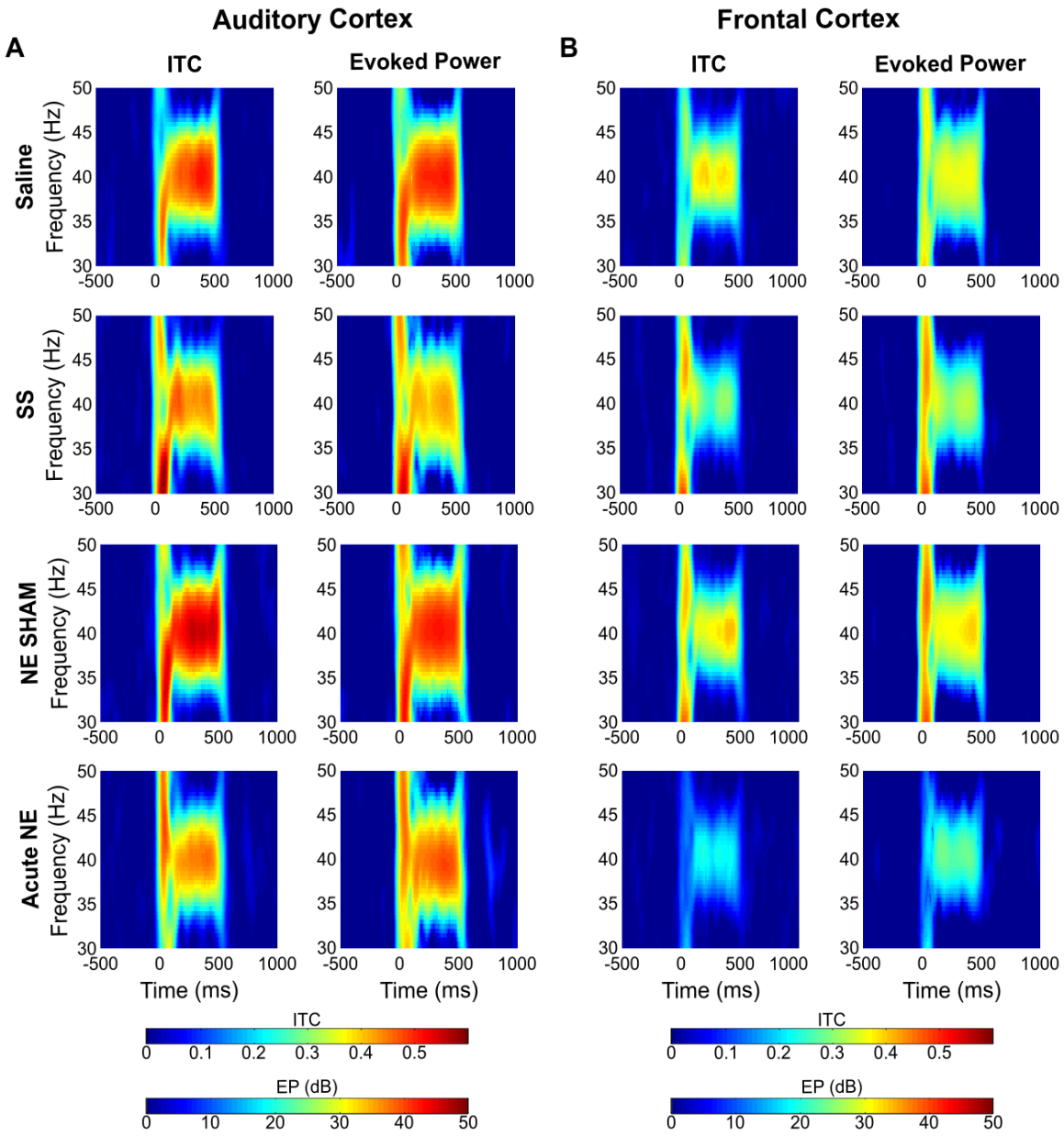

Figure 4. Spectrograms for ITC and EP of the ASSR following systemic treatments. Group averaged data for ITC and EP derived from the AC **(A)** and FC **(B)** 40 Hz ASSR is presented for saline, SS (250 mg/kg, i.p.), sham NE, and acute NE treatments. Both ITC and EP are plotted from 30 Hz to 50 Hz and -500 ms to 1000 ms with respect to stimulus onset (occurs at 0 ms). Scale bars denote magnitudes of ITC and EP values with warmer colours indicating larger magnitudes. Refer to Materials and Methods for details on the derivation of these measurements. N = 11 for all treatments.

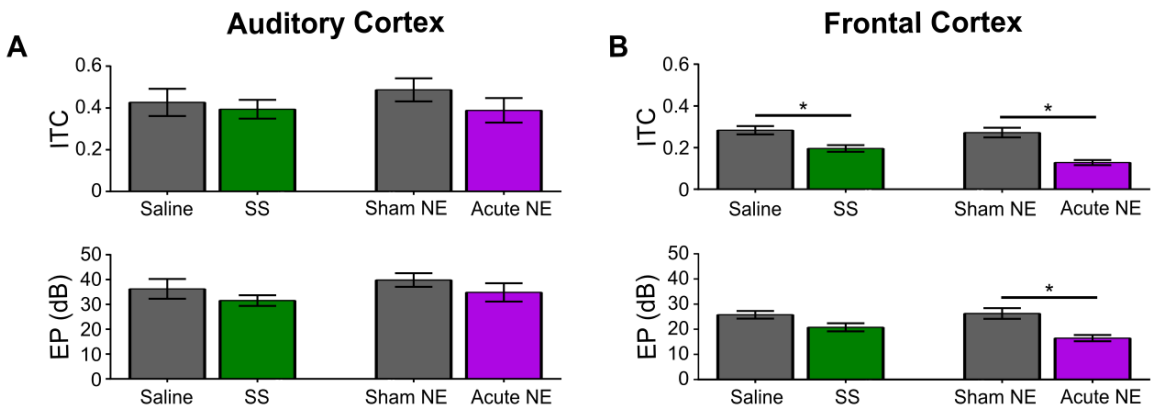

Figure 5. Tinnitus-inducing treatments cause FC ASSR deficits, but do not affect the AC ASSR. Group averaged ITC and EP values for the AC **(A)** and FC **(B)** 40 Hz ASSRs derived from each rat's ITC and EP region of maximum response (i.e., 35 Hz to 45 Hz and 100 ms to 400 ms). Data is presented as group mean ± SEM for the saline, SS (250 mg/kg, i.p.), sham NE, and acute NE treatments. Neither SS (250 mg/kg, i.p.) nor acute NE treatments significantly altered the AC ITC or EP relative to their respective control conditions (saline and shame NE). In contrast, the FC ITC for both SS-treated rats and acute NE-treated rats was significantly decreased ($p < 0.05$) compared to their respective controls. Acute NE-treated rats also exhibited significantly decreased ($p < 0.05$) FC EP. Refer to Results for details on statistical tests used. N = 11 for both treatments. *$p < 0.05$.

Summary of Experimental Series 1B – Electrophysiology. Unexpectedly, treatment with either SS (250 mg/kg, i.p.) or acute NE, while sufficient to induce behavioural evidence of tinnitus, had dissimilar effects on spontaneous AC and FC oscillations. Specifically, SS (250 mg/kg, i.p.) treatment resulted in significantly decreased alpha and increased gamma activity for both AC and FC, in line with our predictions, but acute NE failed to significantly alter spontaneous AC or FC oscillations compared to the sham NE treatment. Interestingly, despite their differing effects on spontaneous oscillatory activity, the two tinnitus-inducing treatments caused similar deficits in the ITC of the FC 40 Hz ASSR, compared to their respective control treatments. Taken together, these aforementioned findings suggest that altered spontaneous cortical oscillations may not be necessary for tinnitus; however, deficits in the FC neuronal circuits that are important for maintaining evoked 40 Hz activity may offer insight into the manifestation of the tinnitus percept.

3.2 Experimental Series 2

Separate from exposing rats to known tinnitus inducers (as described above), the second Experimental Series was conducted to provide an investigation into the electrophysiological (Series 2A) and behavioural (Series 2B) consequences of manipulating the level of inhibition in the medial geniculate body (MGB) through drug micro-infusions. Importantly, to our knowledge, this was the first direct investigation of the TCD model of tinnitus using an animal model.

