Response Properties of the Inferior Colliculus Following Unilateral Noise Induced Hearing Loss: The Effects of Cholinergic Enhancement and Auditory Training

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STATEMENT OF AUTHENTICATION

I, Joan Nguyen, hereby declare the work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution. This thesis was completed during my enrolment with the department of Anatomy and Cell Biology, from February 2016 to July 2020, under supervisory panel members Dr Sindy Kueh, Professor John Morley, and principle supervisor Dr Carl Parsons.

Signed:_____  _____

Date: 29th July 2020

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ABBREVATIONS

A1	Auditory Cortex
ABR	Auditory Brainstem Response
Ach	Aceytlcholine
Ach-D9	Acetylcholine-d9 Chloride
AChE	Aceytlcholinesterase
AN	Auditory Nerve
ANOVA	Anaylsis of variance
ARC	Animal Resources Centre
AT	Auditory Training
AU	Australia
BW	Bandwidth
CA	Canada
CA	California
CC	Corticocollicular
CAP	Compound Action Potenial
CF	Characteristic Frequency
ChAT	Choline acetyltransferase
ChE	Cholinesterase
CIST	Constraint Induced Sound Therapy
CN	Cochlear Nucleus
CNIC	Central Nucleus of the Inferior colliculus
dB	Decible
DC	Dorsal Cortex of the Inferior colliculus
DCN	Dorsal Cochlear Nucleus
DEU	Germany
EAE	Enriched acoustic enviroment
EC	External Cortex of the Inferior colliculus
EE	Excitatory-Excitatory
EI	Excitatory-Inhibitory
EO	Excitatorty-null
ESI	Electrospray ionization source
FA	Formic acid
FL	Florida
FRA	Frequency Response Area
FTC	Frequency Tuning Curve
GABA	Gamma Aminobutyric Acid

GAD	Glutamic Acid Decarboxylase
HRP	Horseradish Peroxidase
IC	Inferior Colliculus
IHC	Inner hair cell
IOF	Input-output function
IVC	Individually ventilated cages
kHz	kilohertz
kV	Kilo Volt
LAeq	A-weighted equivalent
LC	Lateral Cortex of the Inferior colliculus
LDT	Laterodorsal tegmental nucleus
LPZ	Lesion Projection Zone
mAChR	Muscarinic acetylcholine receptor
MN	Minnesota
MRM	Multiple reaction monitoring mode
MT	Minimal Threshold
nAChR	Nicotinic aceylcholine receptor
NB	Nucleus Basalis
NSW	New South Wales
NY	New York
OHC	Outer hair cell
ON	Ontario
OR	Oregon
PPT	Peduncolopontine nucleus
PSTH	Peri-stimulus timed Histograms
PVC	Polyvinyl Chloride
RB	Rostral Belt
RLF	Rate Level Function
SCT	Standard Corticosteroid Treatment
SFR	Spontaneous Firing Rates
SPL	Sound Pressure Level
SSA	Sitmulus Specific adaptations
SSNHL	Sudden sensorineural hearing loss
STDP	Stimulus Timed Dependent Plasticity
TDT	Tucker Davis Technologies
UHPLC-MS/MS	Ultra high liquid chromatography -in tandem mass spectrometer
UNHIL	Unilateral Noise Induced Hearing Loss
USA	United States of America
VAChT	vesicular acetylcholine transporter
VIC	Victoria

ABSTRACT

Tinnitus is a common and potentially debilitating condition that is often associated with hearing loss. Noise induced hearing loss is a frequently utilised animal model, in studies investigating the underlying mechanisms of tinnitus. In the central auditory system, the inferior colliculus (IC) is an obligatory nucleus for the ascending processing of auditory signals. It has been comprehensively investigated in animal models of unilateral noise induced hearing loss (UNIHL). Previous studies have mostly investigated the dominant, contralateral pathway with little attention being paid to the ipsilateral path. In normal hearing animals, contralaterally driven IC neurons are primarily excitatory while ipsilaterally driven IC neurons are primarily inhibitory. However, there are also ipsilaterally driven IC neurons that are excitatory. Literature that have investigated the response properties of these neurons, and their consequential response properties within an animal model of UNHIL is very limited. The first study presented in this thesis investigated the consequences of UNIHL of both dominant contralaterally excitatory and non-dominant ipsilaterally excitatory neurons of the IC.

In vivo electrophysiological recordings were performed in the left and right IC of normal hearing control animals and in animals that had been subjected to an acoustic trauma to their left ear (115 SPL dB at 16kHz for 1 h). Recordings were done 12 weeks following the acoustic trauma. The results indicate, that in line with previous authors, contralaterally excited IC neurons are tonotopically affected that is consistent with the lesion frequency. Interestingly, the tonotopic distribution of ipsilaterally excited IC neurons was not affected by the acoustic trauma. In ipsilaterally driven IC neurons, characteristics associated with normal central gains of excitatory activity were diminished and instead increased inhibitory response characteristics were observed. This result highlights the importance of investigating both dominant and non-dominant pathways of the central auditory system because following UNIHL the consequential response properties are indeed different in ipsilateral and contralateral excitatory IC neurons.

In the central auditory system, the cholinergic system is understood to be a potent neuromodulatory system that is associated with neuroplasticity. We investigated the effects of cholinergic enhancement paired with auditory training (AT) in the month immediately after experimentally induced UNIHL, with the aim of guiding the subsequent recovery of response properties. Following an acoustic trauma, rats were given a cholinesterase inhibitor Rivastigmine or saline for a period of four weeks. This was paired with AT, which consisted of plugging the intact unaffected ear and exposing the lesioned ear to training tones (Constraint Induced Sound Therapy; CIST). The results indicated, that in some of the response properties analysed, there were no statistical differences between AT cohorts and normal hearing control animals. Furthermore, cholinergic enhancement profoundly affected ipsilaterally driven IC neurons, with a high proportion of these neurons displaying narrow and tighter receptive fields. Cholinergic enhancement resulted in similar percentages of excitatory-inhibitory binaural interactions between the two ICs, even though there is profound hearing loss to the left side of these animals. Overall, these findings demonstrate that, in contralaterally driven IC neurons, AT can prevent many of the deficits seen in the response properties that are consequential of UNIHL and, with neuromodulation of the cholinergic system, ipsilaterally driven IC neurons are profoundly influenced.

The adult central auditory system has an extraordinary capacity to be manipulated, by regulating the input of a stimulus. Efforts to control neuronal activity by regulating sensory input in vivo have shown that stimulus timing-dependent plasticity (STDP) alters response properties of auditory neurons. Areas of the central auditory system that have been studied include the auditory cortex (A1) and dorsal cochlear nucleus (DCN). Here we provide novel findings of STDP and the effect of cholinergic enhancement in the IC within an animal model of UNIHL. The results showed that (i) IC neurons are indeed sensitive to STDP conditioning in vivo and (ii) cholinergic enhancement increases this capacity, in both negative and positive directions. These results add to the growing body of evidence to suggest that cholinergic modulation generally acts to optimise the processing of signals in attention demanding contexts.

Rivastigmine is an acetylcholinesterase inhibitor and thus increases free acetylcholine for synaptic availability. However, it is unknown if this is a global response or regionally specific within the brain. In the final part of this project, we employed UHPLC-MS/MS to measure the levels of acetylcholine in rat brain homogenates of different regions (frontal lobe, temporal lobe and IC) in response to a four-week treatment of a human equivalent dose of (0.2mg/kg/daily) Rivastigmine and compared to saline control animals. Both groups received AT. The results showed two major findings: there was a significant increase in the levels of acetylcholine in the temporal lobe, an area where A1 is located and there was a significant decrease in the amount of acetylcholine in the IC, when compared to the saline treated cohorts. This observation is in agreement with the findings of previous authors who have reported, that (i) A1 excitatory activity is associated with the release of acetylcholine within the context of cholinergic promotion and (ii) increased levels of acetylcholine in the IC results in a decreased capacity for these to neurons to adapt acutely. In line with previous authors who have used a similar dose and oral administration of Rivastigmine, this study provides further evidence that when paired with training, the chronic low doses of Rivastigmine indeed influences cholinergic activity in the central auditory system.

These findings may have implications for the development of therapies for tinnitus. By using a human equivalent dose of an approved drug, paired with a well-known neuro-rehabilitation intervention, together these interventions can significantly affect the consequential response properties of IC neurons that are observed following acoustic trauma. In a condition where currently there are no reliable cures, further development of these findings may provide insights that could lead to the generation of novel therapeutic approaches.

Chapter 1
General Introduction

1.1 Introduction

Tinnitus is a perplexing disorder defined by the perception of sound in the absence of an external stimulus. It can be classified as objective or subjective. Objective tinnitus is comparatively rare and is defined as a sound which can be perceived and measured by an examiner, often occurring as a result of an internal stimulus producing a physiological sound (Han et al., 2009). The internal stimulus may be due to swallowing, perturbations of the middle ear, vascular bruits or abnormalities, or a dysfunction of the temporomandibular joint (Grewal et al., 2014). On the other hand, subjective tinnitus, which is more prevalent, is when only the sufferer perceives the experience of the phantom sound. The character of the perceived sound can range between an episodic twang or a constant humming to a debilitating and relentless roar, which may be unilateral or bilateral (Dobie & Snow, 2004).

There is currently no known cure for tinnitus. Approximately 15% of the general adult population suffer from this disorder, with the majority of sufferers being over the age of 60 (Dobie, 2003; Heller, 2003). Tinnitus is frequently associated with some form of hearing loss likely due to the acoustic over stimulation. The effect of acoustic energy is dependent on the intensity and the duration and these two factors determine the dose (Tab.1.1A). The highest daily dose allowed safe by SafeWork Australia NSW (2011) is 85 SPL dB for an average 8-hour day. On average, a night club produces sound intensity ranging between 104 to 120 SPL dB and personal music devices are typically played at SPLs between 75-100 dB (Serra et al., 2005). Therefore, by using portable music players at 100SPL dB for 15 minutes, listeners are potentially exposing themselves to the equivalent level of acoustic energy an industrial operative would experience, in an 8-hour day when exposed to sound pressure levels of 85dB (Kageyama, 1999). It is now clear that the prevalence of tinnitus is increasing as the proportion of senior population increases and more young people are voluntarily exposing themselves to the additive impact of recreational and industrial noise.

Table 1.1. A. Equivalent Noise Exposures LAeq,8h = 85 dB and B. Common noise sources. (A) The combinations of various sound levels and associated times that is the sound energy equivalent of an average 8-hour day within an 85dB noisy environment. (B) Common noise sources and their typical sound level. Safe Work Australia 2011.

A. Equivalent Noise Exposure LAeq, 8h = 85 dB	
Sound Level in dB	Exposure Time
80	16 hours
82	12 hours
85	8 hours
88	4 hours
91	2 hours
94	1 hour
97	30 minutes
100	15 minutes
103	7.5 minutes
106	3.8 minutes
109	1.9 minutes
112	57 seconds
115	28.8 seconds
118	14.4 seconds
121	7.2 seconds
124	3.6 seconds
127	1.8 seconds
130	0.9 seconds

B. Common noise sources and their typical sound levels	
Sound Level in dB	Sound Source
140	Jet engine at 30 meters
130	Rivet hammer (Pain can be felt at this threshold)
120	Rock Drill
110	Chainsaw
100	Sheet-metal workshop
90	Lawn mower
85	Front end loader
80	Kerbside heavy traffic
	Lathe
70	Loud conversation
60	Normal conversation
40	Quiet radio music
30	Whispering
0	Hearing threshold

1.1.1 Animal Models of Tinnitus

To date, studies that use animal models of tinnitus can be broadly divided into one of two categories; namely, the behavioural models and the physiological models (Eggermont & Roberts, 2004; Kaltenbach, 2011). Behavioural models are focused on measuring the percept of tinnitus followed by an investigation into the associated psychophysical characteristics of the percept. Physiological models are used to investigate the changes that occur in the nervous system that may underlie tinnitus. Physiological studies are focused on the mechanisms that can lead to tinnitus, measuring signalling and events that occur at the neuronal level.

