THE EFFECTS OF BACLOFEN ON CHRONIC TINNITUS INDUCED BY ACOUSTIC TRAUMA

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Dedicated to my parents and grandparents (Aie and baba)
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ABSTRACT

Tinnitus is characterised by a phantom ringing in the ear. Approximately twelve million Americans experience tinnitus in a form severe enough to require medical attention. Despite this prevalence there is no effective treatment available due to the uncertain pathophysiology underlining tinnitus. Acoustic trauma is the most common cause of tinnitus and is often associated with neuronal hyperactivity in the central auditory system. Hence any drug that increases GABAergic neurotransmission within the central nervous system, such as baclofen, would decrease hyperactivity and thus alleviate tinnitus. To test this, sixteen Wistar rats were divided into sham (n = 8) and tinnitus (n = 8) groups. Tinnitus was induced by unilateral exposure to acoustic trauma and the presence of tinnitus was assessed by a frequency-specific shift in the discrimination function with a conditioned lick suppression paradigm. Hearing thresholds were also examined by acoustic brainstem-evoked responses (ABR). Once tinnitus was confirmed, the animals were injected with saline (vehicle) followed by baclofen at three different doses (1, 3 and 5 mg/kg) and reassessed for the presence or absence of tinnitus. Acoustic trauma significantly increased the ABR thresholds in the exposed ear and caused a significant downshift in the frequency-response curve in the noise exposed animals, which was indicative of tinnitus. Baclofen alleviated tinnitus like behaviour with the best results achieved at the 5mg/kg dose. However future research is required to determine optimal dose range and optimal treatment duration to ensure complete alleviation of tinnitus in both animals and human beings.
CHAPTER 1

INTRODUCTION

- Tinnitus
- Anatomy of the ear
- Auditory brainstem response
- Pathophysiology of tinnitus
- Non classical auditory pathway
- Baclofen
“Tinnitus is described as the perception of sound that results exclusively from activity within the central nervous system without any mechanical or vibratory stimuli within the cochlea” (Simpson and Davies 1999). It is estimated that approximately 12 million Americans experience tinnitus in a form severe enough to require medical attention (Shargorodsky et al. 2010). It often co-exists with any condition that leads to deteoriation of hearing and eventual hearing loss. Despite the prevalence and morbidity of tinnitus, its underlying pathophysiology is uncertain (Kaltenbach 2000). Tinnitus is often misconstrued to be an auditory dysfunction, as it is a perception of sound. However increasing evidence suggests the anatomical location of tinnitus is more likely to be the central nervous system, i.e. even though the peripheral and central auditory system are involved in the symptomology of tinnitus its maintenance is likely to be within the central nervous system. The fact that deaf people and individuals who have had their auditory nerve severed can hear tinnitus is consistent with the notion that tinnitus is not directly related to the ear. It can be caused by age or noised induced trauma, middle ear infections, Ménière’s disease or even non-otological conditions such as stroke, diabetes and allergies. Despite all of the above putative primary causes of tinnitus, how tinnitus is developed and maintained is still a mystery. Consequently this uncertainty has been an obstacle in discovering a reliable drug treatment for tinnitus (Kaltenbach 2000).

Some commonly used treatments are anxiolytics, sedatives, osmotic regulators, anti-spasticity drugs and antidepressants. However most of these drugs have not undergone systematic clinical or pre-clinical trials (Simpson and Davies 1999). Benzodiazepines (BZP) and tricyclic antidepressants (TCA) have shown some promise in clinical trials but none of these can be consistently agreed to provide replicable long
term relief from tinnitus. Reports suggest cessation of BZPs brings back tinnitus to its original severity or sometimes even worse; this along with problems of dependency of these drugs makes their therapeutic potential in treating tinnitus questionable (Dobie 1999). TCAs are most effective in patients who suffer from depression, sleep deprivation, or other psychological factors which may make the perception of tinnitus worse (Dobie, 1999; Goodey, 2007). The success with most anti-tinnitus drugs is variable, some having an efficacious effect, while others are ineffective (Dobie, 1999; Lockwood et al. 2002). It is likely that this variability may be a consequence of the heterogeneous nature of tinnitus in human patients and therefore it may be a good idea to revert to a reliable animal model which allows direct control over a single causal condition.

Even though tinnitus seems to have a multi-factorial aetiology, it is believed that acoustic trauma is the most common cause of tinnitus (Henry et al. 2005). Studies have reported that acoustic trauma results in an increase in spontaneous firing activity in the ascending auditory pathway following cochlear hair cell damage (Chang et al. 2002; Kaltenbach et al. 2004; Kaltenbach et al. 2000). This hyperactivity extends all the way to the higher auditory centres such as the inferior colliculus and the auditory cortex which in turn contributes towards the perception of tinnitus (Eggermont, 2005). Therefore, although tinnitus may originate peripherally, it is likely, once it is established, that it is maintained within the central nervous system; changes in the neural input cause changes in synaptic plasticity which cause alterations in auditory perception.

Since acoustic trauma is the most common cause of tinnitus, the acoustic trauma model would be an ideal animal model to test the efficacy of anti-tinnitus drugs. Noise exposure has been shown to cause a decrease in inhibition in the cochlear nucleus.
(Brozoski, 2001; Cooper, 1994), an increase in the amplitude of auditory evoked potentials recorded from the inferior colliculus (Salvi et al. 1990) and a decrease in GABAergic inhibition in the inferior colliculli neurons (Szczepaniak and Moller, 1996). This is further supported by a clinical study that suggested that the hyperexcitability of the inferior colliculli may be involved in the generation of some forms of severe tinnitus (Moller et al. 1992). Therefore, it is expected that drugs that increase GABAergic transmission within the central nervous system could thereby decrease hyperactivity and alleviate tinnitus. GABA\textsubscript{B} receptors are found in abundance within the ascending auditory pathway (Faingold et al. 1993; Ma et al. 2002, Szczepaniak and Moller, 1996) and baclofen, which is a GABA\textsubscript{B} receptor agonist, has been shown to have a distinct effect on the discharges of single neurons in the cochlear nucleus (Caspary et al. 1984).

THE EAR

The ear is a complex sense organ divided into three main sections, the outer ear, the middle ear and the inner ear (Raphael and Altschuler 2003). The outer ear which comprises of the pinna and external auditory canal collects, amplifies and directs sound. The external auditory canal also secretes cerumen which protects the ear canal and prevents it from drying out (Lass and Woodford 2007). The middle ear which is located inside the temporal bone consists of the tympanic membrane, the auditory ossicles, the tympanic cavity and the eustachian tubes. The tympanic membrane is a fibrous membrane which acts as a border marking
the end of the outer ear and the start of the middle ear; it vibrates in response to sound and reproduces the frequency and form of the sound wave it has received (Moller 2000). The auditory ossicles malleus, incus and stapes are mechanical interlinks that deliver sound vibration and amplify airborne sound. The middle ear primarily transmits sound vibrations received from the outer ear to fluid vibrations of the inner ear (Moller 2000; Lass and Woodford 2007). The inner ear, whose prime function is the transduction of sound pressure into neurochemical impulses for the auditory nerve, is made up of the oval window, round window, cochlea and the semi-circular canals. The oval window acts as a mediator and transfers sound vibrations from the ossicles to the cochlea, while the round window aids fluid motion within the cochlea and serves to equalise the hydraulic pressure. (Lass and Woodford 2007; Moller 2000).