3.2.1 Experimental Series 2A – Electrophysiology

The aim of Experimental Series 2A was to investigate the potential differences in AC and FC oscillatory activity following either an increase (via local infusion of THIP) or decrease (via local infusion of gabazine) in the level of MGB inhibition. There were 12 rats used for this Experimental Series, all of which were confirmed to have unilateral infusion cannulae projecting into the targeted thalamic region (see **Fig. 6**).

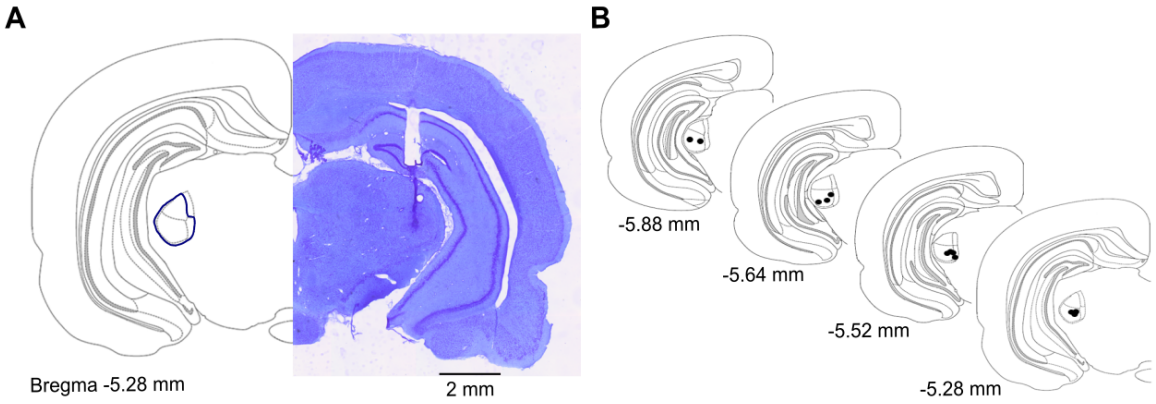

Figure 6. Confirmation of unilateral infusion cannulae projections for rats used in electrophysiology experiments. (A) A representative image of an infusion cannula track projecting into the MGB (outlined) of a brain section of a rat used in electrophysiology experiments. **(B)** A schematic representing the confirmed MGB location of unilateral infusion cannulae tips identified from brain sections of electrophysiology rats (n = 12). Length measurements represent the rostral-caudal distance relative to bregma. Adapted from Paxinos & Watson (2007).

Increased Thalamic Tonic Inhibition. Two-way RM-ANOVAs did not show any significant interactions of treatment x frequency for spontaneous AC (**Fig. 7A, B**) or FC oscillations (**Fig. 7C, D**) following local MGB infusion of either 50μM THIP or aCSF (control condition). As predicted, there was a significant effect of frequency for both AC ($F_{(3,33)} = 179.24$; $p < 0.001$) and FC ($F_{(1.33,14.60)} = 96.86$; $p < 0.001$) oscillations, but *post hoc* testing (paired-samples, two-tailed t tests) showed that there were no significant differences between the 50 μM THIP and aCSF infusions within any of the frequency bins for either AC or FC. Importantly, when the concentration of locally infused THIP was increased from 50 μM to 100 μM, a significant interaction of treatment x frequency was revealed for spontaneous AC oscillations (**Fig. 7E, F**) when comparing the 100 μM THIP and aCSF infusions (two-way RM-ANOVA: $F_{(3,33)} = 4.38$; $p < 0.05$). Additionally, *post hoc* paired-samples, two-tailed t tests revealed that infusion with 100 μM THIP, relative to the aCSF control condition, caused increased AC delta (aCSF: 2.79 ± 0.20 vs 100 μM THIP: 3.26 ± 0.28; $p = 0.039$), increased AC theta (aCSF: 3.84 ± 0.22 vs 100 μM THIP: 4.44 ± 0.26; $p = 0.044$), and decreased AC gamma activity (aCSF: 0.34 ± 0.023 vs 100 μM THIP: 0.27 ± 0.018; $p = 0.020$). For spontaneous FC oscillations (**Fig. 7G, H**), a two-way RM-ANOVA failed to reveal a significant treatment x frequency interaction between the 100 μM THIP and aCSF infusions. However, a predictable significant effect of frequency was found between the two aforementioned thalamic infusions ($F_{(1.60,17.63)} = 111.06$; $p < 0.001$), but *post hoc* paired-samples, two-tailed t tests showed that there were no significant differences between the 100 μM and aCSF infusions for any of the FC frequency bins.

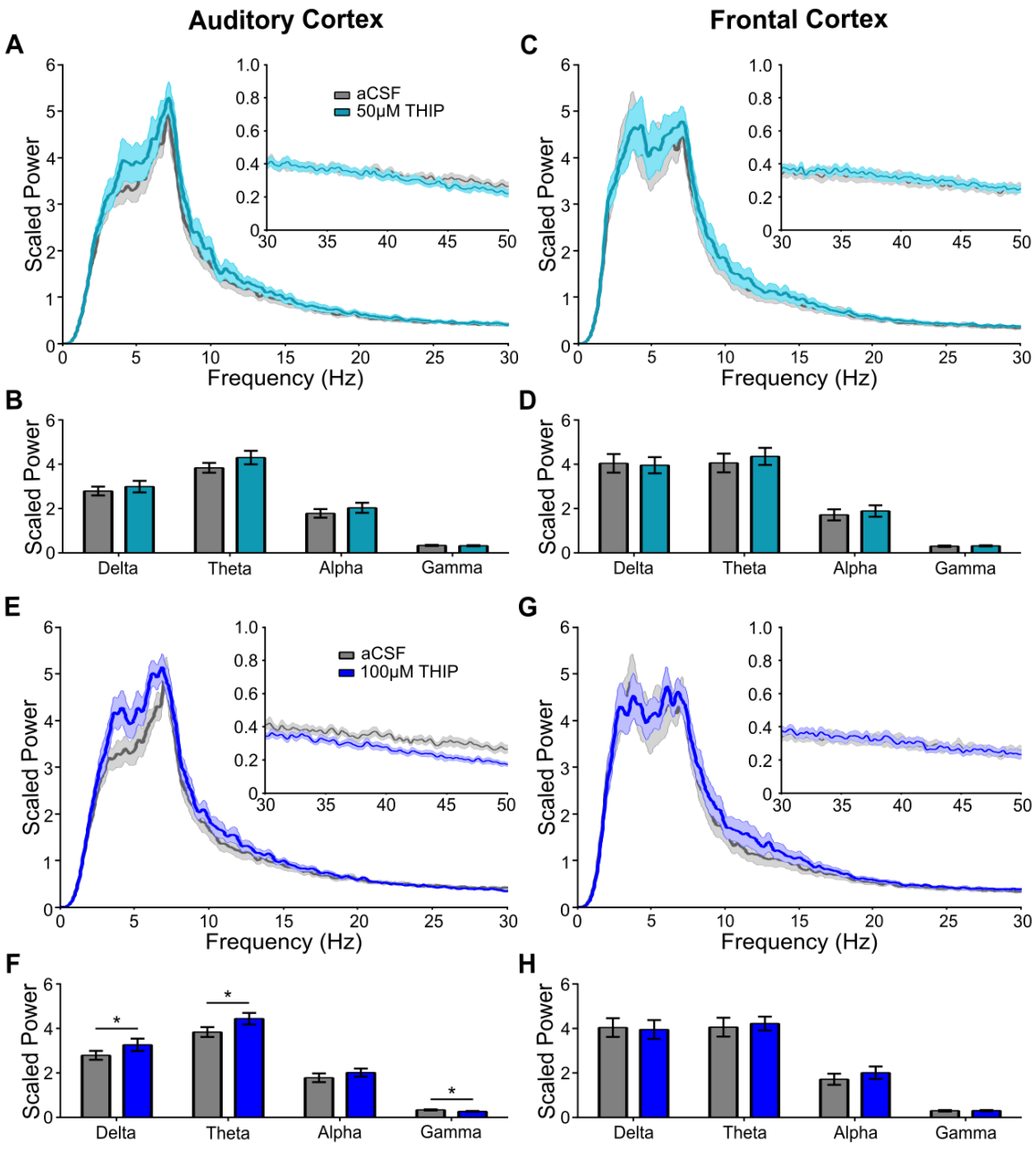

Figure 7. Unilateral thalamic infusion of 100 μM THIP, but not 50 μM THIP, significantly alters AC spontaneous oscillatory profile relative to aCSF infusion. **(A)** AC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for 50 μM THIP and aCSF treatments. **(B)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous AC oscillatory profile and presented as group mean ± SEM for the aCSF and 50 μM THIP treatments. **(C)** FC mean spontaneous oscillatory profile plotted as scaled power over 0 –