There is a general consensus that environmental manipulations experienced by humans that causes tinnitus can be mirrored in animals, and the animal's percept can be measured. Most behavioural models use paradigms to quantify tinnitus by assessing a motor response (Jastreboff et al., 1988; Eggermont & Roberts, 2004; Turner et al., 2006; Yang et al., 2011; Berger et al., 2013). A behavioural model that has received much favour in the last 15 years, is the gap in noise behavioural model of tinnitus, which is based on the acoustic startle reflex paradigm (Turner et al., 2006; Kaltenbach, 2011). The theory behind this model is based on two facts: that the acoustic startle can by evoked by noise and the magnitude of this startle can be suppressed by a gap of silence in the background sound that precedes the startling noise. If the animal had tinnitus, the silent gap in background sound would be 'filled' by the tinnitus percept, and therefore the animal will exhibit a startle that is not suppressed by the gap in noise. Although this model is useful as it circumvents possible compounding motivational and memory aspects of other behavioural paradigms, the inherent limiting factor of all behavioural models is a lack of insight and information about the underlying mechanisms and events occurring within the nervous system. Indeed, we have the means to measure an animal's tinnitus percept, to assess different tinnitus

causing agents and continuing along this line of reasoning- potential treatments. However, a mechanistic reasoning into why tinnitus has manifested is eluded in this approach. This information is only possible with the lens of a physiological model.

Physiological models are used to assess neural events and changes that are likely to be related or correlated with tinnitus (Kaltenbach, 2011). Typically, methods work directly at the level of neurons in the auditory centres to track differences and changes in animals subjected to tinnitus inducing agents. Studies can employ a before and after approach or compare data from control to treated animals (Szczepaniak & Moller, 1996; Salvi et al., 2000b; Furman et al., 2013; Manzoor et al., 2013; Wang et al., 2013; Coomber et al., 2014; Heeringa & van Dijk, 2016). As tinnitus is an auditory percept in the absence of external acoustic stimuli, the consequential underlying mechanism is dependent on central manifestations that are occurring as if there is indeed an external acoustic trigger. Likely candidates are changes in spontaneous activity or hyperactivity (discussed in section 1.1.4). The advantage of this model is that it allows a direct investigation into where in auditory structures the changes in neuron characteristics are. However, it does not directly prove the animal's percept of tinnitus. The value in this approach is, it demonstrates how the tinnitus agents induce changes and how the mechanistic induction of tinnitus can be set in motion.

From a wider perspective, it is arguable that a behavioural approach is a multidimensional view involving multiple brain regions, whereas a physiological approach is viewed at the auditory level only, often concerning one nucleus at a time (Eggermont & Roberts, 2004; Han et al., 2009; Kaltenbach, 2011). Each have their own value depending on the scientific question at hand. In any attempt to understand and treat tinnitus, there is a need to explore both frameworks. However, this project adopts a physiological approach, assessing changes as a result of noise induced hearing loss. Understanding the limitations of this approach means treading carefully when describing our results and not

directly correlating the results with the tinnitus perception. Given the highly personal and subjective nature of tinnitus, it is difficult to directly study this condition in animal models. Instead, by utilising a physiological animal model we can study changes in neural processing that are inferred to produce the percept. Therefore, more appropriately, this project works within the contemporary view that injury to the peripheral structures in the ear results in upstream plastic adjustments in the central auditory system. In the next section, a focus on hearing loss in the context of peripheral structures in the cochlea will be explored.

1.1.2 The Peripheral Auditory System and Hearing Loss

Most cases of tinnitus are accompanied by some form of hearing loss. It is widely accepted that ototraumatic agents that cause cochlear dysfunction will lead to the central manifestation of tinnitus (Moller, 1992). Acoustic trauma is known to produce multiple types of structural damage to peripheral auditory structures. It is also widely accepted that although abnormal inner ear physiology may cause an upstream impact, the sustainability and perseverance of tinnitus cannot be attributed exclusively to cochlear dysfunction (Eggermont & Roberts, 2004; Saunders, 2007; Han et al., 2009).

The loss of sound evoked activity as a result of damage or elimination of sensory hair cells reduces neural efferent activity of the cochlea nerve. This causes a decrease of firing rate, and a decrease in synaptic transmission (Husbands et al., 1999). The decrease of firing rate can occur due to the compromised structural integrity at the tip links, the stereocilia itself (rootlet, ankle and side) or the bundle ratio of inner hair cells (IHC) and outer hair cells (OHC)(Bashtanov et al., 2004; Saunders, 2007). Hair cells, especially the OHCs, are particularly vulnerable to noise and aging and in mammalians, once they die they do not regenerate. The majority of auditory nerve fibres are IHCs and total loss leads to profound deafness, however subtotal loss does not affect audiometric thresholds until it exceeds 80% (Hickox & Liberman, 2014).

Acoustic overstimulation can result in damage to the tip links, which are extracellular linkages important for maintaining coherence of the bundle. This loss of coherence may lead to greater displacement of the hair cells. Damage to sensory hairs cells such as the shaft, rootlet or the tips would also impact on the extracellular coherence. This lack of bundle coherence or rigidity can serve as a gating mechanism for the already open transduction channels, causing depolarised summation which can lead to excessive release of neurotransmitter at the hair cell and cochlea nerve (Husbands et al., 1999; Maison et al.,

2002). As a result, excess release of glutamate can occur. Damage to OHC can compromise its motility, which in turn impact IHC motility by enhancing the sensitivity of IHC (LePage, 1995). If this loss of mechanical connectivity occurs, it serves to produce tonic depolarisation causing an excess of glutamate release, resulting in glutamate excitotoxicity (LePage, 1995; Patuzzi, 2002) resulting in degenerative death of spiral ganglion neurons.

Cochlear synaptopathy is the loss of connections between sensory cells of the cochlea and the neural elements that carry their information to the brain. It has been observed that temporary threshold shifts as a consequence of noise exposure, did not cause a significant loss of sensory cells but instead caused significant cochlea synaptopathy (Furman et al., 2013). This means, that even though some of the hair cells are intact, the neurons are firing at a lowered threshold, leading to the degeneration of the peripheral terminals and widespread degeneration of the cochlea nerve (Hickox & Liberman, 2014). The disruption of the synapses can be permeant and the subsequent neuronal loss it is thought to be a contributing factor to the diminished output from the cochlea.

Stereocilia degeneration and cochlear neuropathy are possible mechanisms that result in disinhibition and promote the generation of spontaneous activity further upstream in the central auditory pathway. Although tinnitus begins with peripheral hearing loss, several studies have shown that, surgical resection of the auditory nerve almost never cease the tinnitus perception. This persistence indicates that tinnitus is a centrally mediated phenomenon. (Dobie & Snow, 2004; Eggermont & Roberts, 2004; Saunders, 2007). In the next section, a focus on hearing loss in the context of the dorsal cochlear nucleus, a target of the cochlea nerve, will be explored.

1.1.3 Cochlear Nucleus and Disinhibition

The local circuitry of the cochlear nucleus (CN) has a variety of specialised postsynaptic neurons that modulate the initial excitatory input, resulting in a refinement of the primary output of the auditory nerve (Kaltenbach et al., 2005). The processing of basic response properties is a function of cell morphology, which governs their temporal response pattern, spectral selectivity and discharge regularity. For example, fusiform and pyramidal cells of the CN fire at the beginning of a stimulus, abruptly decline, followed by a slow growth in spike rate, then continue to spike after the stimulus (similar to 'tonic response' discussed in section 1.2.1). The octopus' cell of posterior ventricular cochlear nucleus only spike on onset of the stimulus and is not sustained (similar to onset response discussed in section 1.2.1). In the anteroventral cochlear nucleus, the bushy cell, responds to onset-related features of the stimulus and resemble the input continuously throughout the stimulus (sustained cell). This means, compared to the fusiform cells of the CN, the bushy cells minimally transform the stimulus. Its frequency tuning curve and discharge regularity almost mirror the auditory nerve input. This is important as it allows the faithful transmission of stimulus for binaural comparators (Rhode et al., 1983). The bushy and fusiform cells are only a few examples of the specialised cells in the CN. Together, the topographical organisation and highly differentiated neuronal cells within the CN serve to selectively attenuate particular signals while amplifying others. The local circuity of the cochlea nucleus can be seen as a preliminary filter of the temporal, spectral and spatial aspects of the stimulus, drawing on specific principles (response properties) designed to optimise the signal for the next central processing task.

Kaltenbach and colleagues (2005) studied well characterised cells of the DCN under hearing loss conditions. Fusiform cells are the major output cells of the DCN and are inherently spontaneously active. Based on the local circuity, the usually inhibited fusiform cell is potentially being disinhibited, increasing its spontaneous output on to the central nucleus of the inferior colliculus (Kaltenbach et al., 2005) (Fig.1.1). It is speculated that damage to OHCs (Type II afferents) impact the granule-cartwheel cell circuitry; possibly resulting in the decrease of inhibitory output onto the fusiform cell (Kaltenbach et al., 2005) (Fig.1.1). Furthermore, damage to IHCs (Type I afferents) causes a loss of activity in the inhibitory interneuoron, the vertical cell, resulting in dminished inhibtion of the fusiform cell. These circuits all contribute to net losses of inhibition. Dysfunction of the various synaptic inputs associated with converging projections, may influence and contribute to the presentations of increase spontaneous firing rates (SFR) of activity seen in the central nucleus of the IC of animal models of tinnitus. Indeed, Mulders and Robertson (2013), found that in animals subjected to acoustic trauma, a higher incidence of SFR was observed when compared to control groups. It possible there is a change in the local circuitry within the IC. It is also plausible, due that the fact the fusiform cell is the main output cell projecting to the central nucleus of the IC, that the SFR source is originating from the neurons in the DCN.

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Kaltenbach, J.A., Zhang, J. & Finlayson, P. (2005) Tinnitus as a plastic phenomenon and its possible neural underpinnings in the dorsal cochlear nucleus. Hearing research, 206, 200-226.

Figure 1.1. Schematic of the neural wiring of the dorsal cochlear nucleus. Inputs of excitation and inhibition between cell types and interneurons in the DCN circuity. Activity from multiple cells such as the stellate, cartwheel and vertical cells normally inhibit the fusiform cell. The fusiform cell is the major output cell to IC neurons. *Acquired from Kaltenbach et al., 2005.

1.1.4 Neuroplasticity and Neural Correlates of Tinnitus

Sensory circuits within the brain retain the ability to change in response to altered sensory input. Neuronal networks are subjected to experience-dependent changes, such that there is a dynamic relationship between the senses, the environment and the neural wiring of that percept (Hebb, 1947). These events broadly, termed neuroplasticity, are perhaps most observable while acquiring skills and learning. The neuroplastic events are often measurable, marked by molecular, cellular and tissue changes. Increased brain-derived neurotrophic factor (Spires et al., 2004; Zigmond & Smeyne, 2014), neurogenesis (Murphy et al., 2004; Van Praag et al., 2005) and an increase hippocampal density and thickness (Diamond et al., 1964; Diamond et al., 1966) are examples of changes observed during periods where plasticity is promoted. This malleability is advantageous for a broad spectrum of medical conditions from total recovery from mild brain injuries (Will et al., 1976) to the attenuation of Parkinson disease (van Dellen et al., 2000). Neuroplastic events can also be described as maladaptive due to a disturbance to the balance of inhibitory and excitatory activity.

As previously described, hearing loss often precedes tinnitus. This phenomenon parallels pain disorders such as Phantom limb pain, where there is a disorder of the central nervous system (somatosensory) associated with a missing limb (Flor, 2008). This is important as both phenomena have remarkably similar central representations due to a 'gap' of sensory input (Møller, 1997). From a wider perspective, it is hearing loss that is impacting the upstream processing of auditory signalling. This maladaptive phantom auditory disorder manifests centrally as hyperactivity involving the disinhibition of local circuits, increased spontaneous activity, altered neural synchrony and tonotopic reorganisation of the central auditory system (Maison et al., 2002; Dobie, 2003; Eggermont & Roberts, 2004; Han et al., 2009; Mulders & Robertson, 2013). These ideas constitute a model of studying the neurophysiology of tinnitus and are essential concepts that will be discussed in this thesis.

The global presentation of tinnitus in terms of attention, emotionality and in general non-auditory structures further extends the neural complexity of auditory processing however these concepts, although important, are beyond the scope of this study.

1.1.4.1 Hyperactivity: Sound-evoked Activity & Spontaneous Firing Rates

Hyperexcitability can be defined as an increase in sound-driven responses or increases in spontaneous firing rates. Cochlear dysfunction may result in a deterioration of output which can lead to the loss of normal inhibitory action leading to hyperactivity. (Abbott et al., 1999; Salvi et al., 2000a). Following acoustic trauma, there is decreased normal excitatory activity of the auditory nerve (AN) fibres followed by an increase of hyperactivity in the dorsal cochlear nucleus (DCN), inferior colliculus (IC) and auditory cortex (A1) (Kaltenbach & Afman, 2000; Salvi et al., 2000a; Norena & Eggermont, 2003; Furman et al., 2013).