The cochlea is a bony snail like ring structure comprising of two and a half rings, it converts stimulus from the outside environment into nerve impulses for transmission within the central nervous system (Lass and Woodford 2007; Raphael and Altschuler 2003). Cochlea is further divided into 3 fluid compartments: scala tympanic, scala media and scala vestibuli. scala tympanic and vestibuli are the two ends of the cochlea and contain perilymph fluid which has a similar composition to that of the extracellular fluid and scala media (which lies in between scala tympanic and vestibuli) comprises of endolymph fluid which has a composition similar to that of intracellular fluid (Moller 2000; Raphael and Altschuler 2003). Scala Media and scala tympani are separated by the basilar membrane. The basilar membrane has different resonant frequencies along its length, with the high frequencies at the base of the cochlea and the low frequencies at the apex of the cochlea. The organ of Corti sits on the basilar membrane and has microscopic hair cells. Each hair cell has many cilia on it. Hair cells are further divided
into inner and outer hair cells; there are three rows of outer hair cells and one row of inner hair cells. When the organ of corti moves in response to a pressure wave it causes a displacement of these hair cells, as the hair cells move the cilia on the hair cells are deflected which in turn exposes ion channels located on the hair cells. The opening of the these ion channels and the change in ion concentration initiates an action potential along the cochlear vestibular nerve (Moller 2000; Raphael and Altschuler 2003).

AUDITORY BRAINSTEM RESPONSES

Auditory Brainstem Responses or ABRs are far-field evoked potentials that occur within the first 10msec after presentation of a transient sound such as a click (Moller 2000). They are predominantly used for diagnostic purposes and for intraoperative neurophysiological monitoring. Since ABRs provide information about functionality in contrast to imaging techniques which only provide insight into structural changes, ABRs are very useful in detecting acoustic tumours as well as studying developmental ototoxicity.

The first published evidence of ABRs was reported in 1969 by Jewett et al who recorded ABRs in cats and suggested the use of this technique to study a sensory system using electrodes outside the system of interest (Jewett, 1969). The electrode arrangement introduced by Jewett et al in 1969 is still the most commonly used technique to record far-field potentials. Recordings are made using atleast two electrodes one to the mastoid and the other to the vertex. Sound is generated using a speaker and the equipment is calibrated so that the desired stimulus intensities are produced (Moller 2000). Stimulus presented in the recording ear, initiates a cascade of neural activity starting from the auditory nerve all the way into the brainstem. However these recorded voltages are incredibly small and buried under a great deal of
physiological noise hence ABRs are recorded averages in response to repeatedly presented stimuli. Since auditory evoked potentials nearly always elicit a similar waveform, they slowly add up as the recordings are made while the background EEG changes, which are more random, cancel out. (Jewett and Williston 1971; Moller 2000). ABRs can also be used to assess hearing sensitivity in animals. Animals are usually anaesthetised to record ABRs as this decreases excess physiological noise and ABRs have been shown to be unaffected by anaesthesia in both humans and animals (Samra et al. 1984; Shaw 1985) Hearing sensitivity is determined by ABR thresholds, which is the minimum intensity that elicits an observable ABR response. It is important to note, there is no such thing as a true ABR threshold that can be generalised across various testing situations as particular recording and sound generation set up can vary as can the observer making judgements on thresholds (Moller 2000). Thresholds have been shown to increase following noise trauma and this increase is mostly evident above the trauma frequency (Lataye and Campo 1997).

ABRs are characterised by 5-7 waves, but only the first five waves in humans and the first four waves in animals (Fig 2) are consistent, the lower peaks are harder to reproduce as their source gets further away from the recording electrodes (Shaw 1985).
The discrepancy in the number of consistent ABR peaks recorded between animals and humans is likely to be because the auditory pathway is smaller in animals than that in humans and therefore the first two waves in a human are equivalent to the first wave in an animal (Moore 1987). Each ABR peak has been associated with an approximate anatomical location within the ascending auditory pathway. Wave 1 originates as result of activity within the auditory nerve, wave 2 from the cochlear nucleus, wave 3 from the medial superior olivary nucleus and wave 4 from the inferior colliculli (Buchwald and Huang 1975; Jewett 1969; Jewett and Williston 1971). It is important to note that only the positive ABR peaks are labelled and mentioned, negative peaks have not yet been thoroughly studied and they could have specific neural generators which provide further insight into the auditory pathway (Shaw 1985; Moller 2000).

ANIMAL MODELS

Animal models offer a way of testing new therapies reliably and inexpensively. However animal models for tinnitus are especially challenging as it is important to determine a method for tinnitus induction that reliably and consistently produces tinnitus in animals each time and following that determine whether or not an animal is experiencing tinnitus which is difficult as it is a psychophysical phenomenon and animals such as rats are unlikely to be able to communicate this information to the experimenter. The two most frequently used models that overcome these problems successfully are the salicylate (SA) and the acoustic trauma model (Darlington 2009; Jastreboff et al. 1988).

The salicylate model reliably induces tinnitus following a single 350mg/kg dose (Kaltenbach 2000, Zheng et al 2008) while the acoustic trauma model uses unilateral noise to induce tinnitus (Bauer and Brozoski 2001). Both models allow control for
tinnitus induction and allow for quantification of tinnitus using psychophysical methods, circumventing the problem of a heterogeneous subject base which is often a concern within the human tinnitus population. Control over a single causal condition removes confounding and allows for a more realistic analysis of possible mechanisms of action or potential drug treatments. Tinnitus is then assessed based on a Pavlovian suppression paradigm introduced by Jastreboff in the 1980s where animals are conditioned to suppress a specific behaviour at the offset of a sound that resembles their tinnitus. Animals are usually trained using a mild foot shock as an unconditioned stimulus to achieve the conditioned response, i.e. suppression of a specific behaviour. However if an animal is experiencing tinnitus it is unlikely to hear the silence (offset of the tone) and hence does not produce the conditioned response, it was trained to perform (Jastreboff et al. 1988; Bauer and Brozoski 2001; Darlington 2009).

The SA model is replicable and inexpensive however it is not a true representation of human tinnitus as it produces acute tinnitus that is reversed following cessation of the drug. Also it is worth noting, SA induced tinnitus is rare in humans and often not a cause of concern. (Eggermont 2005). It has also been shown that SA induced tinnitus is associated with hyperactivity within the inferior colliculus while the acoustic trauma model has been shown to increase hyperactivity within the auditory cortex and dorsal cochlear nucleus (Eggermont 2005) hence drugs that may prove to be efficacious in a salicylate model may not necessarily produce similar results in an acoustic trauma animal model or within a human population where tinnitus is often caused by other factors besides salicylate. It is also worth noting the SA dose required to induce tinnitus reliably in animals is moderate to high (Jastreboff et al 1986) and hence SA toxicity may be a potential risk to animals especially with long term studies. It also limits drug
testing as putative anti-tinnitus drugs may interact with salicylate (Darlington et al. 2009).