30 Hz and 30 – 50 Hz (inset) for 50 μM THIP and aCSF treatments. **(D)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous FC oscillatory profile and presented as group mean ± SEM for the aCSF and 50 μM THIP treatments. **(E)** AC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for 100 μM THIP and aCSF treatments. **(F)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous AC oscillatory profile and presented as group mean ± SEM for the aCSF and 100 μM THIP treatments. Note that there is a significant interaction for treatment x frequency ($p < 0.05$), and that the delta, theta, and gamma bins are all different ($p < 0.05$) between the 100 μM THIP and aCSF treatments. **(G)** FC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for 100 μM THIP and aCSF treatments. **(H)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous FC oscillatory profile and presented as group mean ± SEM for the aCSF and 100 μM THIP treatments. Note that the scaled power axes shown in A, C, E, and G are different between the 0 – 30 Hz and 30 – 50 Hz spontaneous oscillatory profiles. Refer to Materials and Methods for derivation of these measurements and Results for statistical tests used. N = 12 for all treatments. *$p < 0.05$.

Decreased Thalamic Inhibition. For spontaneous AC oscillations (**Fig. 8A, B**), local MGB infusion of 50 μM gabazine, compared to the aCSF control infusion, resulted in a significant treatment x frequency interaction (two-way RM-ANOVA: $F_{(3,33)} = 13.27$; $p < 0.001$). Furthermore, *post hoc* paired-samples, two-tailed t tests revealed that the 50 μM gabazine infusion, relative to the aCSF infusion, caused significantly increased AC theta (aCSF: 3.84 ± 0.22 vs 50 μM gabazine: 6.01 ± 0.46; $p < 0.01$) and AC alpha activity (aCSF: 1.78 ± 0.20 vs 50 μM gabazine: 2.95 ± 0.28, $p < 0.01$), as well as an increase in AC delta activity (aCSF: 2.79 ± 0.20 vs 50 μM gabazine: 3.52 ± 0.20; $p = 0.031$). Similar to the THIP infusions (described above), a two-way RM-ANOVA did not reveal a significant treatment x frequency interaction for spontaneous FC oscillations between the 50 μM gabazine and aCSF infusions (**Fig. 8C, D**), but did reveal a significant effect of frequency ($F_{(1.82,20.06)} = 101.61$; $p < 0.001$). Despite this significant frequency effect, *post hoc* paired-samples, two-tailed t tests did not find any significant differences between the 50 μM gabazine and aCSF infusions for any FC frequency bins.

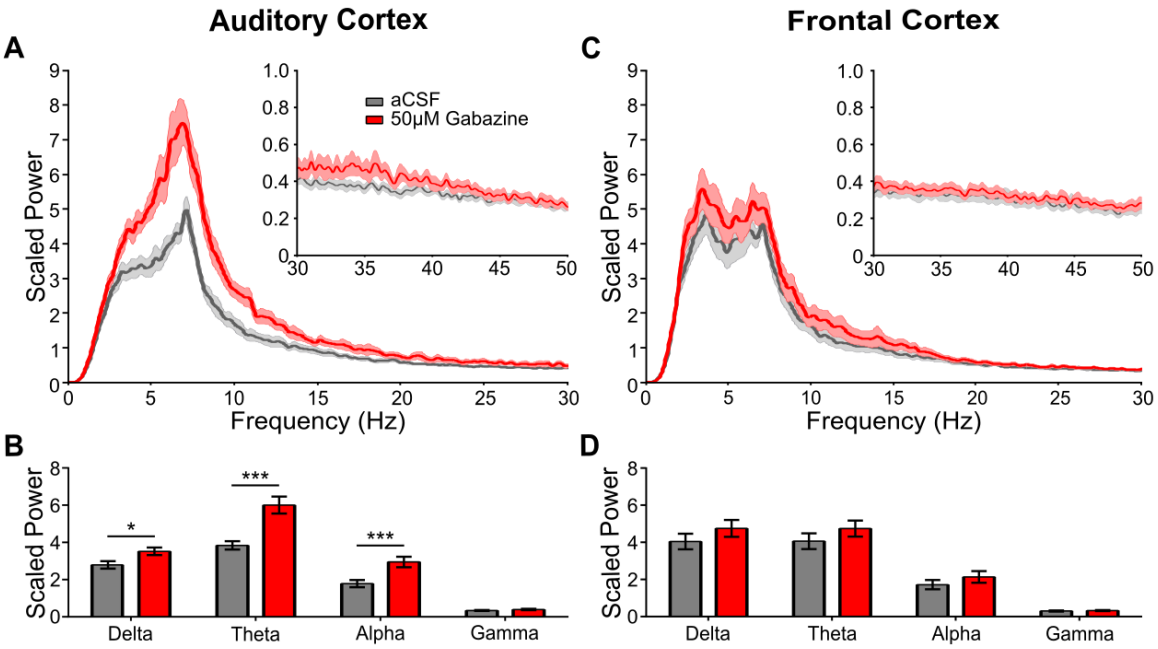

Figure 8. Unilateral thalamic infusion of 50 μM gabazine significantly alters AC spontaneous oscillatory profile relative to aCSF infusion. (A) AC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for 50 μM gabazine and aCSF treatments. **(B)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous AC oscillatory profile and presented as group mean ± SEM for the aCSF and 50 μM gabazine treatments. Note that the theta and alpha bins are both significantly different ($p < 0.0125$) and the delta bin is different ($p < 0.05$) between the 50 μM gabazine and aCSF treatments. **(C)** FC mean spontaneous oscillatory profile plotted as scaled power over 0 – 30 Hz and 30 – 50 Hz (inset) for 50 μM gabazine and aCSF treatments. **(D)** Mean power within the delta, theta, alpha, and gamma frequency bins calculated from each rat's spontaneous FC oscillatory profile and presented as group mean ± SEM for the aCSF and 50 μM gabazine treatments. Note that the scaled power axes for both A and C are different between the 0 – 30 Hz and 30 – 50 Hz spontaneous oscillatory profiles. Refer to Materials and Methods for derivation of these measurements and Results for statistical tests used. N = 12 for all treatments. ***$p < 0.0125$ (Bonferroni-corrected α), *$p < 0.05$.

In addition to uncovering the spontaneous cortical oscillatory effects following a drug-induced increase or decrease in local MGB inhibition, we investigated the effects that the aforementioned thalamic infusions had on the ITC and EP of the AC and FC 40 Hz ASSR. For the comparison of AC ITC (**Fig. 9A, Fig. 10A**) among all thalamic infusions (i.e., aCSF, 50 μM THIP, 100 μM THIP, 50 μM gabazine), a one-way RM-ANOVA revealed a significant treatment effect ($F_{(2.15,23.62)} = 4.078$; $p < 0.05$). Moreover, *post hoc* paired-samples, two-tailed t tests further revealed a significant increase in AC ITC for the 100 μM THIP infusion, compared to the aCSF control condition (100 μM THIP: 0.52 ± 0.036 vs aCSF: 0.36 ± 0.062; $p < 0.01$). Unexpectedly, there was also an increase in AC ITC for the 50 μM gabazine infusion (0.48 ± 0.034; $p = 0.048$), compared to the infusion of aCSF. Additional one-way RM-ANOVAs for AC EP (**Fig. 9A, Fig. 10A**) and FC ITC and EP (**Fig. 9B, Fig. 10B**) did not reveal any significant treatment effects between the thalamic infusions.