Following acute noise trauma in chinchilla, Salvi et al. (2000a) showed a reduction in amplitude of the compound action potential (CAP) from the AN which resulted in the expansion of local field potentials in the IC (Fig.1.2). In the IC, the activity resulted in a greater yield of spikes at all intensities. Furthermore, about 40% of IC neurons showed an expansion of tuning curves below the characteristic frequency (CF) (Salvi et al., 2000a) (Fig.1.2).

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Salvi, R.J., Wang, J. & Ding, D. (2000) Auditory plasticity and hyperactivity following cochlear damage. Hearing research, 147, 261-274.

Figure 1.2. Frequency response area and frequency threshold tuning curves from a neuron in the IC pre- (bold) and post-exposure (light). The traumatizing exposure consisted of 20 min, 16.1 kHz tone at 107 dB SPL. As a result, a threshold increased occured along the low frequency tail, and not at characteristic frequency. *Acquired from Salvi et al. 2000

Similarly, Norena and Eggermont (2003) showed that in cats, following acoustic trauma, neurons in the A1 showed a significant increase in discharge rates. This is suggestive of a loss of sideband inhibition, which resulted in an increase of stimulus driven activity and an enhanced evoked activity. These functional characteristics are thought to impact auditory functions by contributing to loudness recruitment and poor speech discrimination, sometimes associated with tinnitus. Sound-driven hyperactivity is considered a sign of plastic changes. The gain of the central auditory system is increased in order to compensate for the disruption of the cochlea partition (Rauschecker, 1999).

Another form of hyperactivity is an increase of SFR. Spontaneous firing occurs in the absence of a specific stimulus, and occurs due to a stochastic release of neurotransmitters. This stochastic release is considered an inherent and intrinsic property of the cellular membrane of neurons. Devoid of external stimuli basal leakage occurs, the rate of which is distinctively unique for different neurons (Seki & Eggermont, 2003; Saunders, 2007).

In hamster, following an exposure to a 10 kHz tone at a 125-130dB SPL for a period of 4 hours, there was a significant reduction in SFRs in the DCN, 2 days post hearing loss. This was followed by a significant increase of the SFR which occurred at day 5. The authors reported a continuous slight increase in peaks in the DCN for up to 6 months (Fig.1.3) (Kaltenbach et al., 2000). The resultant hyperactivity can be due to either an increase in excitatory synaptic transmission or a decrease in inhibitory transmission (Bilak et al., 1997; Kim et al., 1997). It is possible that the temporal correlation of hyperactivity may be related to trends and patterns of degeneration in the DCN. Post sound exposure, new fibres were seen in the DCN after 2 months (Bilak et al., 1997), whilst the degeneration of fibres were seen between 4 and 8 days (Kim et al., 1997). While the pattern of degeneration appears to fall within the timeframe of the onset of hyperactivity, the relationship between regeneration remains unclear. Further information is needed to determine if there is

a correlation between hyperactivity and the patterns of fibre re- and de-generation. It appears that the impact of sound exposure on the DCN is chronic, where small continuous changes emerge over time, following the acute peripheral peturbation (Fig.1.3) (Kaltenbach et al., 2000).

Interestingly, while increases in SFR were seen in the DCN 5 days after acoustic trauma (Kaltenbach & Afman, 2000), SFRs were apparent in the AC within hours (Seki & Eggermont, 2003). It is possible that this difference may be due to the different species of animals used. However, this temporal relationship of SFR manifestation in these different brain regions may be of significance. Also, following the removal of DCN, the animals still exhibited behavioural tinnitus, suggesting that there are influences from higher order nuclei contributing to the chronic perception of tinnitus (Brozoski & Bauer, 2005).

In the IC of CBA/J mice treated with acoustic overstimulation and acute salicylate intoxication, different SFR trends were seen (Ma et al., 2006). Ma and colleagues reported that salicylate toxicity broadened excitatory receptive fields and suppressed SFRs. On the other hand, in the acoustic overstimulation groups, when the data was combined and organised along the tonotopic axis, there was no statistical difference in SFR when compared to controls. However, when the data was restricted within and around the bandwidth (BW) of the acoustic trauma frequency, an increase of SFR was seen. This was most evident in mice over exposed to bilateral sounds compared to unilaterally exposed animals. The authors suggested that, the relatively smaller sample size of the unilateral exposed mice may account for this discrepancy. In contrast, Mulders and Robertson (2013) observed, in unilateral exposed guinea pigs, there was indeed a significant increase in hyperactivity in the IC. The differences of these results are likely due to differences in the timeline of experimental sampling and species variation. Enhanced evoked responses and spontaneous firing rates fall under the same electrophysiological profile of hyperactivity,

however it is seen that the characteristic profiles of each differ considerably in different brain regions.

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Kaltenbach, J.A., Zhang, J. & Finlayson, P. (2005) Tinnitus as a plastic phenomenon and its possible neural underpinnings in the dorsal cochlear nucleus. Hearing research, 206, 200-226.

Figure 1.3. Time course of changes in maximal spontaneous rates of the dorsal cochlear nucleus over a 6-month period following intense sound exposure. The induction of hyperactivity occurred mainly during the first week after tone exposure (between 2 and 5-day post-exposure) and slight increases in the degree of hyperactivity continued to occur over the entire 6-month period.

* Acquired from Kaltechbach et al. 2000

1.1.4.2 Neural Synchrony and Tonotopic Reorganisation

Neuronal synchrony is described as the presence of a temporal trend of otherwise stochastic spontaneous activity that is seen in multiple neuron clusters (Saunders, 2007). Using a spiking neuron model, increased spontaneous activity and neural synchronisation of cortical AC neurons are seen in animals subjected to acoustic trauma (Seki & Eggermont, 2003; Dominguez et al., 2006). These neuroplastic events in the A1 materialised as a loss of inhibition causing lateral excitation, which resulted in a change of cortical connections (Dominguez et al., 2006). Tonotopic organisation is defined by the orderly organisation of neuron populations that respond specifically to ranges of spectral frequencies. Spectral –frequency representation in the auditory cortex follows a rostral (high frequencies)- caudal (low frequencies) direction, faithful to the tonotopic partition of the cochlear nucleus (Oliver, 2005).

Following damaging noise exposure that results in a hearing loss, there is reduction in input to neurons along the auditory path. The extent of the hearing loss will determine the auditory territory that is affected by the lesion and has been termed the lesion projection zone (LPZ) (Calford et al., 1999). Within the LPZ, there is often a change in characteristic frequency (CF) (Norena & Eggermont, 2003). The LPZ is partially or entirely represented by an expanded receptive field by lesion-edge frequencies (Irvine, 2007). In normal hearing, a peripheral stimulus of a specific frequency simultaneously excites its corresponding cortical area and inhibits edge frequencies. Following hearing loss, disinhibition of edge frequency occurs, causing the neurons to expand their receptive field (Fig.1.4). This 'unmasking' of edge neurons causes higher levels of activity along the tonotopic axis, with the edge frequency imposing its own frequency-selective input on cortical cells within the LPZ. (Norena & Eggermont, 2003). Furthermore, following acoustic overstimulation, although spontaneous rates of cortical neurons increased inside and outside of the frequency ranges that were affected by the hearing loss, in areas of

re-organisation, there was further increase of neural synchrony and spontaneous activity (Dominguez et al., 2006)(Fig. 1.5).

It is sensible to summarise with the observation that tinnitus and the underlying neurophysiology is complex, which reflects the difficulty of finding an effective treatment. Future treatment strategies may have to target multiple factors simultaneously.

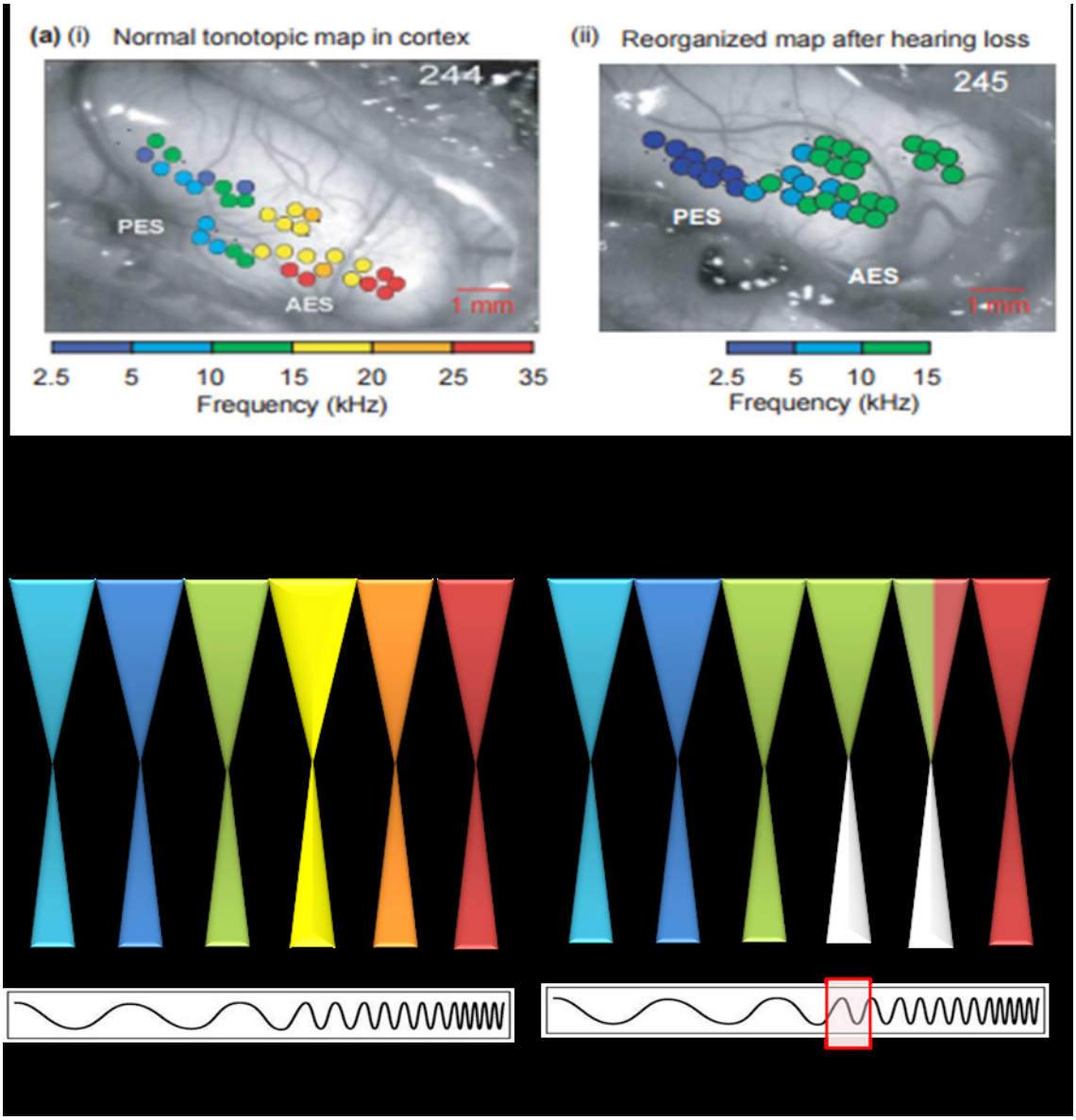


Figure 1.4. Normal and reorganised tonotopic maps in the auditory cortex.