The Acoustic trauma model on the other hand, uses the animal’s auditory discrimination ability which is affected by both the surrounding objective stimulus as well as internal physiological conditions like tinnitus to assess for the presence or absence of tinnitus and it also allows one to measure qualitative aspects of the condition by manipulating objective auditory test stimuli. Since acoustic trauma is the most common cause of tinnitus in humans (Henry et al. 2005), this model provides a realistic representation of human tinnitus. Similar to human tinnitus which is likely to be present for the majority of the individual’s life span (Bauer and Brozoski 2001) the animals induced with acoustic trauma induced tinnitus have also shown to exhibit tinnitus like behaviour up to 17 months post noise exposure (Bauer and Brozoski 2001). Using unilateral noise exposure also ensures the achieved drug effect positive or negative is a dependent on the tinnitus and not associated hearing loss, which is always a concern with tinnitus.

Hence even though SA can consistently induce tinnitus in animals, the acoustic trauma model may be a better representation of the human condition and therefore a better model to test for novel anti-tinnitus therapies.
PATHOPHYSIOLOGY OF TINNITUS

Tinnitus is most likely caused by maladaptive neural plasticity. Neural plasticity is the ability of the central nervous system to change or adapt in response to its environment. The brain has the ability to create or eliminate synapses, unmask dormant synapses or even mask effective synapses rendering them ineffective, all in response to a change in its environment. (Moller 2007; De Ridder and Heyning 2007). This is most pronounced in the developing brain but can also be seen in the mature auditory system where tonotopic auditory maps have been shown to reorganise in response to physiological and pathological sensory stimuli (Gao and Suga 1998; Suga et al. 2000; Kilgard and Merzenich 1998). Expression of neural plasticity may follow the Darwinian principal of survival of the fittest, where neural connections fit for the environment survive and can be strengthened or weakened in response to its physiological surroundings. In tinnitus where deprived neurons have lost sensory input they may extend out to the “undeprived neurons” to form connections and process their information in order to survive (De Ridder and Heyning 2007). This plasticity is usually adaptive as in case of repairs or meeting the changing demands of the CNS such as in the case of cochlear implants. However this can become maladaptive plasticity resulting in the perception of tinnitus (Kral and Tillein 2006; De Ridder and Heyning 2007). Neural plasticity is triggered by deprivation of input (Gerken et al 1991) and even though no consistent morphological evidence has been detected, functional imaging methods such as fMRI and PET scans have shown functionally related changes within the auditory system in patients with tinnitus (Plewnia et al. 2007; Talavage et al. 2000). Neural plasticity may be intended to work as a corrective mechanism to restore normal neural afferent innervation but in some cases may overcompensate and cause hyperactivity.
This is consistent with why electric current passed through the cochlear also helps reduce tinnitus in some patients (Aran and Cazals 1981). Tinnitus is known to worsen with time; the longer the neural plasticity is allowed to consolidate itself the more difficult it is to reverse it (Moller 2007). Tinnitus retraining therapy attempts to treat tinnitus using this rationale, appropriate sound stimulation is used to potentially reverse the functional changes caused by neural plasticity (Jastreboff P and Jastreboff M 2000). Neural plasticity initiates tinnitus but what functional changes develop and maintain this tinnitus is still unknown. Neural plasticity could either be a result of activating the non-classical auditory pathway or disrupting the imbalance between excitation and inhibition within the auditory pathway.

The classical auditory pathway comprises of both excitatory and inhibitory input, which work together to process and interpret auditory input (Caspar et al. 2005). Injury or age can impair this balance between inhibition and excitation, causing hyperactivity. Inhibitory input such as GABA and glycine release have been shown to be more affected than the excitatory input, which in turn alters the overall inhibitory/excitatory balance and associated protein synthesis. (Caspar et al. 2005; Sie and Rubel 1992).
NON CLASSICAL AUDITORY PATHWAY

The non-classical auditory pathway also called the extralemniscal pathway runs parallel to the classical auditory pathway. The non-classical auditory pathway involves a cross modal interaction between the auditory system and the somatosensory system (Moller et al. 1992). The observation that electrical stimulation of the skin or a change in gaze can reduce tinnitus in some patients is indicative of the activation of the non-classical auditory pathway; as the classical auditory pathway has no somatosensory input (Engelberg and Bauer 1985; Levine 1999). The non-classical auditory pathway has been shown to be active in children and may be involved in loudness perception however this gradually decreases with age and the activation of this pathway is rarely seen in individuals over the age of 20 years. Anatomically the non-classical and the classical pathways separate at the inferior colliculli; the non-classical pathway leads into the secondary auditory and association cortices via the medial and dorsal thalamus,
bypassing the primary auditory cortex while the classical auditory pathway projects into the primary auditory cortex via the ventral thalamus (Fig. 1). The neurons in the classical pathway respond specifically to sound and are tuned to respond to sharp frequencies, thus this pathway is called the slow and accurate pathway. By contrast the neurons in the non-classical pathway are less specific to sound and receive input from other sensory modalities besides the ear; this pathway is called the “fast and dirty” pathway. The dorsal and medial thalamus also project directly into the amygdala, the “emotional centre” of the brain, which may explain other affective components of tinnitus such as fear reactions, anxiety, phonophobia and depression (Moller et al. 1992; Moller and Rollins 2002). Functional imaging studies have shown abnormal limbic system involvement in some patients with tinnitus (Lockwood et al. 1998). Classical auditory information can also reach the amygdala but through a circuitous pathway and hence the information that reaches the amygdala through this route has been intensively processed (Moller 1992).
**BACLOFEN**

Baclofen is an anti-spasticity drug used to treat central nervous system (CNS) conditions such as multiple sclerosis and trigeminal nuclei. It is a GABA\(_B\) agonist and is known to have an inhibitory effect within the CNS and an increasing body of evidence suggests that baclofen activates pre-synaptic GABA\(_B\) receptors to produce this inhibitory effect (Ma CL et al. 2002; Sun et al. 2006). Most of the evidence for the mechanism through which baclofen works comes from whole cell patch clamp recordings however whether this inhibitory effect is a consequence of inhibiting excitation or augmenting inhibition is still uncertain.

*Inhibiting Excitation*

Baclofen is stipulated to act pre-synaptically on glutamergic neurons to modulate glutamate release and thus decrease excitation. Sun et al (2006) used whole cell patch clamp recordings to demonstrate the effects of baclofen in the inferior colliculus. They examined the effect of baclofen and CGP35348 (gabab antagonist) on excitatory post-synaptic potentials (EPSPs). Baclofen showed a concentration dependent (0.5-100\(\mu\)M) decrease in EPSPs producing a 50% reduction in EPSPs at 2-5\(\mu\)M. Adding CGP to the baclofen solution reversed the effect of baclofen and increased the mean amplitude of the EPSPs. To further confirm if baclofen was acting pre or post synaptically, they used a pair-pulse stimulus paradigm and demonstrated a greater suppression of the first EPSP compared to the second confirming the pre-synaptic action of baclofen. Sun et al blocked AMPA and NMDA elicited EPSPs individually in an attempt to further elucidate the action of baclofen. Both AMPA and NMDA EPSPs were significantly and reversibly blocked by baclofen however NMDA EPSPs showed a more significant reduction (mean reduction 75.9%) following baclofen application compared
to AMPA EPSPs (mean reduction of 51.4%) (Sun et al. 2006). This is consistent with whole cell recordings from pre-synaptic terminals of the Calyx of Held which has both GABA<sub>B</sub> and metabotropic glumate receptors. Bath application of baclofen suppressed EPSCs in a concentration dependent manner and CGP (Gaba<sub>B</sub> antagonist) attenuated this inhibitory effect of baclofen. Paired whole cell recordings also showed a similar suppression of calcium currents following baclofen application. Baclofen not only inhibits EPSPs but also calcium channels, indicating baclofen could activate G protein coupled pre-synaptic GABA<sub>B</sub> receptors that are linked to calcium channels and thus decrease transmitter release. It is worth noting baclofen had no effect on the inwardly gated potassium currents. Instead baclofen mediated inhibition of the EPSCs remained unaffected despite the G-protein coupled inwardly rectifying potassium channels (GIRKS) blocker (Takahashi et al. 1998)