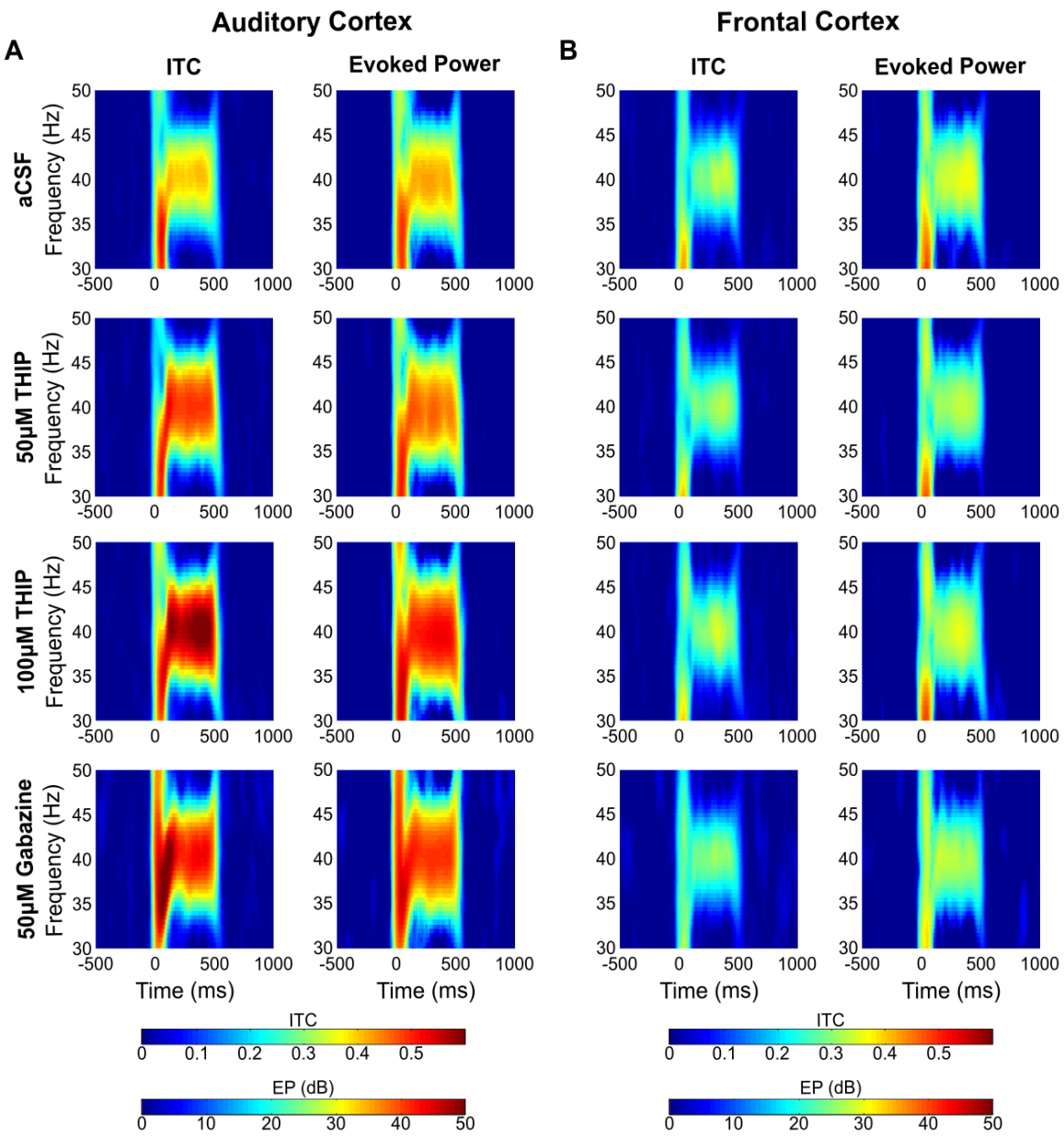

Figure 9. Spectrograms for ITC and EP of the ASSR following thalamic drug infusions. Group averaged data for ITC and EP derived from the AC **(A)** and FC **(B)** 40 Hz ASSR is presented for aCSF, 50 μM THIP, 100 μM THIP, and 50 μM gabazine treatments. Both ITC and EP are plotted from 30 Hz to 50 Hz and -500 ms to 1000 ms with respect to stimulus onset (occurs at 0 ms). Scale bars denote magnitudes of ITC and EP values with warmer colours indicating larger magnitudes. Refer to Materials and Methods for details on the derivation of these measurements. N = 12 for all treatments.

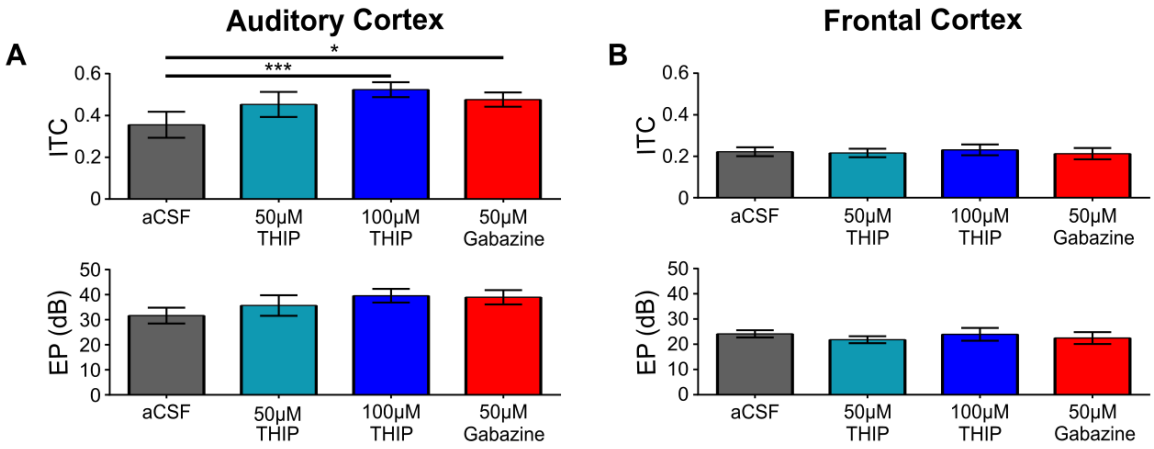

Figure 10. Thalamic infusion of 100 μM THIP augments ITC of the AC ASSR. Group averaged ITC and EP values for the AC **(A)** and FC **(B)** 40 Hz ASSRs derived from each rat's ITC and EP region of maximum response (i.e., 35 Hz to 45 Hz and 100 ms to 400 ms). Data is presented as group mean ± SEM for the aCSF, 50 μM THIP, 100 μM THIP, and 50 μM gabazine treatments. Compared to the AC ITC for the aCSF-treated rats, 100 μM THIP-treated rats exhibited a significant increase ($p < 0.0167$) and 50 μM gabazine-treated rats exhibited an increase ($p < 0.05$) in AC ITC. There were no significant differences between treatments for AC EP, FC ITC, or FC EP. Refer to Results for details about statistical tests used. N = 12 for all treatments. ***$p < 0.0167$ (Bonferroni-corrected α), *$p < 0.05$.

Summary of Experimental Series 2A – Electrophysiology. Partially in line with our predictions, there was a dose-response effect for altered spontaneous AC oscillations as the concentration of locally-infused THIP increased (i.e., significant changes to AC oscillations at 100 μM THIP, but not 50 μM THIP, relative to the aCSF control condition). Despite infusion of 100 μM THIP significantly altering spontaneous AC oscillatory activity (as revealed by two-way RM-ANOVA), the frequency bin-specific changes following 100 μM THIP were only partially supportive of the TCD model of tinnitus. Specifically, 100 μM THIP infusion, compared to infusion of aCSF, caused a predicted increase in AC delta and AC theta activity, but no change in AC alpha activity, and, unexpectedly, a decrease in AC gamma activity. Interestingly, thalamic infusion of 50 μM gabazine, compared to aCSF infusion, resulted in a robust increase in low-frequency AC oscillations (i.e., significant increase in theta and alpha activity, increase in delta activity) with no change in AC gamma activity; a finding that was contrary to our predictions. Investigation into altered evoked cortically oscillatory activity, through the utilization of the 40 Hz ASSR, revealed that, in line with our predictions, 100 μM THIP infusion was sufficient to increase AC ITC. Unexpectedly, infusion of 50 μM gabazine caused a slight increase in AC ITC, suggesting that targeting GABAergic activity within the MGB in opposing ways does not bring about opposing effects on the AC 40 Hz ASSR. Lastly, in line with our predictions, none of the thalamic infusions, compared to the aCSF control condition, significantly altered FC spontaneous or evoked oscillatory activity, supporting the idea that altered activity of TC relay cells from the MGB exerts specific AC oscillatory changes, and not widespread cortical effects.