(a) The cortical surface of a control cat with superimposed colour-coded recording sites, each colour indicating the characteristic frequency for each site. Normal hearing cat (i) compared to a cat subjected to noise induced hearing loss (ii). b) A schematic of normal hearing. c) Acoustic overstimulation of a specific frequency is termed the trauma frequency as indicated by the red box; this corresponds centrally as the LPZ. Edge-frequencies expand and become 'unmasked', expanding their receptive field over the lesion projection zone (green and red). *(A) Adapted from Eggermont & Roberts 2004

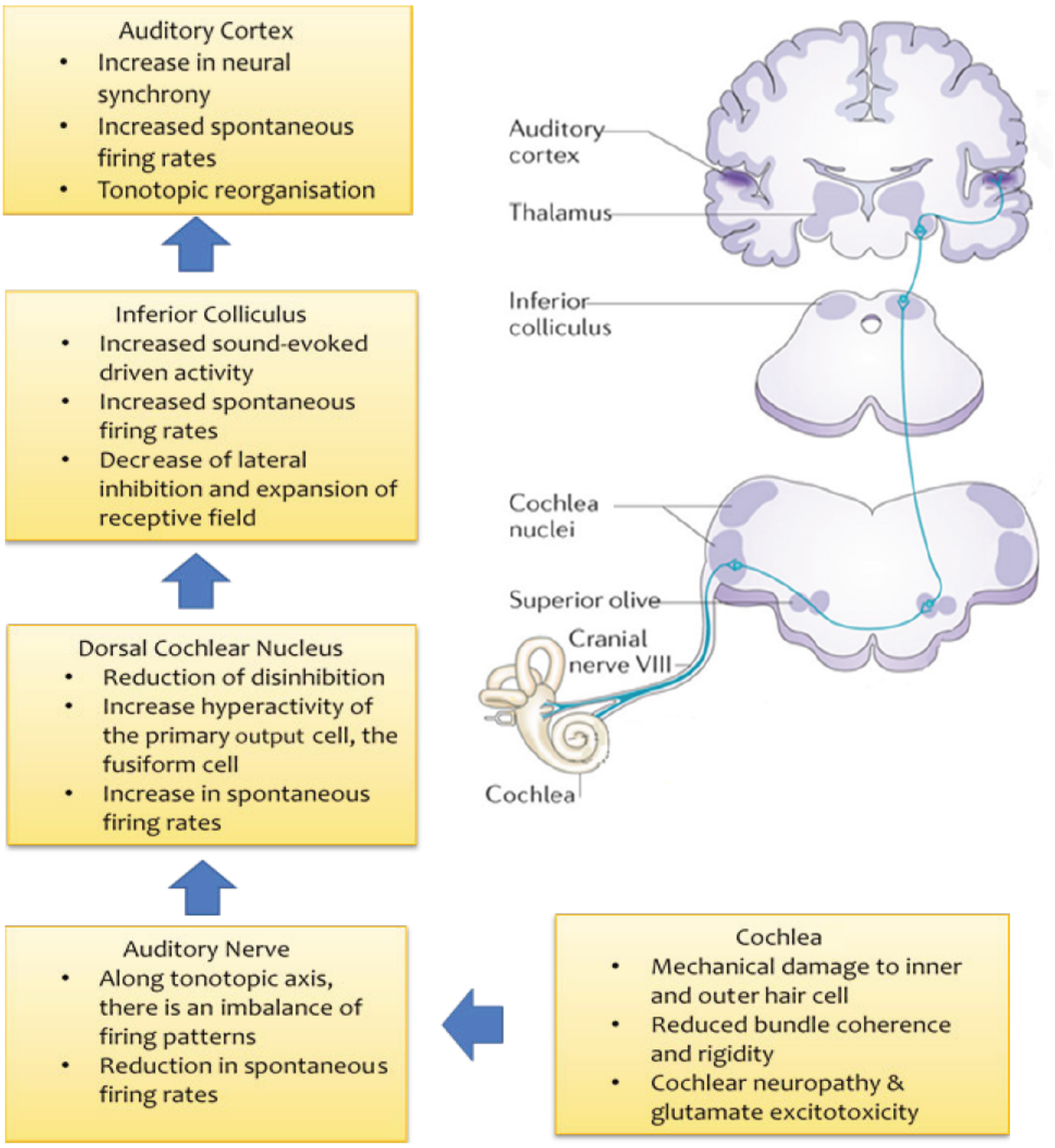


Figure 1.5. Summarised changes in the auditory pathway following exposure to acoustic trauma.

Acoustic overstimulation cause damage to the organ of corti and the hair cells are mechanically damaged, which result in a lack of coherence and rigidity of the hair bundle. This can cause

Figure 1.5 *Continued*

cochlear neuropathy and glutamate excitotoxity (LePage, 1995; Patuzzi, 2002; Bashtanov et al., 2004; Hickox & Liberman, 2014). This results in an imbalance of firing patterns and reduction of firing rates in the auditory nerve (Furman et al., 2013). As a result, dishibition follows, causing central gains of hyperactivity of sound-evoked activity and spontaneous firing rates in the DCN, IC and A1. Tonotopic re-organisation and increase neural synchrony are also manifest in the A1. (Kaltenbach & Afman, 2000; Salvi et al., 2000a; Norena & Eggermont, 2003; Mulders & Robertson, 2013).

1.2 The Inferior Colliculus

The inferior colliculus (IC) (La. Small posterior hill) is a dome-shaped structure located on the posterior aspect of the mesencephalon. In the vertebrate brain, it is considered one of the largest auditory nuclei and is a synaptic terminus for most (if not all), ascending auditory sub-collicular projections. It is a mostly obligatory nucleus for ascending projections to the medial geniculate body (MGB)(Schreiner & Winer, 2005).

The inferior colliculus is anatomically large in size and has convergent connections both in the ascending and descending auditory pathways of the central auditory system. Electrophysiologically, the IC plays a critical role in central auditory processing (Aitkin and Phillips 1984b). Projections into the IC include ascending input from almost all parts of the cochlear nucleus, most of the olivary complex and bilaterally from each lateral lemniscus nucleus as well as descending projections from all auditory cortical areas. Furthermore, the IC itself projects to all brainstem nuclei that project to it, and also sends axons bilaterally to the auditory thalamus. The organisation of afferent input is tonotopically maintained. The cochlea spiral basilar to apex, high to low, frequency orientation is congruous with the tonotopic arrangement of characteristic frequencies within the central nucleus of the IC.

The IC is made-up of three nuclei and the central nucleus (CNIC) receives most, if not all, of the projections from sub-collicular auditory nuclei. Therefore, it is the site that has received the most experimental attention and investigation regarding noise induced hearing loss. As previously described, the consequences seen in other auditory brain regions such as an increase in spontaneous activity and hyperactivity is also seen in the IC (Meltser & Canlon, 2010; Manzoor et al., 2013; Mulders & Robertson, 2013). In order to capture the consequences of the noise induced hearing loss in the IC an understanding of normal physiology is necessary. The purpose of the following section is to review the functional response properties of IC neurons.

1.2.1 Classification of Inferior Colliculi Neuronal Response Properties

In the CN, hyperactivity is investigated as a function of cell type. Morphologically, the IC has only two cell types (Malmierca et al., 1993), and does not reflect the physiological complexities of the IC. Current efforts to further subclass IC cell types based on molecular idenities have been investigated (Goyer et al, 2019). However, an agreement of methodically classifying response characteristics of IC neurons is yet to be universally established. For the purpose of this project, a selection criterion specific to acoustic response properties similar to those used by previous authors will be employed. The following section will review response properties within the IC in the context of normal neurophysiology.

1.2.1.1 Frequency-Response Areas

The IC can be organised along two axes: a frequency axis and a SPL axis. The frequency axis has a low to high bandwidth gradient that is topographical. Low frequencies are seen dorsal to lateral locations and high frequencies are seen ventral to medial locations in rats (Fig.1.6A). A second gradient is also seen where the one-dimensional cochlear frequency map is transposed in a three-dimensional map in the CNIC (Fig.1.6A) as band widths are 'sheets' with a shallow continuum termed 'frequency-band laminae'.

(A) This figure was removed and can be accessed

Stiebler, I. & Ehret, G. (1985) Inferior colliculus of the house mouse. I. A quantitative study of tonotopic organization, frequency representation, and tone-threshold distribution. Journal of Comparative Neurology, 238, 65-76

(B) This figure was removed and can be accessed

Schreiner, C. & Winer, J.A. (2005) The inferior colliculus. Springer.

Figure 1.6. Organisation of the inferior colliculus in a Mouse (A) and Cat (B). (A) A three-dimensional plot of frequency-band laminae of the CNIC, the long thick lines on the 10- and 30-kHz lamina indicate the direction of the shallow gradient of frequency increases (medial to lateral) within a lamina. The concentric increase in darkness of shading (10-Khz) lamina corresponds to the increase of minimal threshold (MT) of neurons on a lamina. (B) Electrode penetration from dorsal to ventral through the ICC showing a step-wise progression of characteristic frequencies. * Acquired from Stiebler & Ehret, 1985 (A) & * Schreiner & Winer, 2005 (B).

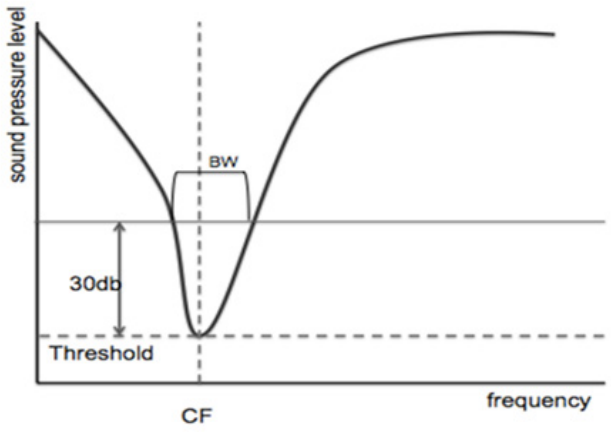


Figure 1.7. A schematic plot showing frequency response area (FRA). Characteristic Frequency (CF), minimal threshold (MT) and bandwidth (BW). Bandwidth is used to determine the Q value. Q_{30} is calculated by dividing the CF by the band width of frequencies 30dB above the CF (Q30= CF/BW30). *Adapted from Ma et al., 2006

The frequency response area (FRA) is a map of a neuron's receptive field. Plots show the receptive field of neurons in three dimensions: a) spike activity b) frequency and c) sound pressure levels. It shows spike activity in responses to varying SPL and frequency combinations and the intensity of spiking activity within each SPL-frequency combination, visualised as a heat map. A hallmark of neurons in the auditory system is its tonotopic organisation. When investigating acoustic trauma-induced changes, an insight into the receptive field of neurons is useful to determine changes in tonotopic organisation. Characteristic frequency (CF) is defined as the frequency with the minimal intensity threshold (MT) needed to elicit a response (Fig.1.7). The two-dimensional matrix of response magnitudes to all frequency-intensity pairs can determine the frequency tuning properties of neurons. The boundary of an excitatory response area is termed a frequency tuning curve (FTC), and to the left of the CF is the low-frequency bandwidth and the right, the CF high-frequency bandwidth; together they express the overall value of the bandwidth.

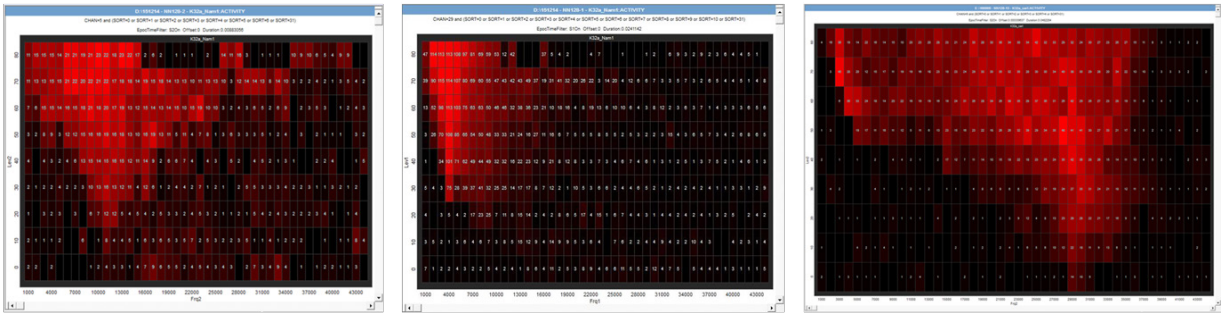
The shape of FTCs can be categorised as a function of response type (Ramachandran et al., 1999; LeBeau et al., 2001). Response types of IC neurons broadly fall into one of two categories, V-shaped (Fig.1.8A) and non-V shaped (Fig.1.8B) (LeBeau et al., 2001). In Le Beau's study (2001), the latter response types were established on isolated single units and FRAs were determined audiovisually, using contralateral stimulus parameters. V-shaped FRAs are considered the general 'classic' response type whilst non-V shaped FRAs are reflective of more specialised and specific aspects of sound processing (Palmer et al., 2013).

V-shaped neurons can be classed as either monotonic or non-monotonic according to their rate-intensity function. Monotonic V-shaped neurons typically have narrow tips at the CF and progressively widen laterally in response to higher SPLs. The wider response areas, on the lateral sides of the CF are generally not symmetrical. Low or high frequency

response areas can 'tail-off', that is the slope of the low (Fig.1.8A.ii) or high (Fig.1.8A.iii) frequency component can be shallower. In addition, in response to increased intensities, an increase of spikes can also occur.