*Augmenting Inhibition*

Whole cell patch clamp recordings from the inferior colliculus of the rat have demonstrated an overall concentration dependent decrease in inhibitory post-synaptic potentials (IPSPs) following baclofen application, and this inhibitory effect was reversed by phaclofen (MA cl et al. 2002) Similar results were obtained in the median eminence where once again baclofen reduced IPSP amplitude but did not increase membrane conductance (Anderson and Mitchell 1985)

Baclofen modulated inhibition of excitatory transmission and/or augmentation of inhibitory transmission has also been shown in other areas of the brain such as the hippocampus and the amygdala (Yamada et al. 1999; Lei and McBain 2003). Baclofen was shown to inhibit EPSCs and IPSCs in a concentration dependent manner in the basolateral amygdala which was reversed with CGP55845A (GABA<sub>B</sub> antagonists). To
elucidate the site of action of baclofen, pair pulse ratio as well as the frequency of miniature synaptic currents were examined. Baclofen was found to increase the pulse pair ratio; and this change in ratio was similar to the change observed when the extracellular calcium was lowered. It is worth noting, modifications of the post-synaptic receptors by GABA<sub>A</sub> and glutamate antagonist did not significantly alter the pulse pair ratio. Baclofen also decreased the frequency but not the mean amplitude of miniature synaptic currents to both IPSCs and EPSCs, thereby suggesting baclofen acts on pre-synaptic GABA<sub>B</sub> receptors, assuming the role of autoreceptors at the gabaergic terminals or hetroreceptors at the glutemergic terminals(Yamada et al, 1999). Similarly baclofen was once again shown to inhibit stimulus evoked EPSCs and IPSCs at both the inhibitory gabaergic and excitatory glutamerigic synapses in the CA3 stratum raditum of Sprague Dawley rats. Replicating the Yamada et al (1999) study, this paper too showed an increase in the pair pulse ratio and decrease in the frequency of miniature synaptic currents following baclofen application, which is consistent with baclofen’s pre-synaptic mechanism of action (Lei and McBain 2003).

Recapitulating baclofen has been shown to increase inhibition within the central auditory pathway however the jury is still out on whether this inhibition is a consequence of augmenting inhibition or inhibiting excitation.

**AIM**

The aim of this project was to determine the efficacy of L-baclofen in treating acoustic trauma induced tinnitus in rats using the unilateral acoustic trauma model.
CHAPTER 2

METHOD

• Animals
• Tinnitus induction
• Hearing levels
• Tinnitus Assessment
• Statistics
ANIMALS

Sixteen male Wistar rats (300 – 350 g) were obtained from the animal breeding station, Dunedin, New Zealand. They were housed in groups of two or three and maintained on a 12:12 hour light:dark cycle, at 22°C. Rats were given free access to food but were water deprived throughout the behavioural testing period in accordance with the conditioned lick suppression paradigm. The protocol was in compliance with the regulations of the Otago University Committee on Ethics in the Care and Use of Laboratory Animals. The animals were divided into two groups 1) Noise-exposed group or 2) Non-exposed (Sham) group. The study followed a repeated measures design which meant that each animal was tested with vehicle and L-baclofen at each of the low (1mg/kg), medium (3mg/kg) and high (5mg/kg) doses (Table 1).

EFFECTS OF BACLOFEN ON NOISE INDUCED TINNITUS

Tinnitus Induction: Rats were induced with tinnitus using the unilateral acoustic trauma model (Bauer et al. 2001). Briefly, each rat was anaesthetised with a mixture of ketamine HCl (50 mg/kg, s.c.) and medetomidine hydrochloride (300 µg/kg, s.c.) and placed in a modified stereotaxic head-frame inside a sound attenuation chamber. The exposed animals received a 16 kHz tone at 110 dB SPL (Fig4) produced by a NI 4461

![Spectrum of 16kHz tone used to induce acoustic trauma](https://example.com/spectrum.png)
dynamic signal acquisition and generation system for one hour (National Instruments New Zealand Ltd. Auckland New Zealand) while the sham animals received the same anaesthetics but no acoustic trauma exposure. Acoustic trauma was delivered through a close field magnetic speaker with a tapered tip (Tucker Davies Technologies, Alachua, FL, USA) attached to a 3 mm cone shaped speculum which fitted into the external auditory canal. A 438 pressure field microphone was also placed within the chamber to ensure that the sound field was effectively contained by the inserted speculum. Acoustic values were calibrated prior to the noise exposure by connecting the speaker to a ¼ inch pre polarised free field microphone (Type 40BE, GRAS Sound & Vibrations, Holte, Denmark) via the speculum used to fit into the external auditory canal. An ear plug was inserted in the contralateral ear to protect the ear from any residual sound exposure (Fig. 5).
### TABLE 1: EXPERIMENTAL TIME LINE