Collectively, these aforementioned findings confirm that the local increase (via 100 μM THIP) or decrease (via 50 μM gabazine) in the level of MGB GABAergic inhibition is sufficient to bring about changes that are specific to AC oscillatory activity. Importantly, these findings partially support the role for increased thalamic tonic inhibition inducing a TCD-like AC oscillatory profile, allowing us to proceed to the behavioural investigations of Experimental Series 2B, in order to directly test whether these alterations in AC oscillatory activity are indeed sufficient to induce behavioural evidence of tinnitus.

3.2.2 Experimental Series 2B – Behaviour

Armed with the electrophysiological confirmation (Experimental Series 2A, above) that manipulation of local MGB inhibition disrupts AC oscillatory activity, we tested whether these changes in AC oscillations were sufficient to induce a tinnitus-like behavioural profile, as would be predicted by the TCD model of tinnitus. There were 8 rats used for this Experimental Series, all of which were confirmed to have their bilateral infusion cannulae projecting into the targeted thalamic region (see **Fig. 11**).

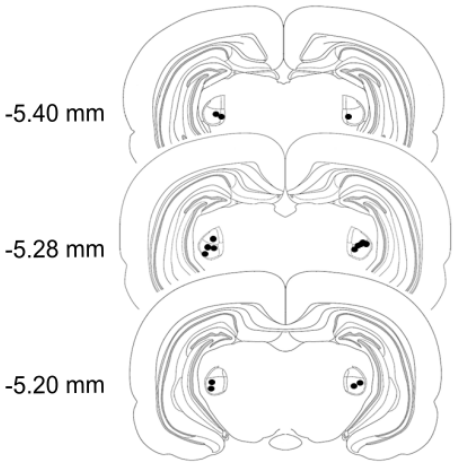

Figure 11. Schematized representation of confirmed bilateral infusion cannulae tips located in the MGBs from the recovered brain tissues of rats used in behavioural experiments (n = 8). Length measurements represent the rostral-caudal distance relative to bregma. Adapted from Paxinos & Watson (2007).

50 μM THIP vs aCSF. For both the quiet (left panel of **Fig. 12A**) and AM (middle panel of **Fig. 12A**) stimuli trials, separate two-way RM-ANOVAs did not show any significant interactions or effects for either of the acoustic conditions. For the NBN stimulus trials (right panel of **Fig. 12A**), a two-way RM-ANOVA failed to reveal a significant treatment x time interaction; however, a significant effect of time was found ($F_{(2,14)} = 5.90$; $p < 0.05$). Additionally, *post hoc* paired-samples, two-tailed t tests revealed two differences: namely, (1) the proportion of correctly identified NBN trials decreased following infusion of 50 μM THIP (50 μM THIP Test: 94.56 ± 1.19 %) compared with the day prior to 50 μM THIP infusion (50 μM THIP Pre: 98.01 ± 0.38 %; $p = 0.011$) and (2) this measure increased on the day following 50 μM THIP infusion (50 μM THIP Post: 98.66 ± 0.46 %) compared with the day following aCSF infusion (aCSF Post: 96.14 ± 0.98 %; $p = 0.01$).

100 μM THIP vs aCSF. Similar to the behavioural results described above, two-way RM-ANOVAs failed to reveal any significant interactions or effects for either the quiet (left panel of **Fig. 12B**) or the AM (middle panel of **Fig. 12B**) stimuli trials. While there was no significant interaction for the NBN stimulus trials (right panel of **Fig. 12B**), a two-way RM-ANOVA did reveal a significant effect of time ($F_{(2,14)} = 4.23$; $p < 0.05$), and *post hoc* paired-samples, two-tailed t tests showed a decrease in the proportion of correctly identified NBN trials following infusion of 100 μM THIP (100 μM THIP Test: 93.47 ± 1.61 %) compared with the day prior to 100 μM THIP infusion (100μM THIP Pre: 96.98 ± 0.86; $p = 0.027$).

50 μM Gabazine vs aCSF. For this last comparison of thalamic infusions, two-way RM-ANOVAs for the quiet (left panel of **Fig. 12C**) and NBN (right panel of **Fig. 12C**) stimuli trials failed to reveal any significant interactions or effects for either of the acoustic conditions. For the AM stimulus trials (middle panel of **Fig. 12C**), a two-way RM-ANOVA failed to reveal a significant interaction of treatment x time, but did reveal a significant effect of time ($F_{(2,14)} = 4.40$; $p < 0.05$). Additionally, *post hoc* paired-samples, two-tailed t tests revealed that the proportion of AM trials identified as NBN was significantly different between the day prior to aCSF infusion (aCSF Pre: 5.91 ± 1.03 %) and the day prior to 50 μM gabazine infusion (50 μM gabazine Pre: 2.10 ± 0.75; $p < 0.01$). Lastly, *post hoc* paired-samples, two-tailed t tests showed that the proportion of

AM trials identified as NBN following infusion of 50uM gabazine (50 μM gabazine Test: 11.58 ± 2.99 %) was increased ($p = 0.019$) compared with the day prior to 50 μM gabazine infusion (50 μM gabazine Pre).