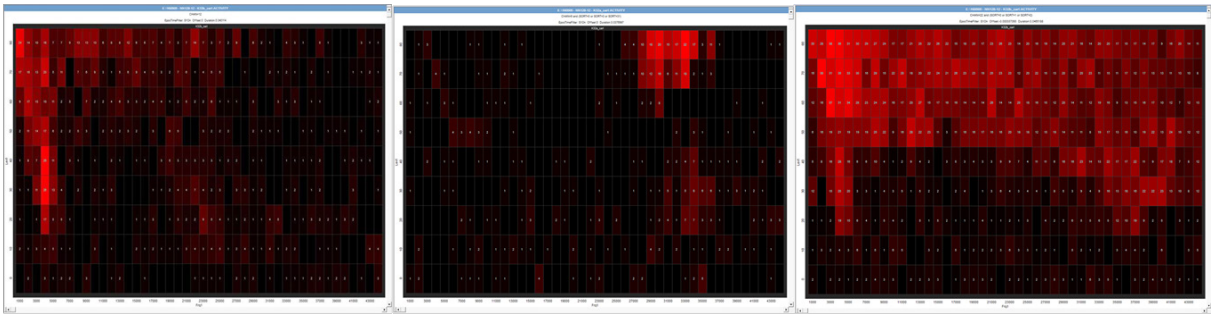
Non-V shaped IC neurons include I-shaped or narrow (Fig.1.8B.iv), O-shaped or closed (Fig.8B.v) and W-shaped or double peaked (Fig.1.8B.iv), (LeBeau et al., 2001). Interestingly, in the presence of $GABA_A$ antagonist bicuculline, these non-V shaped neurons showed more of a V-shape FRA. The authors suggest that this is indicative of the response types for many of these non-V shaped neurons and may be a site of convergence from other pathways (LeBeau et al., 2001). Closed responses are well-defined, circumscribed areas of excitation with strict isolation of frequency-intensity combinations; this follows a similar description of type O neurons (Fig.1.8B.v) (Ramachandran 1999). Double-peaked response shows a 'W' shape, where there are 'two tips' separated by an area lacking excitation (Fig.1.8B.vi). Type I or narrow units have a narrow excitatory area, usually centred within a very small frequency range (Fig.1.8B.iv).

A: V-shaped FRAs



(i) V-shaped (ii) V-shaped high-frequency tail-off (iii) V-shaped low-frequency tail-off

B: Non V-shaped FRAs



(iv) Narrow shaped (v) Closed shaped (vi) W-shaped

Figure 1.8. Raw representative examples of V-shaped and non V-shaped FRAs of IC neurons. (A) V-shaped FRAs. (i) Is a typical V-shaped response (ii) is a V-shape response which has a high-frequency 'tail-off' and (iii) is a V-shaped response showing a low frequency 'tail-off'. (B) Non-V shaped FRAs. (iv) is a narrow or I-shaped response type, (v) is a closed response type or O-shaped response and (vi) is a double peak or W-shaped response, where two 'peaks' are discernible and between and an area with no excitation. Plots show the receptive field of neurons in three dimensions: a) spike activity (red intensity) b) frequency (Y-axis) and c) sound pressure levels (X-axis).

1.2.1.2. Peri-Stimulus Time Histograms and Input-output Function

Peri-stimulus time histograms (PSTH) show temporal relationships between a stimulus and the resulting spike pattern. Typically, in the IC, two spike patterns are observed: Tonic and onset (Fig.1.9A&B). These neurons can be further investigated using rate/level functions or input output function (IOF), providing additional information of the response of a neuron to varying stimulus parameters. Most commonly, frequency is held constant while SPL is varied, and this shows the spiking output of a neuron. This metric is most useful when presenting stimuli at a neurons CF, but may be applied to other frequencies, or indeed other stimuli such as noise or clicks. The IOF of IC neurons can be categorised as monotonic or near monotonic with the rate-intensity function as either saturating or non-saturating (Semple & Kitzes, 1985; Lumani & Zhang, 2010) (Fig.1.9C&D). A response is saturated when increasing SPL fail to produce further increases in firing rate but instead maintain the maximal firing (Fig.1.9C.Blue). A non-saturating monotonic response is when the IOF elicit continual increase of spikes as SPL presentations increase but is limited to the system (i.e. physiological range and stimulus parameters) (Fig.1.9C.Red) (Semple & Kitzes, 1985; Mulders & Robertson, 2013). A classic non-monotonic response is an inverted U-shaped function (Fig.1.9D). Increases in SPL produce an initial increase in firing, but with further increases in SPL the firing rate declines.

In the context of acoustic trauma, Mulders and Robertson (2013) reported that there was no significant difference in the proportion of onset and tonic PSTHs in the CF of IC neurons between the sham and acoustic trauma groups (Fig.1.10A). A significant difference was seen in the IOF, where the trauma group showed a larger portion of monotonic responses and smaller number of non-monotonic response and the inverse was seen in the sham group (Fig.1.10B). It is possible that the difference in proportion could be due to a loss of peripheral inhibition (Salvi et al., 2000a). The typically non-monotonic

profile of a continuous incremental decrease preceded by a maximal firing rate is an inhibitory driven pattern. As a result of no inhibitory pressure, an incremental decrease does not occur, thus the response profile is observed to be monotonic.

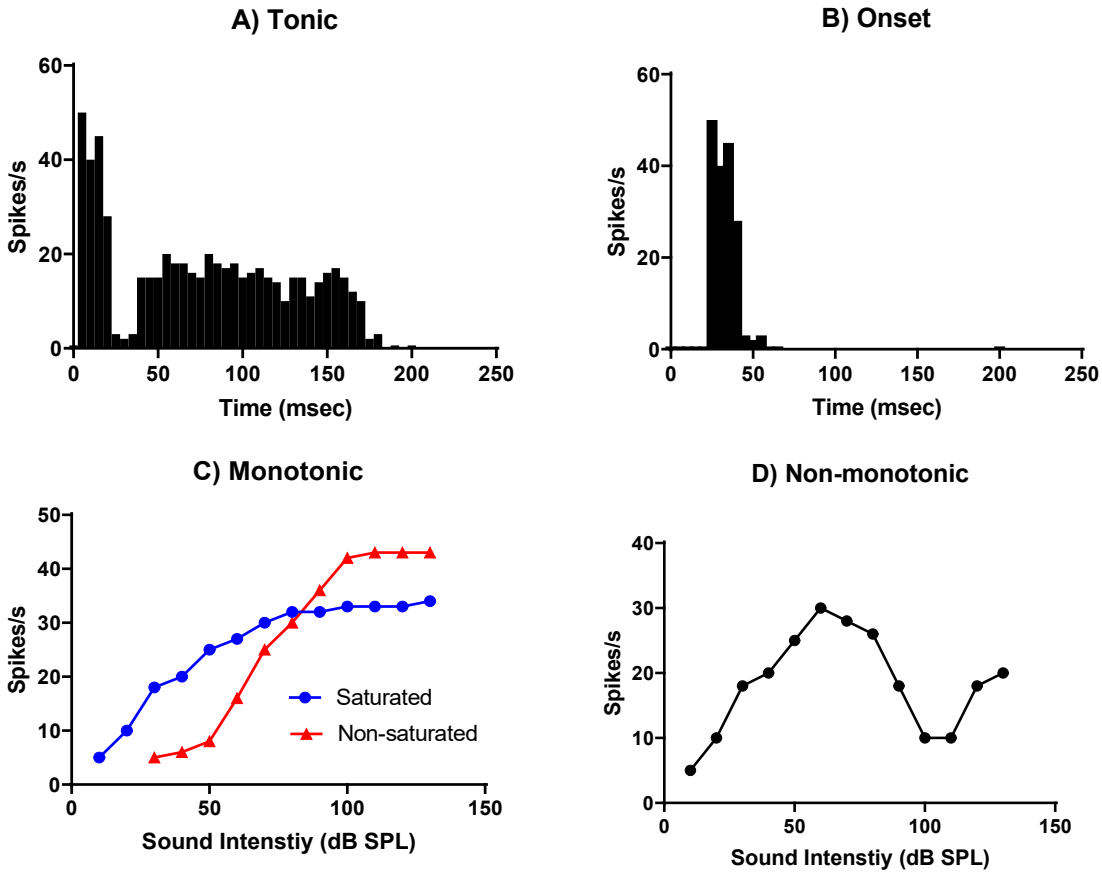


Figure 1.9. Representative examples of peri-stimulus timed histograms (PSTH) and input-output functions. Peri-stimulus timed histograms examples of tonic (A) and onset (B). stimulus. Representative examples of input-output functions (C) shows a saturated (blue) and non-saturated (red) monotonic responses, (D) shows a non-monotonic response.

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Mulders, W. & Robertson, D. (2013) Development of hyperactivity after acoustic trauma in the guinea pig inferior colliculus. Hearing research, 298, 104-108.

Figure 1.10. Percentage of IC neurons with response properties, (A) PSTH types (B) IOF types, (C) FRA types, (D) binaural interactions. Compares sham (white bars) and acoustic trauma animals (black bars) proportions. In response to acoustic trauma, a significant difference is seen in the proportion of IC neurons, specifically the IOF and binaural response characteristics. *Acquired from Mulders and Robertson (2013)

1.2.1.3 Binaural Interactions in Inferior Colliculus

The superior olivary complex is traditionally thought to be the first site where the right and left cochlear pathways converge. It is from here that the majority of neurons beyond this site are binaurally influenced (Oliver & Shneiderman, 1991). When investigating and distinguishing binaural input and interaction characteristics, categorizing neural interactions into broadly defined groups is useful when comparing a change in the proportion of specific binaural responses. Binaural processing of different cues is considerably complex and therefore there is a degree of variability in classification systems. The following section of this chapter will feature aspects of binaural excitatory-inhibitory processes; exploring a commonly used classification scheme employed to quantify and investigate binaural interactions.

The CNIC is a major integrative centre and the vast majority of these neurons respond to binaural stimulation (Oliver & Shneiderman, 1991). This structure receives converging ipsilateral and contralateral projections from lower auditory structures, which can be monaural or binaural. The IC has a bias of neurons exhibiting binaural inhibition. When sound presentations stimulate both ears, CNIC neurons typically generate an excitatory-inhibitory (EI) response – that is contralaterally excited neurons are inhibited by ipsilateral presentations (Fig.1.11A). A smaller proportion of neurons show an excitatory-excitatory (EE) response: neurons are excited by both contralateral and ipsilateral sound presentations (Fig.1.11C). EO binaural responses are non-binaural interactions that are exclusively monaural (Fig.1.11B)(Goldberg & Brown, 1969; Flammino & Clopton, 1975; Irvine & Gago, 1990).

Table 1.2. Possible combinations of binaural interactions. The most common binaural interactions are EI, EE and EO. OE or OE is purely monaurally responsive neurons. II, IO, OI and OO are generally considered non-auditory responsive neurons. (Irvine and Gago 1990)

	Ipsilateral Stimulation		
Contralateral stimulation	Excitation	Inhibition	No effect
Excitation	EE	EI	EO
Inhibition	IE	II	IO
No effect	OE	OI	OO

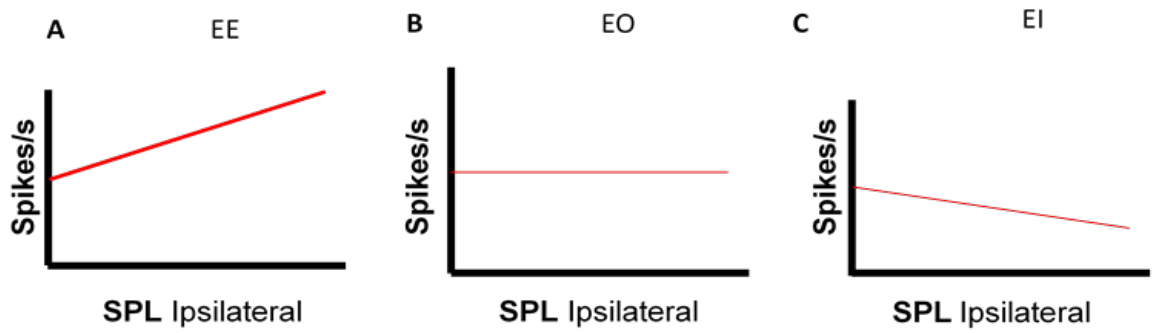


Figure 1.11. Schematic examples of binaural response types. A) EE response type, when there is an increase of spikes/s as there is an increase of ipsilateral SPL intensities. B) EO response type, when there are no changes in spike/s. C) EI response type, when there is a decrease of spike/s with increasing ipsilateral SPL intensities.