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<td>53-70</td>
<td>Discrimination Phase – Administered the saline Vehicle</td>
<td>Vehicle Administered to all rats. 6 days at each of the three frequencies.</td>
</tr>
<tr>
<td>71-88</td>
<td>Discrimination Phase – Baclofen 1mg/kg (Low dose)</td>
<td>L-baclofen (1mg/kg) administered to all rats. 6 days at each of the three frequencies.</td>
</tr>
<tr>
<td>89-106</td>
<td>Discrimination Phase - Baclofen 3mg/kg (Medium dose)</td>
<td>L-baclofen (3mg/kg) administered to all rats. 6 days at each of the three frequencies.</td>
</tr>
<tr>
<td>107-124</td>
<td>Discrimination Phase – Washout 1 (Break) Period</td>
<td>Washout Period. Animals withdrawn from drug. 6 days at each of the three frequencies.</td>
</tr>
<tr>
<td>125-142</td>
<td>Discrimination Phase - Baclofen 5mg/kg (High dose)</td>
<td>L-baclofen (5mg/kg) administered to all rats. 6 days at each of the three frequencies</td>
</tr>
<tr>
<td>143-160</td>
<td>Discrimination Phase – Washout2 Period</td>
<td>Washout Period. Animals withdrawn from drug. 6 days at each of the three frequencies.</td>
</tr>
</tbody>
</table>
**Hearing Levels:** Hearing levels were tested using acoustic brainstem-evoked response (ABR) thresholds and were measured before and immediately after noise exposure in both ears. Animals were anaesthetised as described above and an acoustic stimulus was presented directly at the entrance of the ear canal. ABRs were recorded using stainless steel subdermal needle electrodes. Recording electrodes were placed subcutaneously at the vertex and over the bullae of the recording ear along with a reference electrode over the occiput. ABR thresholds were tested using 5 ms duration tone bursts (2 ms rise/decay, 1 ms plateau) presented at a rate of 21 tone bursts per second. Tone bursts were presented in decreasing intensities beginning with levels that elicited distinct evoked potentials. Thresholds were indicated by the lowest intensity that produced visually distinct potentials progressing in 20 and 10 dB steps. Hearing thresholds were tested at 8, 16, 20 kHz, respectively.
**Tinnitus Assessment:** Tinnitus assessment was performed two weeks following acoustic trauma, in an operant chamber (ENV – 007, Med Associates Inc., St Albans, VT, USA) inside a sound attenuation box (40.6 x 15.9 x 21.3 cm). The chamber was illuminated by a house light with a drinking tube, which was positioned on one side of the chamber 5 cm above the floor through a V-shaped restrictor plate. The animal’s drinking activity was quantified by the number of licks made by the animal and was recorded using a lickometer with a photobeam (ENV-251L, Med Associates Inc.) positioned in front of the drinking tube. A camera mounted on the ceiling monitored the animal’s behaviour within the chamber and the speaker (ENV-224DM, Med Associates Inc., St Albans, VT, USA) in the chamber produced tones at three different frequencies - broad band noise (BBN which was white noise ranging from 3 to 20 kHz with no equalisation using a NI 4461 Dynamic Signal Acquisition and Generation card (National Instruments New Zealand, Auckland, New Zealand) and was calibrated as dB (SPL)), 10 kHz and 20 kHz tones (at various intensities) via a sound generator (ANL926, Med Associates Inc., St Albans, VT, USA). BBN was white noise and covered a range of frequencies, while 10 kHz and 20 kHz were below and above the trauma frequency respectively. The chamber floor was lined with stainless steel bars (0.48 cm), spaced 1.6 cm apart and evoked an electric shock of 0.1-
0.5 mA by a constant current shock source (ENV-410B, Med Associates Inc., St Albans, VT, USA) through a scrambler (ENV-412, Med Associates Inc., St Albans, VT, USA).

Tinnitus assessment was performed using a frequency specific shift in the discrimination function in a conditioned lick suppression paradigm. Each animal was placed in the chamber for 15 minutes each day and subjected to three phases: the acclimation phase, the suppression phase and the discrimination phase. During the acclimation phase BBN was turned on before placing the animal in the chamber and this acted as a background noise throughout the 15 minute session. This 15 minute session was interrupted by ten 15 second sessions. Two of the ten 15 sec sessions were the “tone off” periods i.e. the background noise went off and there was silence in the chamber while during the other eight 15 sec sessions the background noise was substituted by acoustic presentations of either BBN, 10 kHz or 20 kHz (at one of four different intensities each repeated once; stimulus presentation period, BBN = 30, 40, 50, 70 dB; 10 kHz = 60, 70, 80, 90dB and 20 kHz = 70, 80, 90, 100 dB). The ten 15 sec sessions occurred in a random order but not within the first and last one minute of the full fifteen minute session. The acclimation phase allowed the animal to get accustomed to the sound chamber (Fig 7A) and each animal was given two days at each frequency to acclimate. Following acclimation each rat received six days of suppression training (Two days at each of the three frequencies, i.e. BBN, 10kHz and 20kHz) during which the last three seconds of the silent periods were paired with a mild foot shock (0.1 - 0.5mA). The foot shock acted as an unconditioned stimulus and the silence or “tone off” period acted as a conditioned stimulus which meant that the animals learnt to associate the foot shock with the silence, i.e., the tone off periods and therefore reacted by suppressing their drinking during those silent periods (Fig. 7B). The drinking
suppression was quantified using suppression ratios (SRs) which took into account the number of licks the animal made in the preceding 15 secs relative to the number of licks the animal made in the current 15 secs (when they were presented with tones of different intensities (Equation 1)). A suppression ratio of less than 0.2 (SR < 0.2) was representative of the conditioned suppression the animals were trained to achieve.

\[
SR = \frac{B}{(A + B)}
\]

\[
\begin{align*}
A &= \text{Number of licks in the preceding period.} \\
B &= \text{Number of licks in the current period}
\end{align*}
\]

Equation 1: Suppression Ratio.

Suppression training was followed by the discrimination phase during which rats were shocked only if they continued to drink in the silent periods (Fig. 7C), thereby resulting in an SR > 0.2. Hence the sham rats suppressed their drinking during the silent or “tone off” periods to avoid the foot shock and drank normally during the eight acoustic stimulus presentation periods. However, in animals with acoustic trauma-induced tinnitus, the tinnitus rather than the silence was associated with the foot shock (during the silent period) and therefore, an acoustic stimulus at the frequency resembling their tinnitus produced greater licking suppression during the stimulus presentations whilst having no effect on the drinking activity in sham animals. Each animal received 18 days of discrimination (i.e. six days at each frequency BBN, 10 kHz and 20 kHz) to confirm for tinnitus and subsequently 18 days of discrimination for each of the treatment phases,
i.e. vehicle, L-baclofen low dose (1mg/kg), L-baclofen medium dose (3mg/kg), washout period, L-baclofen high dose (5mg/kg) and washout period (Table 1 for details) to assess drug effect.

**Drug treatment:** Animals were administered with either vehicle (saline) or L-baclofen depending on the treatment phase, one hour before tinnitus assessment. Both saline and L-baclofen were administered subcutaneously (s.c.). L-Baclofen (Sigma) was dissolved in distilled water to make a stock solution of 25 mg/ml weekly and stored at 4°C. The stock solution was further diluted with saline each day to 2.5 mg/ml for a daily working solution. Baclofen was administered each day corresponding to the appropriate treatment phase at the time, i.e. low dose (1mg/kg), medium dose (3mg/kg) or high dose (5mg/kg). Besides vehicle and L-baclofen, the animals also had two washout periods – one after the medium dose and the second washout period following the high dose treatment. During the washout periods the animals were withdrawn from L-baclofen and reassessed for tinnitus to confirm that any change in the tinnitus like behaviour was due to the effects of L-baclofen not the lack of persistence of tinnitus.

**STATISTICS**

All data were tested for the parametric assumptions of normality and homogeneity of variance (Kutner et al. 2005). Where these assumptions were violated, the data were natural log or square root transformed and re-tested. The ABR and suppression ratio data were analysed using multilevel ANOVAs.
**Fig 7:** Tinnitus assessment phases. Acclimation (2 days at each frequency), Suppression (2 days at each frequency), Discrimination (6 days a each frequency).
RESULTS

• Auditory brainstem evoked responses

• Tinnitus assessment and drug treatment
AUDITORY BRAINSTEM EVOKED RESPONSES

Auditory brainstem evoked responses (ABRs) were tested before and immediately after acoustic trauma at three different frequencies (8 kHz, 16 kHz and 20 kHz). ABR thresholds were then used to determine hearing levels at each frequency, i.e. the lowest intensity at which a visible auditory potential could be detected. Acoustic trauma significantly elevated ABR thresholds of the exposed ear (F (2,168) = 5.03, P = 0.008) at all three frequencies (Fig. 8). The greatest increase in the ABR threshold after acoustic trauma was observed at 20 kHz, averaging 72.5 dB. No significant difference in thresholds was observed for the contralateral ears of the exposed animals following acoustic trauma. Sham animals showed no significant change in ABR thresholds before or after noise exposure for both ears.