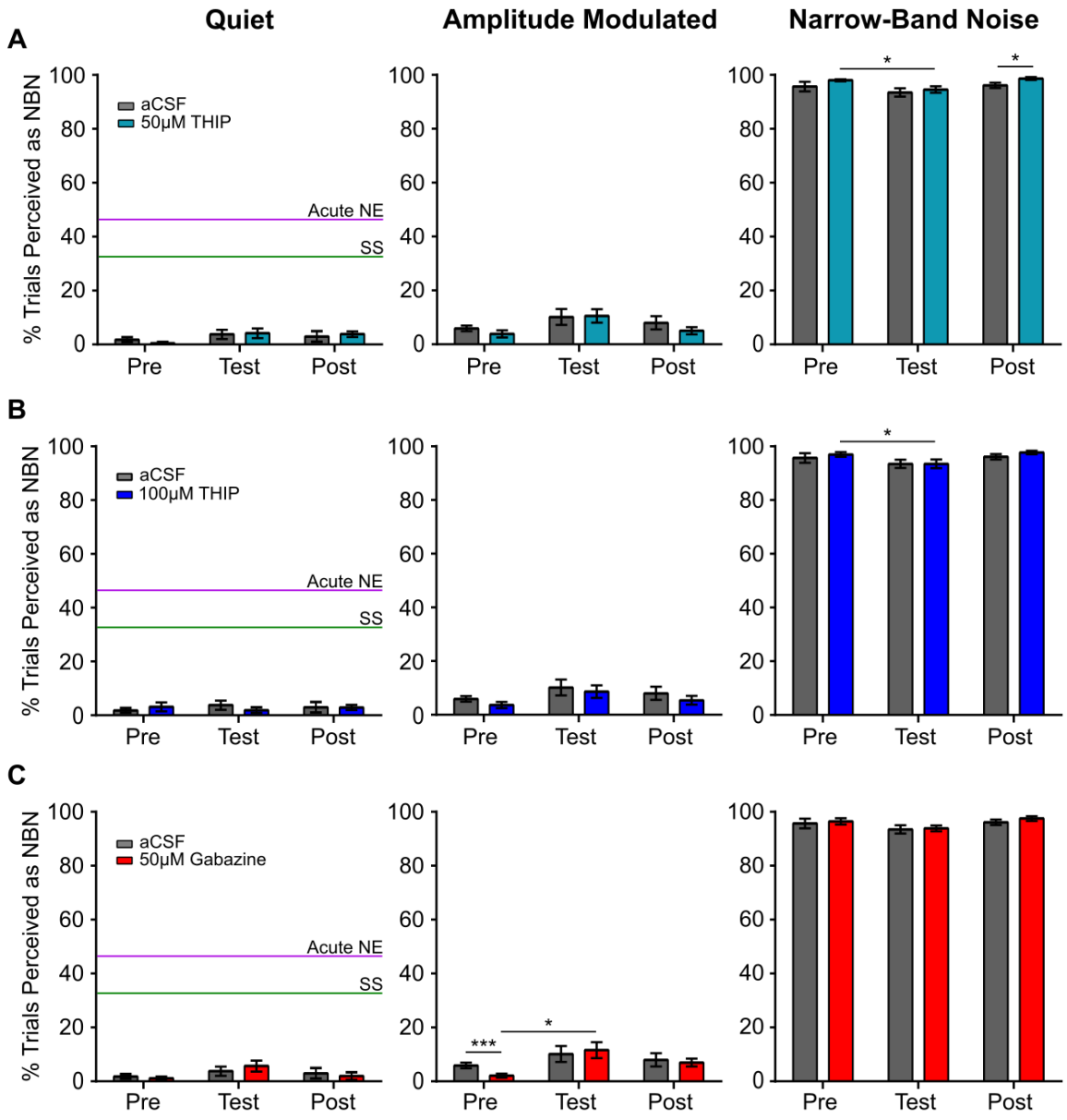

Figure 12. Rats trained on two-choice operant conditioning paradigm do not exhibit behavioural evidence of tinnitus following any of the bilateral thalamic infusions. Data are shown as group mean ± SEM for behavioural performance on the day before ("Pre"), the day of ("Test"), and the day after ("Post") treatment. Rats treated with either **(A)** 50 μM THIP, **(B)** 100 μM THIP, or **(C)** 50 μM gabazine do not exhibit a tinnitus-like behavioural profile as evidenced by the very low proportion of quiet trials identified as NBN following treatment with any of these thalamic infusions compared with aCSF infusion (control) or the group's Pre day performance. Statistical analyses revealed minor changes to AM and NBN stimulus trial performance; refer to Results for details. The

proportion of quiet trials identified by these same rats as NBN following SS and acute NE treatments (as shown in Fig. 2) are shown here for reference. N = 8 for all treatments. ***$p < 0.01$ (Bonferroni-corrected α), *$p < 0.05$.

Summary of Experimental Series 2B – Behaviour. In contrast to our predictions, bilateral thalamic infusion of either 50 μM THIP or 100 μM THIP did not cause behavioural evidence of tinnitus. Ultimately, the confirmation that thalamic infusions of 100 μM THIP was sufficient to alter AC oscillatory activity (Experimental Series 2A, above), but did not induce behavioural evidence of tinnitus, fails to support the causal relationship between elevated thalamic tonic inhibition and generation of tinnitus, which is central to the TCD model of tinnitus. In line with our predictions, bilateral thalamic infusion of either aCSF or 50 μM gabazine did not result in behavioural evidence of tinnitus. Collectively, the findings obtained in Experimental Series 2, in line with the findings of Experimental Series 1, fail to support a clear link between altered cortical oscillatory activity and the manifestation of tinnitus.

Chapter 4

4 Discussion

The overarching goal of this thesis was to provide further insight into the relationship between altered cortical oscillations and tinnitus. In order to carry out this objective, two Experimental Series – both employing behavioural and *in vivo* electrophysiological techniques in rat models – were performed. The objective of Experimental Series 1 was to investigate the potential changes in oscillatory activity, as recorded from the auditory (AC) and frontal (FC) cortices, following exposure to either 250 mg/kg (i.p.) sodium salicylate (SS) or a transient bout of loud noise (acute NE); both of which are known tinnitus inducers. Importantly, this Experimental Series served as one of the few studies into the relationship between altered cortical oscillations and tinnitus using animal models. The objective of Experimental Series 2 was to investigate the cortical oscillatory and behavioural effects of manipulating local inhibition within the MGB – an investigation, which, to our knowledge, was the first direct study of the TCD model of tinnitus. Through performing these Experimental Series, three novel findings were discovered: (1) While SS (250 mg/kg, i.p.) and acute NE treatments were sufficient to induce behavioural evidence of tinnitus, these two treatments caused dissimilar effects on spontaneous cortical oscillations; (2) Manipulation of MGB inhibition, through local drug infusion, was capable of altering spontaneous AC oscillations, but did not result in behavioural evidence of tinnitus; and (3) SS (250 mg/kg, i.p.) and acute NE treatments caused 40 Hz ASSR deficits that were restricted to the FC, whereas effects on the ASSR following manipulation of local MGB inhibition was restricted to the AC. The interpretation of these novel findings, how they relate to our current understanding of the relationship between altered cortical oscillations and tinnitus, and their implications for future studies are discussed in detail throughout this chapter.

4.1 Altered Spontaneous Oscillations are not Solely Predictive of the Presence of Tinnitus

In the present study, we found that exposure to either 250 mg/kg (i.p.) sodium salicylate (SS) or a single bout of loud noise (acute NE) – two well-known inducers of tinnitus –

caused dissimilar effects on spontaneous cortical oscillations. Specifically, treatment with SS (250 mg/kg, i.p.), relative to the saline control condition, resulted in a significant decrease in auditory cortex (AC) delta and alpha activity and a significant increase in gamma activity. In stark contrast, treatment with acute NE failed to cause any significant differences in spontaneous oscillatory activity compared to its control condition, sham NE.

When interpreting the findings obtained in this study in the context of results obtained in previous studies, it is important to note that the majority of previous studies have been carried out in human subjects. These human-based studies often compare spontaneous oscillatory activity of chronic tinnitus sufferers to normal controls. Evidence from these studies over the past few decades have provided some support for a characteristic AC spontaneous oscillatory profile exhibited by tinnitus subjects, which includes increased delta-theta (Llinás et al. 1999; Weisz et al. 2005; Adjamian et al. 2012), decreased alpha (Llinás et al. 1999; Weisz et al. 2005; Lorenz et al. 2009), and increased gamma activity (Weisz et al. 2007; Ashton et al. 2007; Lorenz et al. 2009; Ortmann et al. 2011). The changes in spontaneous AC oscillations following SS (250 mg/kg, i.p.) treatment observed here are partially in line with this characteristic oscillatory profile; specifically, a decrease in alpha and increase in gamma activity. While there have been no human-based investigations into potential cortical oscillations changes following SS-induced tinnitus, previous animal studies that have investigated this relationship have yielded results that are in agreement with those obtained in this study. For example, Stolzberg et al. (2013) observed an SS-induced decrease in alpha activity and increase in gamma activity, while Berger et al. (2017) found an SS-induced decrease in alpha activity. Therefore, the SS-induced decrease in AC alpha and increase in AC gamma activity are in line with what would be predicted by the literature.

Enhanced delta-theta activity has been reported in some EEG/MEG studies of tinnitus subjects (Llinás et al. 1999; Weisz et al. 2005; Adjamian et al. 2012). These increased low-frequency oscillations are widely considered to reflect aberrant neural activity, consequent on peripheral deafferentation, which is necessary to induce the alterations in other frequency bins (e.g., alpha, gamma) that ultimately give rise to tinnitus (Llinás et al.

2005; Weisz, Dohrmann, and Elbert 2007; Weisz et al. 2011). Despite being observed in some human neural recording studies, animal electrophysiological studies have yet to confirm enhanced delta-theta activity following SS treatment: e.g., delta-theta activity has been observed to either remain unchanged (Noreña et al. 2010; Berger et al. 2017) or decrease (Stolzberg et al. 2013, as well as the present study) following exposure to high doses of SS. One possibility for this discrepancy is that the mechanisms responsible for elevated delta-theta activity [e.g., proposed thalamocortical burst firing (Llinás et al. 1999; Llinás et al. 2005)] that is observed in some tinnitus subjects takes time to develop following peripheral deafferentation, whereas tinnitus occurs relative quickly following excessive exposure to SS (e.g., within a couple hours, as evidenced by the behavioural results of Experimental Series 1A).