Uniformed sampling location is an inherent obstacle (via audio-visual feedback) for in vivo electrophysiology. Therefore, a large sampling size as well as post hoc histology is necessary to draw inferences of a change due to an intervention versus physiological variations. In regard to tonotopic organisation, when the electrode penetrations lie in a dorsal ventral direction, there is a positive correlation between the characteristic frequencies and depth of recording (Fig.1.6B). Functionally, the dorsomedial region corresponds to low frequency neurons and the ventromedial regions correspond to high frequency neurons (Malmierca et al., 1995)(Fig.1.6A). Irvine & Gago 1986, proposed that along with the topographical organisation, there is also evidence suggesting organisation of binaural input. In ventral regions EI and monaural EO neurons are found in clusters (200-300um) in an 'intermingled' pattern. Rostrally, EI populations are found in caudal and lateral electrode positions. In horizontal penetrations of a specific frequency lamina, rostral vs caudal location influenced the strength of characterised inhibition. Caudally, isolated neurons had weaker ipsilateral inhibitory responses, while rostral neurons have strong ipsilateral inhibition. This binaural segregation possibly reflects the converging projections from lower auditory structures, governed by the changes in the strength of firing responses of the excitatory input.

It is expected that, in normal hearing animals, binaural EI interactions will be more commonly seen in the CNIC. This physiological bias is likely to be from inhibitory projections from the ipsilateral lateral superior olive, the ipsilateral ventral complex of the lateral lemniscus and contralateral dorsal nucleus of the lateral lemniscus (Malmierca et al., 1995; LeBeau et al., 2001). It is expected that in control condition, binaural interactions EI, EO and then EE respectively will be seen in the highest to lowest portions (Flammino & Clopton, 1975; Silverman & Clopton, 1977; Zhang & Kelly, 2010).

In line with the notion that increased ipsilateral activity is a result of decreased contralateral activity, McAlpine et al. (1997) showed that in deafened ferrets (surgical ablation of the right cochlea) the response properties of the ipsilateral IC were indeed altered. In intact animals, an ipsilateral excitatory response was seen in 33% of recording loci. In contrast, in acutely ablated ferrets, 70% ipsilateral responses were excitatory and in chronically ablated ferrets (2-3 days after), 80-90% of ipsilateral responses were excitatory. The intact contralateral IC was largely unaffected. This study shows that, ipsilateral activity can increase due to a loss of contralateral input. Studies that investigate the binaural interaction of ipsilaterally driven excitatory ICs are scant and largely deduced by studies that focuses on the consequences that occur in contralaterally driven IC neurons (Irvine & Gago, 1990; Oliver & Shneiderman, 1991).

1.3 Cholinergic Modulation

Acetylcholine is a critical neuromodulator, that permits long lasting experience-dependent cortical plasticity. Interventions that specifically cause dysfunction in the central cholinergic system antagonise training induced plasticity in the motor and sensory cortices and by consequence, inhibit the recovery from brain damage (Metherate & Weinberger, 1990; Conner et al., 2005; Ramanathan et al., 2009; Gawel et al., 2014). On the other hand, cholinergic promotion combined with sensory stimuli directly affects the receptive field of cortical auditory neurons and thus enhances an animal's discriminatory ability (Kilgard & Merzenich, 2001; Kilgard et al., 2007; Shepard et al., 2013; Voss et al., 2016). There is a growing body of evidence to suggest that, cholinergic modulation generally acts to optimise the processing of signals in attention demanding contexts (Hasselmo & McGaughy, 2004; Sarter et al., 2005; Jääskeläinen et al., 2007; Ayala & Malmierca, 2015).

Taken together, these studies suggest that acetylcholine plays a modulatory role in the central auditory system, potentially affecting the auditory system in ways that primes the system to consider certain sounds to be behaviourally or physiologically relevant. These studies also highlighted the great potential for exploiting cholinergic modulation in central maladaptive conditions that manifests as a result of peripheral sensory deficits.

1.4 Hypothesis and Aims

1.4.1 Hypothesis

Previous studies have observed significant changes in the IC following UNIHL. These studies have predominately been conducted on IC neurons that are excited by the contralateral ear. However, the central auditory system is a bilateral system and to date there are no studies looking at the consequences of UNIHL on ipsilaterally driven IC neurons. In fact, in normal hearing animals, the monaural response properties of IC neurons ipsilaterally excited is relatively scant. Much of the information has been deduced from studies that investigate binaural interactions (Goldberg & Brown, 1969; Flammino & Clopton, 1975; Semple & Kitzes, 1985; Irvine & Gago, 1990; McAlpine et al., 1997; Graña et al., 2017). From these studies; it is known that ipsilaterally excited non-dominant IC neurons are predominately inhibitory neurons. It is speculative that due to this physiological predisposition, research on excitatory IC neurons in the non-dominant pathway has not been well characterised when compared to their contralateral counterparts. However, McAlpine et al. (1997), have previously shown that after unilateral cochlea ablation, there is a significant increase in the population of ipsilaterally driven IC neurons that are excitatory. There is currently no evidence if a similar outcome occurs following UNIHL, where partial hearing loss is present. We hypothesised that there are indeed changes in the response properties of ipsilaterally driven excitatory IC neurons following UNIHL.

1.4.2 Aims

As the auditory system is a bilateral system, the first aim of the study is to understand the consequence of UNIHL on both non-dominant and dominant excitatory IC neurons. By utilising commonly used IC response properties on both contralaterally and ipsilaterally driven IC neurons, one can begin to understand and compare the consequences that can occur on both left and right sides of the inferior colliculi.

Having established a detailed criterion of response properties of IC neurons pre and post UNIHL, the second aim was to manipulate those consequential patterns. In consideration to dominant and non-dominant changes that occurred, auditory training and cholinergic enhancement was used as a means to alter the consequences observed. Furthermore, within this animal model of cholinergic enhancement, the third aim was to investigate the short-term acute neuroplastic capacity of IC neurons having been primed in an enhanced state. Rivastigmine, an acetylcholine esterase inhibitor, was used to enhance the cholinergic activity of the auditory system. The fourth and final aim was to determine if the effects of this drug were regionally specific within the brain.

In this study,

- In vivo electrophysiological recordings were performed in left and right IC in control and UNIHL animals.
- The results of this study provided a sensitive criterion to compare consequences that occur in both contralaterally and ipsilaterally driven exciatatory IC neurons.
- A therapeutic intervention which consisted of cholinergic enhancement paired with auditory training influenced the consequential response properties of the IC following UNIHL.
- Using in vivo electrophysiology, an investigation of the short term acute neuroplastic capacity was investigated by means of a stimulus time dependent plasticity (STDP) paradigm.
- The effect of Rivastigmine on acetylcholine levels in the frontal cortex, temporal cortex and inferior colliculus was determined by utilising ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS/MS).

Chapter Two
General Methods

2.1 Animal Preparation

2.1.1 Animals and Ethics

The experiments described in this thesis complied with the Australian code for the care and use of animals for scientific purposes, the New South Wales Animal Research Act and were approved by the Western Sydney University Animal Care and Ethics Committee. Male Wistar rats were used in these studies (Australian Resource Centre, Perth Australia). Animals were a minimum of three months old, at the start of each experiment. The animals were housed in individually ventilated cages (IVC), 2-3 per cage and given food and water ad lib. The animals were on a 12 hr light/dark cycle with environmental enrichment consisting of large tissue boxes, polyvinyl chloride (PCV) tubes and a ledge to hide in and climb on. Individual rats were identified using an AVID identity microchip that was place subcutaneously on the dorsal aspect of the neck whilst under anaesthesia. All experiments described were completed in the Auditory Neuroscience Laboratory (30.2.69) at the School of Medicine, Western Sydney University.

2.1.2 Auditory Brainstem Response

The auditory brainstem response (ABR) test measures averaged auditory evoked potentials extracted from electrical activity in the brainstem, which originates from the ascending auditory structures. The resulting recording is a series of waves (Fig.2.1). In rats, peak 1 (P1) represents evoked potentials from the cochlear hair cells or the auditory nerve. Peak 2 (P2) represents the cochlear nucleus activity. Peak 4 (P4) in rodents is analogous to Peak V in human ABR, and this wave arises in the locality of the inferior colliculus or lateral lemniscus. An ABR was performed to estimate the natural hearing thresholds, in order to determine if the animal had normal hearing prior to the allocation of animals into an experimental group (Fig.2.2).

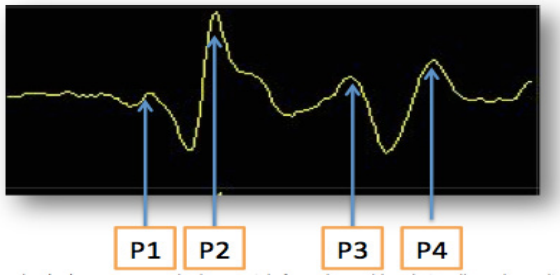


Figure 2.1. An auditory brainstem response from a rat. Generally, early peaks represent activity peripheral and later peaks represent centrally located activity. In rats, peak 1 (P1) represent evoked potentials from the cochlear hair cells or the auditory nerve. Peak 2 (P2) represent the cochlear nucleus activity. Peak 4 (P4) in rodents is analogous Peak V in humans ABR, this wave arises in the locality of the inferior colliculus or lateral lemniscus.

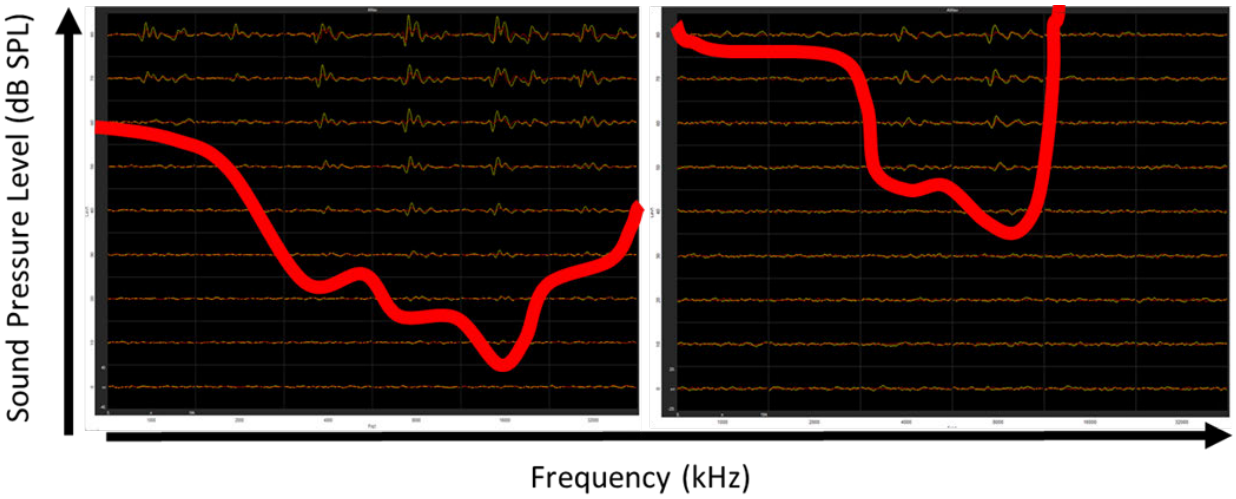


Figure 2.2. Representative examples left sided auditory brainstem responses from a rat. The left ABR is representative example of normal hearing prior UNIHL and the right ABR is a representative example of the same animals hearing 12 weeks post UNIHL. It is not unusual for very high levels of acoustic stimulation to provide some hearing loss over frequencies outside the 16kHz. trauma tone. Plots show three dimensions: a) Outline of hearing activity (bold red line), b) frequency (Y-axis) and c) sound pressure levels (X-axis).