![Graph showing hearing thresholds before and after exposure in isipilateral and contralateral ears.](image)

**Fig 8:** Hearing thresholds before exposure and after exposure in isipilateral and contralateral ears. Rats in the exposed group were exposed to noise once unilaterally for 60 mins using a 16 kHz tone at 110 dB. Rats in the sham group had no noise exposure. All animals were anaesthetized when recording ABRs.
TINNITUS ASSESSMENT AND DRUG TREATMENT

Following acoustic trauma the exposed animals demonstrated positive psychophysical evidence of tinnitus with a significant downshift in their tone discrimination function (i.e. decreased suppression ratios (SRs)) relative to the unexposed group at all the presented stimuli (BBN: F(1,4.02) = 9.23, P = 0.04; Fig 9A) 10 kHz (F(1,5) = 10.71, P = 0.03; Fig 9B) and 20 kHz (F(1,5.01) = 84.25, P = 0.000; Fig 9C). The suppression ratios take into account the number of licks the animal made in the preceding 15 secs relative to the number of licks the animal made in the current 15 secs when they were presented with tones of different intensities (equation 1). Animals were conditioned to suppress drinking during the silent periods generating a suppression ratio of less than 0.2 during these “tone off” periods while drinking normally during the eight acoustic stimulus presentations. However, animals in the exposed group associated their tinnitus rather than the silence with the foot shock and hence produced a greater licking suppression for an acoustic stimulus at the frequency resembling their tinnitus. This meant that the exposed group produced overall lower suppression ratios compared to the sham group. Suppression ratios were proportional to change in intensities (P = 0.000) for all the treatment phases.

Following tinnitus confirmation all the animals were administered the saline vehicle and re-tested for the evidence of tinnitus. Exposed animals continued to perceive tinnitus i.e. exhibited a significantly greater suppression in their drinking behaviour compared to the unexposed animals, at all the test stimuli (BBN: F(1,5) = 11.01, P = 0.02; Fig 10A, 10 kHz: F(1,5) = 14.64, P = 0.01; Fig 10B and 20 kHz: F(1,5) = 67.57, P = 0.000; Fig 10C).
Animals treated with low dose (1mg/kg) L-baclofen showed no psychophysical evidence of tinnitus at BBN (Fig 11A) and 10 kHz (Fig 11B) i.e. there was no significant difference between the two curves, but at 20 kHz the exposed animals continued to exhibit significantly lower suppression ratios compared to sham animals (F(1,5) = 24.62, P = 0.004; Fig 11C).
Following the low dose, L-baclofen was increased to 3mg/kg (medium dose). At the 3 mg/kg dose, no evidence of tinnitus was found at 10 kHz or 20 kHz i.e. discrimination performance for all stimuli was identical for both the groups (Fig 12B and 12C). However for BBN the suppression ratios were significantly lower in the exposed group compared to the sham group (BBN: F(1,5) = 10.45, P = 0.02; Fig 12A).

Fig 10 Tinnitus like behaviour following 1ml of saline. Subjects and test parameters were as described in Figure 9. There was a significant difference in discrimination performance between the exposed and unexposed subjects at all three frequencies.
The animals were withdrawn from L-baclofen following the medium dose for a washout period to ensure any change observed in tinnitus-like behaviour was a result of the drug. During this period exposed animals exhibited tinnitus like behaviour for BBN and 20 kHz stimuli (BBN: F(1,4) = 11.04, P = 0.03 Fig 13A; 20 kHz: F(1,4) = 36.99, P = 0.004 Fig 13C.). However, for the 10 kHz stimulus animals in both groups showed equivalent tone discrimination (Fig 13B).
Figure 12: Tinnitus assessment after all subjects were treated with baclofen at 3mg/kg daily for 18 days. Subjects and test parameters were as described in Figure 9. There was a significant difference in discrimination performance between the exposed and unexposed subjects at BBN (A) but no difference between the exposed and unexposed at 10 kHz (B) and 20 kHz (C).
Fig 13 Tinnitus assessment after all subjects were withdrawn from baclofen for 5-6 days. Subjects and test parameters were as described in Figure 1. There was a significant difference in discriminative performance between the exposed and unexposed subjects at BBN (A) and 20 kHz (C) but not at 10 kHz (B).
The high dose (5mg/kg) of L-baclofen was administered after the washout period and the dose increment alleviated tinnitus in exposed animals at all frequencies, i.e. the exposed and unexposed groups both showed equivalent discrimination for all stimuli; BBN (Fig 14A), 10 kHz (Fig 14B) and 20 kHz (Fig 14C).

Animals were once again withdrawn from L-baclofen for another washout period following the 5mg/kg dose. No difference in suppression ratios was detected between the exposed and sham groups across all the presented frequencies BBN (Fig15A), 10 kHz (Fig 15B) and 20 kHz (Fig 15C.)
Fig. 14 Tinnitus assessment after all subjects were treated with baclofen at 5mg/kg daily for 18 days. Subjects and test parameters were as described in Figure 9. There was no significant difference in discrimination performance between the exposed and unexposed subjects at all three frequencies.
Fig. 15 Tinnitus assessment after all subjects were withdrawn from baclofen for a washout period. Subjects and test parameters were as described in Figure 9. There was no significant difference in discrimination performance between the exposed and unexposed subjects at all three frequencies.
CHAPTER 4

DISCUSSION

• Interpretation of results
• Comparison to present day literature
• Significance of this study
• Clinical implications
• Critical evaluation of the experimental design
• Future research
• Conclusion.
INTRODUCTION OF RESULTS

Auditory brainstem evoked responses measured post noise exposure demonstrated significantly elevated ABR thresholds at all of the three frequencies, i.e. 8, 16 and 20 kHz with the greatest increase at 20 kHz. Studies have shown that the dorsal cochlear nucleus (DCN) reorganises in response to physiological stimuli (Suga et al 2000; Gao and Suga 1998) and so acoustic trauma, which acts as a tetanus stimulus, is likely to reorganise this tonotopic map within the DCN. Following acoustic trauma even as the afferent input decreases, the efferent input remains unaffected cementing the change in the tonotopic DCN map, causing a subsequent increase in ABR thresholds in the ipsilateral ear. Since the trauma frequency used was 16 kHz it is likely that the most prominent change in the DCN would occur at and around that frequency.

Acoustic trauma produced tinnitus-like behaviour in all the exposed animals, at all the presented frequencies (i.e. BBN, 10 and 20 kHz Fig. 9). Previous studies have linked acoustic trauma to hyperactivity within the ascending auditory pathway (Kaltenbach et al. 2000; Moller 2000) and tinnitus has been associated with maladaptive neural plasticity (Ridder and Heyning 2007; Kral and Tillein 2006). Hence it is likely that acoustic trauma increases the spontaneous firing rates within the ascending auditory pathway and this physiological change triggers neural plasticity as a corrective mechanism to restore the excitation/inhibition balance within the auditory pathway, however in some cases it may overcompensate and result in the perception of tinnitus.

The change in intensity was proportional to the change in suppression ratios i.e. higher intensities produced higher suppression ratios across all three frequencies for all
the treatment phases. Since animals were taught to suppress drinking in silence, the higher amplitude tones were considered a “safe haven,” as these made the distinction between the speaker off and the tone periods more apparent for both groups of animals.