In the present study, we found that exposure to transient loud noise, while sufficient to induce behavioural evidence of tinnitus, failed to significantly alter spontaneous cortical oscillations. Importantly, this finding contradicts the notion that altered spontaneous oscillatory activity is necessary for the generation of tinnitus – a belief that is strongly held (Llinás et al. 2005; Weisz et al. 2011; De Ridder et al. 2015). To date, there has been one human-based study and one animal model study investigating the effects of loud noise exposure on cortical oscillatory activity. The former study, performed by Ortmann et al. (2011), observed an increase in AC gamma activity in amateur rock musicians that experienced tinnitus following band practice. The discrepancies between the findings of the present study and those of Ortmann et al. (2011) could be due to the fact that our study was performed in rats. Additionally, the methods for noise-induced tinnitus differed between the two studies: our study used a tonal noise exposure, whereas the study by Ortmann et al. (2011) induced tinnitus through exposure to loud music. The latter study, performed by Noreña et al. (2010), found a general decrease in 10 – 30 Hz AC activity following loud noise exposure in guinea pigs. It is important to note that there were several methodological differences between the present study and the study conducted by Noreña et al. (2010). For example, the protocol used for loud noise exposure differed drastically between the two studies: i.e., a $1/3^{rd}$ octave band centered on 8 kHz presented at 115 dB SPL for 2 hours (Noreña et al. 2010) compared to a 12 kHz tone presented at 112 dB SPL for 15 min (present study). Additionally, Noreña et al. (2010) failed to

compare their changes in cortical oscillations following noise exposure to a relevant control condition, such as the sham NE treatment as used here. Therefore, it is difficult to interpret their observed changes in oscillations as solely due to the noise exposure, and not due to some other confounding factor. Given the methodological differences, it is not entirely surprising that the results obtained in our study differed from the aforementioned study.

A potential limitation in our study is that the behavioural and electrophysiological experiments used separate cohorts of animals. Nevertheless, it is highly probable that rats given the SS (250 mg/kg, i.p.) and acute NE treatments for electrophysiological experiments were indeed experiencing tinnitus, as it was the same experimental parameters (e.g., dosage and waiting period of SS; loudness and duration of acute NE) that were used in the electrophysiological and behavioural experiments. Furthermore, our two-choice operant conditioning paradigm was confirmed to be robust against false positives: i.e., both SS (250 mg/kg, i.p.) and acute NE treatments caused tinnitus-like behaviour, whereas the control conditions (i.e., saline and sham NE) did not (see Results from Experimental Series 1A). Finally, the study performed by Stolzberg et al. (2013) observed SS-induced alterations in AC oscillatory activity that are directly in agreement with our findings. Importantly, Stolzberg et al. (2013) observed these oscillatory changes in rats that were concurrently performing on a two-choice operant conditioning task and exhibiting a tinnitus-like behavioural profile. Taken together, it is reasonable to suggest that the rats used for the electrophysiological experiments in our study were indeed experiencing tinnitus following treatment with either SS (250 mg/kg, i.p.) or acute NE, and were not experiencing tinnitus following treatment with the control conditions.

Collectively, the aforementioned findings of our study confirm that tinnitus-like behaviour can be induced in the presence (i.e., SS (250 mg/kg, i.p.) treatment) or absence (i.e., acute NE treatment) of significantly altered spontaneous cortical oscillations. Therefore, based on these findings, there does not appear to be a clear link between altered oscillatory activity and the manifestation of tinnitus.

4.2 Manipulation of MGB GABAergic Inhibition Alters Spontaneous Cortical Oscillations, but does not Induce Behavioural Evidence of Tinnitus

To our knowledge, our study was the first to report that putatively increasing MGB tonic inhibition results in altered spontaneous AC oscillations. Moreover, this increase in thalamic tonic inhibition, mediated by local infusion of 100 μM THIP, caused an increase in AC delta-theta activity that is in line with the TCD model of tinnitus (Llinás et al. 1999; Llinás et al. 2005). This enhanced delta-theta activity likely reflects an increased proportion of MGB burst firing onto the AC, as THIP, through its preferential agonism of δ-subunit-containing $GABA_A$ receptors, has been shown to trigger TC relay cells to undergo burst firing *in vitro* (Cope, Hughes, and Crunelli 2005; Sametsky et al. 2015). While these oscillatory changes give support to the TCD model of tinnitus, we found that bilateral MGB infusion of 100 μM THIP failed to induce behavioural evidence of tinnitus. Taken together, these electrophysiological and behavioural findings suggest that enhanced delta-theta activity within the AC (likely reflecting aberrant MGB burst firing) is not involved in the manifestation of a tinnitus percept.

A potential limitation of this study concerns the concentration of THIP used to elicit altered AC oscillatory activity. Specifically, whether or not this concentration was preferentially exerting its effects at extrasynaptic, δ-subunit-containing $GABA_A$ receptors, or whether it was eliciting widespread agonism of both synaptic and extrasynaptic $GABA_A$ receptors. Studies investigating the EC_{50} of THIP at extrasynaptic $GABA_A$ receptors typically found in TC relay cells (i.e., containing α_4 and δ subunits) have reported this value to be within the range of mM [35 mM (Meera, Wallner, and Otis 2011)] to μM concentrations [~ 10 μM (Brown et al. 2002; Mortensen et al. 2010), ~ 50 μM (Jia et al. 2005; Stórustovu and Ebert 2006)]. In contrast, the EC_{50} of THIP at the synaptic, γ-subunit-containing $GABA_A$ receptors, which mediate phasic inhibitory Cl^- currents (Farrant and Nusser 2005; Belelli et al. 2009), have been found to be much higher [100 – 200 μM (Brown et al. 2002; Jia et al. 2005; Meera, Wallner, and Otis 2011)] than at the extrasynaptic receptors. Moreover, 50 μM THIP has been reported to cause behaviourally- (Paydar et al. 2014) and electrophysiologically-relevant (Mesbah-

Oskui, Orser, and Horner 2014) changes when infused into thalamic nuclei. Taking these *in vitro* and *in vivo* results into account, we decided to use a low concentration of 50 μM THIP and a high concentration of 100 μM THIP for local thalamic infusions. As predicted, MGB infusion of 50 μM THIP was not sufficient to significantly alter AC oscillations, but the 100 μM THIP was sufficient for this purpose. Therefore, it is likely that the 100 μM THIP infusion was exerting its effects preferentially at extrasynaptic $GABA_A$ receptors because (1) this concentration was sufficient to alter AC oscillations in a way that is in agreement with the predictions of enhanced thalamic tonic inhibition (Llinás et al. 1999; Llinás et al. 2005) and (2) if the 50 μM THIP was more selective towards extrasynaptic $GABA_A$ receptors, it would be expected that this lower concentration would exert significant changes to AC oscillations, which was not found. It might be worthwhile for future studies to characterize the effects that local MGB infusion of a nonspecific $GABA_A$ receptor agonist, such as muscimol, has on AC oscillations, from which it can be inferred whether or not 100 μM THIP is causing altered AC oscillations through enhanced thalamic tonic inhibition or through a widespread agonism of both synaptic and extrasynaptic $GABA_A$ receptors in the MGB.

While it would be tempting to claim that TCD does not induce behavioural evidence of tinnitus, the oscillatory changes reported here for 100 μM THIP infusion only partially support the role for enhanced thalamic tonic inhibition in causing TCD. Interestingly, AC delta-theta activity was increased, but we also found a THIP-induced *decrease* in AC gamma activity – an oscillatory change that directly opposes the TCD model of tinnitus. It is possible that enhanced thalamic burst firing does not induce regions of aberrant gamma oscillations within the AC; however, the present study cannot verify this possibility, as our electrophysiological recordings were restricted to the cortex, and thus, we are not able to confirm whether or not thalamic burst firing activity was actually affected following our thalamic drug infusions. That being said, it will be crucial for future studies into the TCD model of tinnitus to confirm that enhanced thalamic tonic inhibition indeed causes an increase in burst firing of MGB TC relay cells *in vivo*, and that the enhanced burst firing component gives rise to the altered spontaneous AC oscillations that are characteristic of TCD.

In addition to investigating the role of enhanced thalamic tonic inhibition in the context of TCD and tinnitus, we were also interested in characterizing the effects of decreased thalamic inhibition on spontaneous cortical oscillations, as we were unaware of prior studies that have investigated this relationship. Through local MGB infusion of 50 μM gabazine, a $GABA_A$ receptor antagonist, we found a robust increase in low frequency AC oscillations; e.g., increased power can be seen for oscillations < 20 Hz. Despite this profound disruption in low frequency AC oscillations, bilateral infusion of 50 μM gabazine failed to significantly affect performance on the two-choice operant conditioning paradigm. Taken together with the results from the 100 μM THIP thalamic infusions, manipulation of MGB GABAergic inhibition can result in either a specific disruption of AC delta-theta activity (100 μM THIP) or a broad disruption of AC low-frequency oscillations (50 μM gabazine), both of which can occur in the absence of tinnitus-like behaviour. It is important to note that the cohort of rats used for the behavioural thalamic infusion experiments were the same as those used for the tinnitus-inducing treatments described above. Given the sensitivity of our two-choice operant conditioning paradigm for detecting tinnitus-like behaviour in this cohort of rats and its resilience against screening for false positives, there is a high probability that if any of our thalamic drug infusions gave rise to tinnitus, it would have been detected by our behavioural paradigm. Therefore, the findings of our study do not support a role for enhanced low frequency AC oscillations in the generation of tinnitus.