Auditory brainstem responses were also performed at two weeks and at 12 weeks post acoustic trauma to assess temporary and permanent threshold shifts. Both left and right sided hearing was assessed. Animals were anesthetised using Ketamine (75 mg/kg) and medetomidine (0.5 mg/kg), delivered intraperitoneally. The depth of anaesthesia was determined by assessing pedal reflex, with its absence indicating sufficient depth. Auditory brainstem response tests were performed in a sound-attenuating chamber. The evoked potentials were measured using subcutaneous stainless-steel electrodes, inserted at the midline 2.5 cm anterior to the intra-aural axis (reference), caudal to the left and right pinna (active x 2) and the right hind leg (ground). Acoustic stimulation and electrophysiological recordings were performed with Tucker Davis Technologies (TDT) system 3 Software (OpenEx) and hardware (Tucker-Davis Technologies, Alachua, FL, USA). A small 3.5 mm inner diameter tube, originating from a left or right speaker was placed in each of the animal's ear canal. Stimuli were delivered using a closed MF1 magnetic speaker driver (Tucker-Davis Technologies). Stimuli were generated using an RX6 multifunction processor (Tucker-Davis Technologies) with a sampling rate of 100 kHz. The acoustic stimuli were attenuated using PA5 attenuators (Tucker-Davis Technologies). Acoustic stimuli consisted of tones at 1, 2, 4, 8, 16 and 32 kHz at a range of 0–85 dB SPL. Animals with hearing loss had an adjusted SPL range of 0-100 dB SPL. Tone bursts were 3 ms in duration, presented every 17 ms. Each frequency/SPL combination was presented 500 times sequentially from lowest to highest. During the ABR recording, the rat's temperature was monitored using a rectal thermometer and maintained at 37.5 ± 1 °C using the Physitemp system (Physitemp Instruments, LLC, Clifton, NJ, USA). At the conclusion of the experiment, the electrodes were removed. The effects of the anaesthesia were reversed with, atipamezole (1 mg/kg) delivered subcutaneously at the dorsal aspect of the neck. Animals were monitored until disorientated behaviour ceased, such as limping and slow movements, before they were returned to the animal facility.

2.1.3 Unilateral Noise Exposure

Rats were anaesthetised with the same protocol as described in the ABR section (Section 2.1.2). Temperature was maintained as described in Section 2.1.2. The rat's right ear was blocked using a foam ear plug for the entire duration of the acoustic trauma (Moore et al., 1989) and damage to this ear via bone conduction were unlikley due to the levels used. The rat's left ear was exposed to a 16 kHz band pass 1/10th octave noise (115 dB SPL) for one hour, which was produced by a CTS power line speaker (KSN-1188; Piezo Source, San Jose, CA, USA) that was connected to NAD stereo power amplifier (C 268; NAD, Pickering, ON, CA). The signal was generated with a multiprocessor RX6 and was attenuated with a PA5 connect from TDT SA1, which in turn is connected to the stereo amplifier to reach 115 dB SPL. Calibration and confirmation of the trauma SPL was done prior to each experiment with a sound level meter, placed in position of the rat's left external acoustic meatus. The effects of the anaesthesia were reversed as described in Section 2.1.2.

2.1.4 Stereotaxic Surgery

The animal was anesthetised as described in Section 2.1.2. The depth of anaesthesia was determined by assessing pedal reflex, with its absence indicating sufficient depth. Atropine sulphate (0.15mg/kg), delivered intraperitoneally, was used to minimise mucous secretions. Dexamethasone (0.1mg/kg), delivered subcutaneously was used to reduce brain swelling. The animal's core body temperature was maintained in the same manner as previously described in Section 2.1.2. The rat's fur on the dorsal aspect of the head and neck was shaved prior to surgery.

The experiment was carried out in a sound-attenuated room. The anaesthetised rat was placed on a stereotaxic frame, with the head secured in place with ear and bite bars. Ocular lubricant (Novartis Pharmaceutical, Sydney, NSW, AU) was applied to the eyes to prevent drying. A midline scalp incision was made to expose the dorsal surface of the skull. Two anchoring screws were placed anteriorly to Bregma, and a head post was attached to the anchoring screws using dental acrylic. Bilateral craniotomies were performed over the IC regions, with the centres of the craniotomy approximately in line with Lambda (Fig.2.3). Stereotaxic co-ordinates were used to determine the exact position of electrode penetration (Paxinos & Watson, 2006). At the completion of the surgical preparation, ear bars were removed, and replaced by 3.5mm diameter tubes originating from the MF1 speakers.

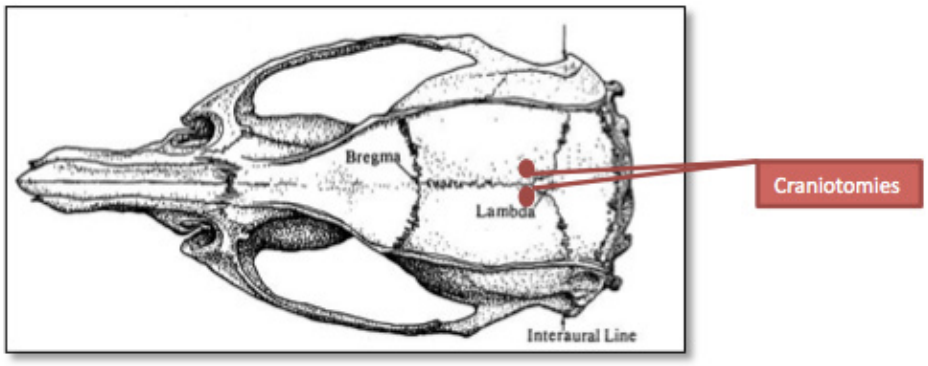


Figure 2.3. A schematic with superimposed closed circles indicating the positions of craniotomies (*Adapted from Paxinos & Watson, 2006).

2.2 Electrophysiological Recording and Acoustic Stimulation

Data were acquired using Tucker Davis Technologies (TDT) system 3 Software (Open Project) and hardware (Tucker-Davis Technologies, Alachua, FL, USA). Stimuli were generated as described in Section 2.1.2.

Single shank electrodes with 32 recording sites were used (A1x32-6mm-50-177-A32; Neuronexus). Electrode impedances were checked prior to each experiment using the Nanoz software and hardware (Plexon Inc, Dallas, TX, USA). Electrodes were coated with DiI (Molecular Probes Inc. Eugene, OR, USA) to allow for a post hoc histological analysis, to visually confirm electrode penetration in CNIC (Fig.2.4). Electrodes, placed on the electrode head stages were attached to Narishige hydraulic micro drivers (Narishige International USA Inc, Amityville, NY, USA). Activity recorded from the electrodes on the head stages were digitised via PZ2-256 256 channel preamp Z-series Bioamp connected to TDT systems 3 hardware and software. The digitised signals were transduced audio-visually using TDT Open Project software.

A broadband noise stimulus delivered to the contralateral ear was used as a search stimulus during electrode penetration. Entry to the CNIC was indicated by the synchronous sound-driven activity of the neural unit, via digitised audio-visual feedback. This was done for both the left and right IC. Three to six electrode penetrations at different sites within each of the CNIC was done for each animal.

The acoustic stimulus protocol consisted of two monaural conditions and one binaural condition. The first recording parameter was a noise stimulus which consisted of a broadband noise at a range of 0-90dB, duration 50 ms, presented in steps of 10dB. Each SPL was presented 50 times sequentially from lowest to highest of both frquency and SPL.

This was done monaurally for left and right ear. The second recording parameter consisted of tones at 1-44 kHz at a range of 0 dB–90 dB SPL, with steps of 1khz and 10dB. Tone bursts were 5 ms in duration, presented every 50 ms, with rise and fall time of 2 ms. Each frequency/SPL combination was presented 50 times sequentially from lowest to highest. This was done monaurally for left and right ear. The third and binaural recording parameter consisted of tones at 0-40 kHz (steps of 4kHz) and 0-100dB (steps of 20dB). Tone bursts were 5 ms in duration, presented every 50 ms. Each frequency/SPL combination was presented 10 times binaurally in a semi-stochastic sequence. Data was collected and stored for analysis to be completed offline. Open sorter (TDT) was used for spike sorting and Open explorer (TDT) was used to visualise data.

2.3 Single-unit classifications

Neuronal responses were defined by an increase of 60% over spontaneous activity within a spiking window as the minimum criteria. Neurons response properties were classified by applying previous described criterias. This study investigated the following response properties: frequency response areas (LeBeau et al., 2001; Ma et al., 2006; Mulders & Robertson, 2013), peri-stimulus timed histogram (Reese et al., 1995; Mulders & Robertson, 2013), input-out function (Semple & Kitzes, 1985; Irvine & Gago, 1990) and binaural responses (Semple & Kitzes, 1985; Irvine, 2007; Mulders & Robertson, 2013). Examples of these response properties will be briefly described, as a more in depth discussion has previously been presented in Chapter one.

The minimal monaural stimulus required to elicit a driven response for each FRA was determined to be the neuron's characteristic frequency (CF) and minimal threshold (MT). Frequency response area (FRA) shape types were categorized as, V-shaped, O-shaped, I-shaped and W-shaped (Refer Section 1.2.1). Type V units display excitatory activity bounded V-shaped tuning curve. Type O units displayed excitatory activity within an

O-shaped tuning curve distinct to a circumscribed area. Type I units have a narrow excitatory area, usually centred within a very small frequency range. Type W units displayed an excitatory area with double-peaks with a distinct area of no activity between the peaks.

The PSTH of a neuron can be determined by observing the overall histogram shape of activity at CF. Tonic cells show an onset response followed by a sustained train of activity throughout the duration of the tone. Tonic firing patterns include A) an onset response immediately followed by lower but sustained activity and B) a burst of firing at the onset, followed by a brief pause and immediately tailed by continuous firing activity. Onset cells show an onset response at the beginning of the stimulus only.

Monaural input-output functions were categorically determined as either monotonic or non-monotonic. A monotonic unit shows a continuous increase of firing rate or a maximal saturation of firing rate with further increase of tone intensity. Non-monotonic neurons show an initial increase in firing rate for increasing tone intensity, followed by a decrease in firing rate in response to additional increases in tone intensity.

Binaural IOF were also determined for monaurally excited ipsilateral and contralateral cells. An excitatory-null (EO) response showed the same firing rate in response to increasing SPL intensity presented to the opposite ear. An excitatory- excitatory (EE) response showed an increase in firing rate in response to increasing SPL intensity presented to the opposite ear. An excitatory- inhibitory (EI) response showed a decrease in firing rate in response to increasing SPL intensity introduced to the opposite ear.

2.4 Statistical analysis

All statistical analysis was done using GraphPad Prism software (Prism 8 Version). Details of the analysis will be provided in each results chapter.

2.5 Histology Sample Preparation and Imaging

2.5.1 Tissue Preparation

At the end of each in vivo electrophysiology experiment, the animal was deeply anesthetised with Pentobarbital sodium (~300mg), intraperitoneally. The depth of anaesthesia was determined by assessing pedal reflex, with its absence indicating sufficient depth. This was followed by exsanguination by means of cardiac perfusion using 150mL of normal saline until the return flow runs clear, before switching to 4% paraformaldehyde solution for 150mL. The head was decapitated, and the brain carefully removed, post-fixed in 4% paraformaldehyde for two days at 4°C, followed by cryoprotection in 20% sucrose solution for an additional two days at 4°C or until the brain has sunk to the bottom within the solution.

The brain was then macro-section into left and right hemispheres and coronally trisected in order to isolate the midbrain. The midbrain section was submerged into Tissue-Tek® O.C.T. Compound (Sakura Finetek USA Inc, Torrance, CA, USA), in a sagittal orientation. The midbrain sections were slowly frozen in liquid nitrogen and stored at -80°C. The brain was sectioned (40 μm sections) using Cryostat Zeiss Hyrax C20 (Carl Zeiss, Jena, DEU) and collected directly on double-coated gelatine slides.

2.5.2 NeuroTrace Fluorescent Nissl Stain Protocol

For NeuroTrace Fluorescent Nissl Stain, sections were rehydrated for 40 minutes in 0.1 M phosphate-buffered saline (PBS), pH 7.2. This was followed by a 10 minutes wash with PBS containing 0.1% Triton® X-100 and then two washes of five minutes each in 0.1 M PBS, pH 7.2. The brain sections on the slides were circumscribed with a hydrophilic gel and approximately 200 µL of the diluted NeuroTrace Fluorescent Nissl Stain (1:300 in 0.1M PBS, Molecular Probes) was applied onto the brain sections and incubated for 20 minutes in a humidified light tight chamber. The stain was removed and brain sections washed for 10 minutes with PBS containing 0.1% Triton® X-100 followed by two times PBS wash for five minutes. A final wash of two hours in PBS was done at room temperature. The sections were cover-slipped using fluorescent compatible mounting medium.

2.5.3 Imaging using confocal microscopy

Sections were imaged using a Confocal Microscope comprising the LSM 5 EXCITER laser scanning microscope with Axiovert 200M inverted optical microscope (Carl Zeiss MicroImaging GmbH 07740 Jena, DEU).