L-baclofen alleviated the acoustic trauma induced tinnitus in rats, achieving the best results at the 5mg/kg dose (high dose). At the 3 mg/kg dose (medium dose), the animals continued to show evidence of tinnitus like behaviour at the BBN frequency and similarly psychophysical evidence of tinnitus was also observed at the 20 kHz frequency with the 1mg/kg dose (low dose). Only the 5mg/kg dose (high dose) showed complete alleviation of tinnitus across all three frequencies. When prescribed, baclofen is individually titrated for each patient starting at the lowest dose and increments are made until the optimum effect is achieved, usually around 40mg with the absolute maximum of 80mg per day (http://www.drugs.com/pro/baclofen.html). The high dose of 5mg/kg used in this study is equivalent to approximately 60mgs in an average human being. While the 3 mg/kg and 1 mg/kg dose amount to approximately 36 mgs and 12 mgs respectively in humans; both well below the optimum therapeutic dose.

Acoustic trauma causes disinhibition within the auditory pathways that results in increased activity (Kaltenbach et al 2000, Eggermont et al 2004), which in turn triggers maladaptive neural plasticity to generate the perception of tinnitus (Caspar et al 2005, Sie and Rubel 1992). Therefore, in theory reversing this disinhibition should decrease hyperactivity and thus alleviate tinnitus. L-baclofen, which is a GABA\(_B\) agonist, is likely to have increased inhibition within the ascending auditory pathways and hence decreased the perception of tinnitus. However, the exact mechanism by which L-baclofen exerts its effect is still unknown. The literature suggests that L-baclofen activates pre-synaptic GABA\(_B\) receptors to increase inhibition within the central
nervous system. However, whether this increase in inhibition is a consequence of augmenting inhibition or decreasing excitation is still debatable. Some studies suggest that L-baclofen activates GABA_B autoreceptors and increases GABA within the CNS (Ma et al 2002; Sun et al 2006; Isaacson 1998); others suggest that GABA_B receptors act as heteroreceptors and decrease glutamate secretion within the nerve terminals (Takahashi and Tsjimoto 1998; Sun et al 2006). It is perhaps more likely that glutamatergic terminals have GABA_B hetroreceptors while GABAergic terminals have GABA_B autoreceptors. Thus, L-baclofen may activate presynaptic GABA_B receptors and depending on the nerve terminals involved either decrease excitatory transmission or increase inhibitory transmission; however the overall effect of baclofen is to modulate pre-synaptic GABA_B receptors and increase inhibition within the CNS. Further investigations are required of the auditory system in particular, as a number of the findings are extrapolated from other regions in the brain and it is possible that the auditory system may only have GABA_B autoreceptors or GABA_B heteroreceptors, not both.

The drug used in our study was L-baclofen, but baclofen is usually found in its racemic form which contains equal amounts of both the L and D enantiomers. However, only the L enantiomer is thought to be active (Szczepaniak and Moller 1995). Previous studies have shown significant decreases in auditory evoked potentials in the cochlear nucleus and the inferior colliculi following treatment with L Baclofen (Szczepaniak and Moller 1995; Caspary et al 1983). However, clinicians who use baclofen as an anti-tinnitus drug, use baclofen that is available clinically and this contains equal amounts of both enantiomers L and D. This raises the possibility that either the D enantiomer is somehow suppressing the therapeutic benefit of L baclofen or perhaps the L baclofen is
required in increased amounts to exert its anti-tinnitus effects. Perhaps using just the L enantiomer, as we used in our study, to test its efficacy as an anti-tinnitus drug would provide further insight and help rule out this possibility.

Two washout periods were used in the study, one between the medium and high dose and another one following the high dose, to ensure any changes observed in tinnitus-like behaviour was a result of the drug and not just a reversal of tinnitus over time. During this time the animals were withdrawn from L-baclofen and re-assessed for tinnitus. Animals exhibited tinnitus-like behaviour for BBN and the 20 kHz frequency following the medium dose, but no evidence of tinnitus was found following the high dose. This raises two possibilities, either the tinnitus was reversed or a longer washout period was needed to confirm the presence of tinnitus. However, it is unlikely that the tinnitus in animals was reversed, as previous studies have shown that once tinnitus is established, it only worsens over time. In addition a previous pilot study in our laboratory has confirmed the presence of tinnitus following acoustic trauma up to 12 months following noise exposure. Nevertheless, a longer washout period is required to conclusively confirm this. To further ensure that the results we achieved were not as a result of sedation because of L-baclofen, the number of licks over the duration of the study were measured and these were consistent, confirming no negative drug effect.

We administered L-baclofen two weeks after induction of tinnitus. As mentioned above, even though tinnitus might originate peripherally, once it is established, it is maintained within the central nervous system and is known to worsen with time making it more difficult to reverse (Moller 2007). Therefore, L-baclofen administered two weeks after tinnitus developed has set in may allow alleviation of tinnitus by interfering with the process, i.e preventing it from becoming established. The only
clinical trial conducted with baclofen (Westerberg et al 1996) used patients who were refractory to other forms of anti-tinnitus treatment. These patients may belong to a subset of patients in whom, persistent tinnitus has been well established and they may not necessarily respond to baclofen alone. These cases might require a more robust combination treatment approach.

**COMPARISON TO THE LITERATURE**

To the authors knowledge there are no reported studies that have tested the efficacy of L-baclofen on noise induced tinnitus in rats. Baclofen has been shown to decrease auditory potentials within various auditory structures such as the cochlear nucleus and the inferior colliculi of various mammals (Szczepaniak and Moller 1995; Caspary et al 1983; Sun et al 2006; Ma et al 2002) but none have systematically investigated the efficacy of baclofen on noise induced tinnitus. The most relevant studies are those that have investigated the effects of baclofen on noise induced increases in amplitudes of click evoked potentials from the surface of the inferior colliculi (Szczepaniak and Moller, 1996) and the double blind placebo controlled trial that investigated the efficacy of baclofen in treating tinnitus (Westerberg et al 1996).

Szczepaniak and Moller (1996) investigated the effects of diazepam, clonazepam and baclofen on the amplitudes of click evoked potentials in the inferior colliculi. Baclofen was the only compound that showed a dose dependent decrease in the amplitudes of click evoked potentials following noise exposure. However, the drug was administered intravenously and the doses used in this study were above the recommended doses used for humans. The maximum prescribed dose for baclofen is 80mg a day while the study used up to 40mg/kg which translates to approximately
486mg in an average human being. Furthermore, the potentials were recorded from the surface of the inferior colliculli, which is not a true physiological representation of what happens when sound is presented to the ear. The inferior colliculli do not work independently to process sound, they are part of an interconnected pathway, and the lower auditory structures provide afferent input to the inferior colliculli. Thus, baclofen may work effectively on the inferior colliculli neurons when administered intravenously but this may not necessarily be useful if baclofen is selective only for these neurons. The effect of baclofen within the ascending auditory pathway as a whole may not necessarily replicate the findings from this study. Hence, any extrapolation from this study needs to be done with caution. However, this study provides evidence that baclofen is effective within the auditory pathway and thus provides the basis for further testing of baclofen in noise-induced tinnitus.