4.3 Is Enhanced Gamma Activity Important in Tinnitus?

Enhanced AC gamma activity has been proposed to be a neural correlate of tinnitus (Llinás et al. 2005; Weisz, Dohrmann, and Elbert 2007). In the present study, we have identified four different relationships between altered AC gamma activity and behavioural performance. These relationships include: (1) significant gamma increase and tinnitus-like behaviour (250 mg/kg (i.p.) SS); (2) slight gamma decrease and tinnitus-like behaviour (acute NE); (3) modest gamma decrease and normal behaviour (100 μM THIP); and (4) no gamma change and normal behaviour (50 μM THIP and 50 μM gabazine). As our findings suggest, the relationship between altered gamma activity and tinnitus is unclear. In fact, within the human EEG/MEG studies, there have been several

findings of increased spontaneous gamma exhibited by tinnitus subjects (Ashton et al. 2007; Weisz et al. 2007; Lorenz et al. 2009; Ortmann et al. 2011) as well as findings that spontaneous gamma activity is not different between tinnitus subjects and normal controls (Adjamian et al. 2012; Zobay et al. 2015). Further troubling for the interpretation of gamma and the manifestation of tinnitus are the EEG/MEG studies that attempt to correlate changes in gamma activity with changes in tinnitus percept severity. For example, van der Loo et al. (2009) found that changes in gamma correlate with tinnitus percept severity, whereas Kahlbrock and Weisz (2008) found no such correlation. Lastly, and most disconcerting, were the findings of Sedley et al. (2012), who found that tinnitus subjects experiencing increased tinnitus severity (following residual excitation) exhibited a *decrease* in gamma activity.

Taken together, the aforementioned results from human neural recording studies and findings from the present study suggests that a complex relationship between altered gamma activity and tinnitus exists. While the present study identified several different combinations of gamma-behaviour relationships (discussed above), there were no treatments associated with enhanced gamma activity in the presence of normal behaviour. Therefore, to gain further insight into the possible importance of increased AC gamma activity in tinnitus generation, it would be useful for future studies to explore the behavioural effects (using a robust behavioural paradigm, such as the two-choice operant conditioning paradigm employed in our study) following confirmed enhancement of AC gamma activity. One potential technique that could be useful in this context would be driving gamma activity within the AC through the use of optogenetics, which has recently been performed in other cortical regions using *in vivo* animal models (Cardin et al. 2009; Sohal et al. 2009).

4.4 40 Hz ASSR: A Potential Screening Tool for the Presence of Tinnitus in Animals?

The 40 Hz ASSR offers an alternate approach to investigating potential changes in gamma oscillations (in this case, evoked oscillations) and may provide insight into functional abnormalities in neuronal circuits important for maintaining gamma activity (Brenner et al. 2009). In the present study, we performed the first tinnitus-related ASSR

investigation in an animal model. We had originally predicted that the AC ASSR would show enhanced ITC and EP, consistent with the proposal that AC gamma activity, and neuronal synchrony in general, is elevated in the tinnitus state. Contrary to our predictions, we found that both tinnitus-inducing insults (i.e., SS (250 mg/kg, i.p.) and acute NE) did not significantly affect the AC ASSR measures compared to their respective controls. Interestingly, local MGB infusion of 100 μM THIP significantly increased the ITC of the AC ASSR. This enhanced AC ITC following 100 μM THIP infusion implies that the functional capacity of AC neuronal circuits that carry out activity within gamma frequencies is augmented. While this is a novel finding, it does not have implications for tinnitus-related abnormal neural activity, as the 100 μM THIP infusion did not significantly affect behavioural performance on our two-choice operant conditioning paradigm. Taken together, these findings suggest that the AC 40 Hz ASSR is not a useful measure for assessing oscillatory abnormalities associated with tinnitus in animals.

In contrast, the changes to the FC ASSR proved to be in line with the presence/absence of tinnitus-like behaviour. For example, despite the disparate effects on spontaneous cortical oscillations, both SS (250 mg/kg, i.p.) and acute NE treatments caused a decrease in FC ITC. Furthermore, treatments that do not induce behavioural evidence of tinnitus (e.g., all thalamic infusions) did not significantly affect the FC ASSR. While this is the first study to probe for tinnitus-related neuropathological activity using the ASSR in animals, our findings suggest that the FC 40 Hz ASSR may be a useful screening tool (with high throughput potential) for the presence of tinnitus. Future studies will need to clarify whether the use of the FC ASSR is appropriate in this context.

A potential limitation here is the argument that the FC ASSR is not a genuine ASSR evoked from the FC, and is instead a result of volume conduction of the AC ASSR. If this were true, then the FC ASSR would be expected to change in the same direction as the AC ASSR following all of the treatments performed in our study. However, this is not the case, as the results of our study demonstrate that the FC ASSR can be significantly altered, while the AC ASSR remains unchanged (e.g., following treatment with either SS (250 mg/kg, i.p.) or acute NE), or the FC can remain unchanged, while the AC ASSR is

significantly altered (e.g., following thalamic infusion of 100 μM THIP). Therefore, it would appear that the FC ASSR observed in this study is a genuine steady-state response. Further support for this notion comes from studies showing that auditory-evoked responses can be elicited from brain regions outside of the central auditory system [e.g., FC (Simpson and Knight 1993), parietal cortex and hippocampus (Sullivan et al. 2015)].

While it is difficult to interpret the FC ASSR deficits associated with the tinnitus-inducing treatments at this time (due to limited previous studies of this nature), these findings are in line with those of Schlee et al. (2008). This group used a 37 Hz ASSR to investigate the coherency between different brain regions (e.g., temporal, frontal, parietal, and anterior cingulate cortices) in order to probe for abnormalities in functional connectivity within human tinnitus subjects (Schlee et al. 2008). Interestingly, they found abnormal connectivity within tinnitus subjects that involved FC regions, suggesting that FC abnormalities may be important in the generation and/or conscious awareness of tinnitus (Schlee et al. 2008). Given the findings of Schlee et al. (2008), along with the findings of the present study, we suggest that future studies explore the relationship between AC and FC coherency using the 40 Hz ASSR to elucidate potential aberrant communication between cortical regions that are implicated in tinnitus. It could very well be that aberrant communication between brain regions (e.g., AC and FC) is necessary for the conscious awareness of tinnitus, as proposed by various network models of tinnitus (Leaver et al. 2011; De Ridder et al. 2011; Rauschecker et al. 2015), and not just abnormal neural activity confined to a single brain region.

4.5 Conclusion

This study was one of the few animal model investigations into the relationship between altered cortical oscillations and tinnitus and, to our knowledge, the first direct investigation of the TCD model of tinnitus. Importantly, this study revealed that, while alterations in cortical oscillations are observed following some tinnitus-inducing insults, these alterations might not be necessary to manifest the tinnitus-like behavioural profile. Additionally, the putative increases or decreases of local MGB inhibition can elicit altered cortical oscillations, but is not sufficient to cause behavioural evidence of tinnitus.

In conclusion, our study has revealed three distinct scenarios in the context of the cortical oscillations-behavioural performance relationship: (1) Significant spontaneous oscillations and behavioural changes (SS (250 mg/kg, i.p.) treatment); (2) Only significant behavioural changes (acute NE treatment); and (3) Only significant spontaneous oscillations changes (100 μM THIP & 50 μM gabazine). These findings suggest that the relationship between altered cortical oscillations and tinnitus is complex, and future studies are needed in order to gain further insight into this relationship. Lastly, the use of the 40 Hz ASSR has revealed cortex-specific deficits in stimulus-driven gamma oscillations, which might be important in tinnitus, and suggests that the FC 40 Hz ASSR might be a future high-throughput screening method for assessing the presence of tinnitus in animal models. Future studies will need to validate this possibility.

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