The double blind placebo controlled trial that tested the efficacy of baclofen in treating tinnitus reported baclofen to be no more useful than placebo (Westerberg et al 1996). However it is important to note this study used a heterogeneous subject base, i.e. the underlying pathophysiology associated with tinnitus varied enormously between the participants. Six subjects in both the baclofen and placebo group suffered from noise-induced tinnitus, which made up less than 20% of the subject base. The other associated causalities among subjects varied from viral upper respiratory illness, Meniere’s syndrome, tempromandibular dysfunction and others. These forms of tinnitus may have different mechanisms and thus it would be naïve to expect one drug to treat all of these different causes of tinnitus. The study also included patients who were on antidepressants for so long that they did not have a change of medication for the duration of this study; this further confounds the data as the perception of tinnitus
is worsened with depression and one of the measures of tinnitus used in this study was a subjective questionnaire called the THI (Tinnitus Handicap Inventory). This study lasted for three weeks and this time frame was too short to assess the efficacy of an anti-tinnitus drug, especially since these patients had suffered from tinnitus for an extended period of time. Another important confound within this study was inadequate power. Power calculations done at the start of the study determined that 45 patients were needed in both the placebo and baclofen group to determine a difference in treatment between the two groups, but the study used only 31 subjects in the baclofen group and 32 in the placebo group, making it even more difficult to determine any difference between the two groups.

**SIGNIFICANCE OF THIS STUDY**

Tinnitus is a major cause of disability within New Zealand and affects approximately 10% of the population (Davis and Rafaie, 2000). This prevalence is likely to increase in the future due to the rise in portable devices that allow individuals to listen to music for long periods of time at high amplitudes. Current tinnitus treatments consist of behavioural therapies, auditory masking techniques and a trial and error drug regimen.

Tinnitus has a multifactorial aetiology and it is likely that different kinds of tinnitus might have different neural substrates and may therefore need different drug treatments. Noise trauma is the most common cause of tinnitus ahead of head and neck injury (Henry et al 2005), and therefore the acoustic trauma model is an effective and reliable model to test the efficacy of potential anti-tinnitus drugs.
The biggest obstacle for drug development is the complex, uncertain pathophysiology underlying tinnitus; therefore a reliable animal model provides further insight into the mechanisms underlying tinnitus. Since L-baclofen had never been systematically investigated, positive or negative results obtained in this project, contribute to an otherwise scarce literature.

**CLINICAL IMPLICATIONS**

L-baclofen was shown to decrease the perception of tinnitus in animals with noise induced tinnitus. Since baclofen is already available clinically as an anti-spasticity drug, expensive drug trials are not required to determine drug safety. Clinicians are well aware of the side effects associated with the drug and hence can make an informed decision about who can and cannot use this drug.

This study has also demonstrated a reliable animal model, which means that other anti-tinnitus drugs can be tested to determine efficacy. Since the acoustic trauma model requires no drug administration to induce tinnitus, one need not worry about possible drug interactions that may distort data. The success of this model also means that further insight can be gained into the neurophysiological changes that may occur in tinnitus. Most tinnitus models induce acute tinnitus, which may not necessarily produce the same neurophysiological or neuroanatomical changes that chronic tinnitus is likely to produce.
CRITICAL EVALUATION OF THE EXPERIMENTAL DESIGN

Hearing Levels

Hearing levels were tested using auditory brainstem-evoked responses before and immediately after acoustic trauma. Hearing thresholds were determined as the lowest intensity which elicited an observable auditory potential. This was done manually and could have resulted in an unconscious bias on the experimenter’s part. Ideally, the experimenter should have been unaware of whether the animals were in the sham or exposed group. However, this was impractical for this study.

Thresholds were recorded before and after noise exposure in every animal; however no ABRs were conducted at the end of the study. Previous literature has shown that elevated hearing thresholds after acoustic trauma are only temporary in a mature auditory pathway (Lataye and Campo 1997; Henley and Rybak 1995) and a pilot study previously done in our laboratory has also shown a complete reversal of these elevated thresholds after a few months of noise exposure. Re-testing ABR thresholds would have provided better insight into the animals’ hearing and further confirmed that the animals’ hearing was unaffected for the duration of the study despite the tinnitus.

Animals in the exposed group received unilateral acoustic trauma, which meant that the contralateral ear was unexposed and thus acted as a control for that animal, decreasing between group variability while determining thresholds. Animals in both groups were subjected to noise exposure (exposed group) or no noise exposure (sham group) in either the right or left ear at random, to avoid any potential lateralisation bias.

Assessing hearing levels confirmed the success of the induced acoustic trauma while still ensuring the animal was able to perform the tinnitus assessment as the
induced trauma only affected one ear. However controlling for above discrepancies would make this study more robust.

**Drug Treatment**

L-baclofen was administered subcutaneously for all doses, which meant that the action of L-baclofen was not limited to the auditory system. It is also important to note that prescribed baclofen in humans is administered orally and this could affect the bioavailability of the drug and hence its therapeutic effect in treating tinnitus. Baclofen in humans is associated with a number of adverse effects such as drowsiness, gastrointestinal (nausea, diarrhoea), cardiovascular (hypotension, chest pain), neuropsychiatric and other (http://www.drugs.com/pro/baclofen.html) side effects and not all of these might extend into other species or not all which may be detected when performing the study. However as mentioned earlier sedation was eliminated as a potential side effect within this study as the number of licks made by the animals was observed and these were shown to be consistent throughout the duration of this study. No observable gastrointestinal side effects were noted however these cannot be eliminated without further examination of the animals. Taking all these factors into account could make baclofen less useful in humans. Paradoxically, one of the side effects reported with baclofen is tinnitus.

However, the doses used in this study were within recommended dose range prescribed for humans; the highest dose of 5mg/kg is approximately equivalent to the 60mg dose prescribed in humans.
Data Collection

The sample size of eight animals per group was based on power calculations done on a pilot study previously conducted in our laboratory. Eight animals per group allowed us to detect a potential difference between the two groups. The repeated measures design allowed each animal to act as a control for itself, which decreased between group variability.

FUTURE RESEARCH

This study should be repeated by administering L-baclofen orally, perhaps using oral gavage, as the drug is given orally to humans. This will provide further insight into L-baclofen's efficacy in treating tinnitus. It would also be worth using L-baclofen as a pre-treatment, before tinnitus is induced and testing to see if tinnitus can be prevented from becoming permanent.

Using D-baclofen and repeating this study could provide insight into reasons why the racemic baclofen available clinically does not show the same positive anti-tinnitus drug effect that is observed with L-baclofen singularly.

Neurochemical studies such as Western blots for GABA receptors may also allow one to study the underlying mechanism of tinnitus. It is likely that tinnitus causes receptor changes within the CNS which may be crucial in maintaining tinnitus. If these changes can be studied, therapeutic treatments for tinnitus could be narrowed down to the target site and thus facilitate further anti-tinnitus drug discovery.
CONCLUSION

L-baclofen alleviated tinnitus in rats achieving optimal results at the 5mg/kg dose, which is within the dose range currently prescribed for humans. Since the drug is already available clinically, a clinical trial using a homogeneous subject base would be the next step to confirm its position as an anti-tinnitus drug. Acoustic trauma-induced chronic tinnitus in rats is a realistic representation of tinnitus observed in humans and thus provides a reliable animal model to test other anti-tinnitus drugs. The animal model can also be used to further study neurochemical changes associated with tinnitus and thus gain insight into its pathophysiology.
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