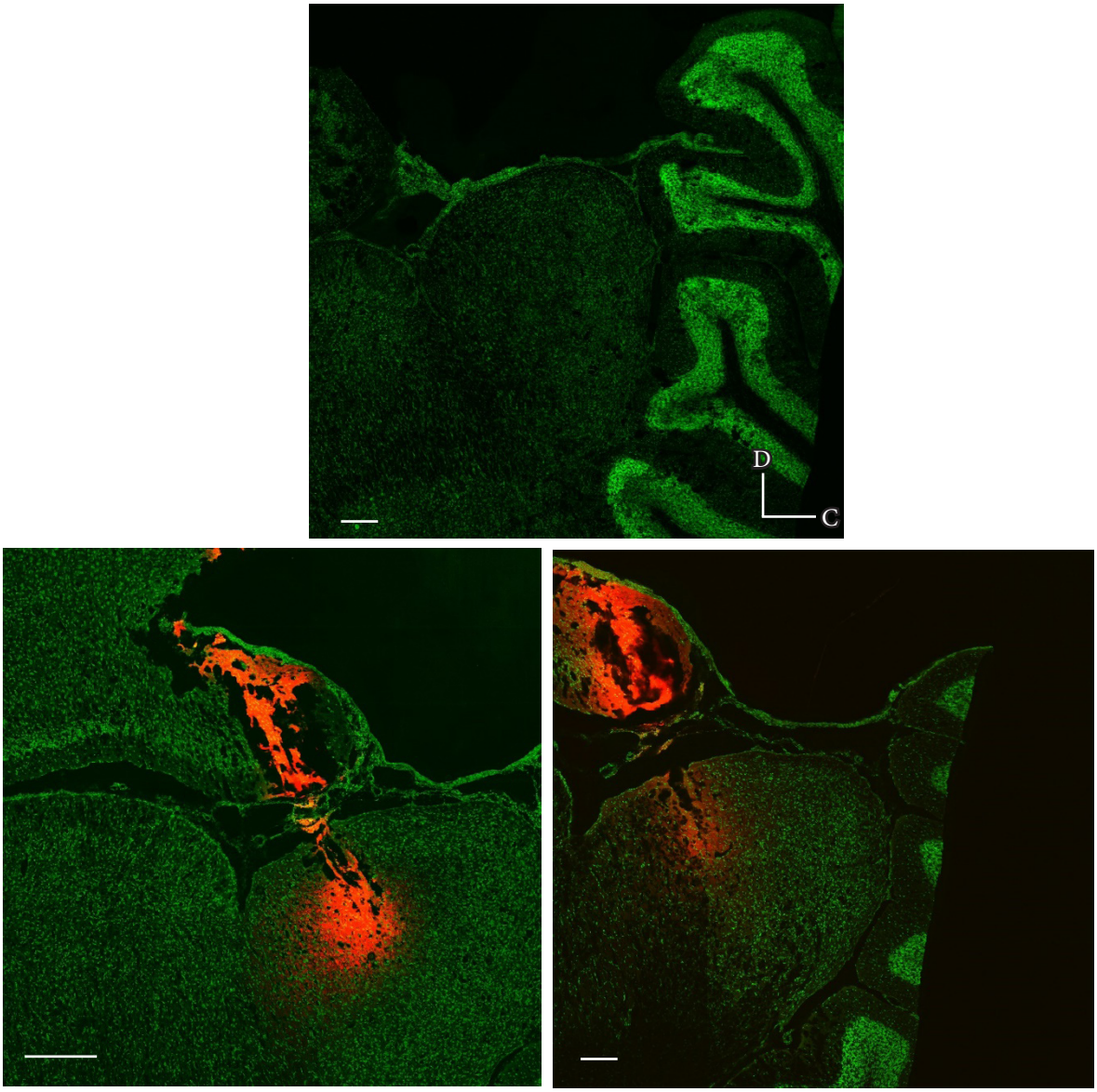


Figure 2.4. Representative examples of the stained images. Top panel is 1:300 green NeuroTrace Nissl stain of the inferior colliculus. The bottom panels the 1:300 NeuroTrace Nissl stain (Green) with electrode tract (DiI; Red) in the inferior colliculus. NeuroTrace was excited with a 488 nm laser and signal was sampled at 525 nm. DiI was excited at with 543 nm and signal was sampled at 590 nm. Scale bar is 500 μm with dorsal and caudal orientation for all three images.

Chapter 3
Binaural Interactions in the Inferior Colliculus following Noise Induced Hearing Loss

3.1 Introduction

Tinnitus is becoming an increasingly prevalent condition and is currently incurable. It is almost always associated with hearing loss (Dobie & Snow, 2004; Roberts et al., 2010). Although the onset of tinnitus can occur due to hearing loss, the persistence and preservation of the tinnitus perception suggests that it is indeed a central auditory phenomenon (Saunders, 2007). The exact underlying neural mechanism is currently unknown; however, previous authors have proposed mechanisms that suggests a loss of regulation between normal excitatory and inhibitory processing (Eggermont & Roberts, 2004). In animal models of hearing loss, the loss of excitatory-inhibitory regulation centrally involves an increase of spontaneous firing rates and hyperactivity (Kaltenbach & Afman, 2000; Norena & Eggermont, 2003; Manzoor et al., 2013; Mulders & Robertson, 2013).

The Inferior Colliculus (IC) is an obligatory nucleus in the central auditory system. It is the site of termination of ascending auditory inputs from almost all lower auditory brainstem nuclei and receives descending projections from thalamocortical centres (Oliver & Shneiderman, 1991; Oliver, 2005). Functionally, it is a critical nucleus, and the consequences of unilateral noise induced hearing loss (UNIHL) in the IC has been studied extensively. Previous authors have shown evidence of neuronal plasticity occurring after UNHIL within the IC (Szczepaniak & Moller, 1996; Hernández et al., 2005; Ropp et al., 2014). In line with other auditory structures, there is an increase of hyperactivity (Ma et al., 2006; Mulders & Robertson, 2013) and spontaneous firing rates (Moore & Kowalchuk, 1988), an expansion of tuning curves (Szczepaniak & Moller, 1996) and differences in the proportion of cell populations expressing specific receptive field types (Wang et al., 2013; Coomber et al., 2014). In addition, there have been studies that emphasise the important role of inhibition operating within the IC itself (LeBeau et al., 2001; Lu & Jen, 2001). In vivo electrophysiology paired with microiontophoresis injections of $GABA_A$ antagonists, show that the frequency response area (FRA) of an IC neuron can be directly altered in response to specific inhibitory influences. For example, when subjected to bicucculine (GABAA receptor antagonist), an IC neuron with an O-shaped FRA displayed a V-shaped FRA, and this effect was reversed when the bicuculine injection was discontinued (LeBeau et al., 2001).

The IC also plays an important role in the bilateral processing of sound. Indeed, as sound is received by both ears, the accurate representation of sounds is dependent on specific dominant (contralateral) and non-dominant (ipsilateral) neural interactions. This interaction is important for binaural functions such as sound localisation (Silverman & Clopton, 1977; Oliver & Shneiderman, 1991). Animals that have evolved with a higher physiological demand for sound location have much larger ICs, such as seen in the barn owl. Indeed, the ability to locate the source of a sound in space is compromised if there is a loss of function to one ear (Schreiner & Winer, 2005). Unilateral noise induced hearing

loss (UNIHL) is a model commonly used to study tinnitus and binaural interactions in this model have been previously investigated (Mulders & Robertson, 2013; Ropp et al., 2014; Shaheen & Liberman, 2018). However, these investigations have paid more attention to the effects of the dominant contralateral pathway. Little is known of the effects of the ipsilateral pathway. McAlpine et al. (1997) have previously shown that after unilateral cochlear ablation, there is an increase of ipsilaterally excited IC neurons. There is currently no evidence for similar outcome following UNIHL, where there is typically only a partial hearing loss.

This study investigated the changes in the binaural response properties of the IC following UNIHL. Using in vivo electrophysiology, the response properties of IC neurons were characterised in response to both contralateral and ipsilateral monaural stimulation. In the same neurons, recorded evoked responses were also investigated under binaural conditions. Responses of the left and right IC were recorded simultaneously.

3.2 Hypothesis & Aims

The aim of this study was to characterise the binaural response profiles of both contralaterally and ipsilaterally driven IC neurons as a consequence of UNIHL.

It was hypothesised that after UNIHL, there will be:

- A shift in the tonotopic organisation of contralaterally and ipsilaterally driven IC neurons.
- Significant changes in the proportion of binaural interactions in contralaterally and ipsilaterally driven IC neurons.
- A shift in the minimal threshold of contralaterally and ipsilaterally driven IC neurons.
- Significant changes in the inhibitory profile of contralaterally and ipsilaterally driven IC neurons.
- Significant changes in the proportion of Frequency response area (FRA) types in IC contralaterally and ipsilaterally driven IC neurons.

3.3 Research method

3.3.1 Animals and Ethics

The experiments described in this study complied with the Australian code for the care and use of animals for scientific purposes, the New South Wales Animal Research Act and were approved by the Western Sydney University Animal Care and Ethics Committee (A11491). Twelve three-month old male Wistar rats were used in these experiments. The animals were housed and cared for in the animal facility at the School of Medicine, Western Sydney University as described in the general methods section (Section 2.1). Rats were subjected to a pre-screen Auditory Brainstem Response (ABR) to quantify normal hearing (Section 2.3) prior to their allocation into control (n=6) or UNIHL (n=6) cohorts (Fig.3.1). Individual rats were identified using an AVID microchip that was placed subcutaneously on the dorsal aspect of the neck whilst under anaesthesia after the ABR protocol (Fig.3.5). All experiments described were completed in the Auditory Neuroscience Laboratory (30.2.69) at the School of Medicine, Western Sydney University.

The in vivo electrophysiology, single unit classification (Fig.3.2, 3.3, 3.4 & 3.6) and histology was completed as described in Section 2. Each electrode penetration was histologically processed, to visually confirm that the electrophysiological data was indeed obtained from the IC. All statistical analysis was done using GraphPad Prism software (Prism 8 Version).

Experiments commenced 12 weeks old for control and (Fig.3.1) after the noise trauma procedure, to ensure a permanent threshold shift (PTS) in hearing. The PTS of an animal's hearing, is dependent upon the level, frequency and the duration of the exposure. Furthermore, below a critical level of about '115 dB SPL', PTS and cell losses are generally related to total energy of continuous exposures (Clark, 1991). In consideration of these

factors, a 16kHz tone at 115dB SPL was used in this experiment to consistently produce a PTS in these animals. When considering the recovery period post acoustic trauma, previous authors have used similar recovery periods of eight to twelve weeks and reported similar changes in frequency extent and threshold increase after acoustic trauma (Salvi et al., 1979; Mulders & Robertson, 2009; Dong et al., 2010; Wang et al., 2013). Mulders and Robertson (2011) investigated hyperactivity in the midbrain after acoustic trauma and measured the effect of the resulting hyperactivity following cochlear ablation. The authors reported recovery time at or greater than 8 weeks, the early stages of plasticity transitions where the elevated firing is intrinsic to central neurons and is not altered by cochlear ablation. That is, with the complete removal of afferent input, the UNIHL consequences of centralised hyperexcitability persisted (Mulders & Robertson, 2011). The value of this study means that a minimum of an 8-week recovery period will likely capture events that are intrinsically associated with central manifestations occurring as a consequence of UNIHL.

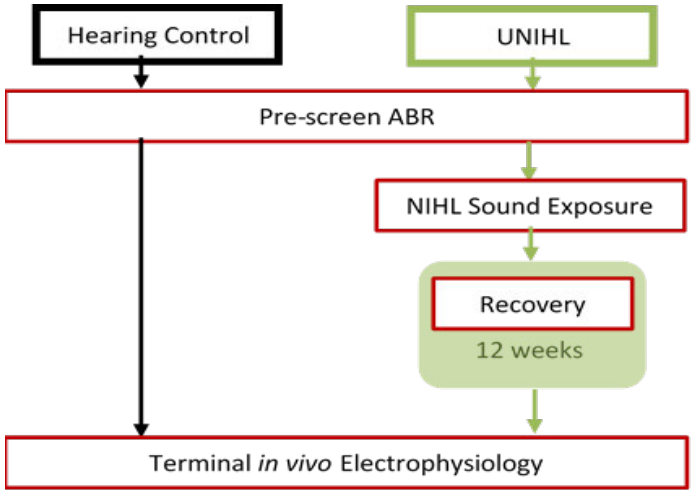


Figure 3.1. Schematic diagram of experimental timeline.

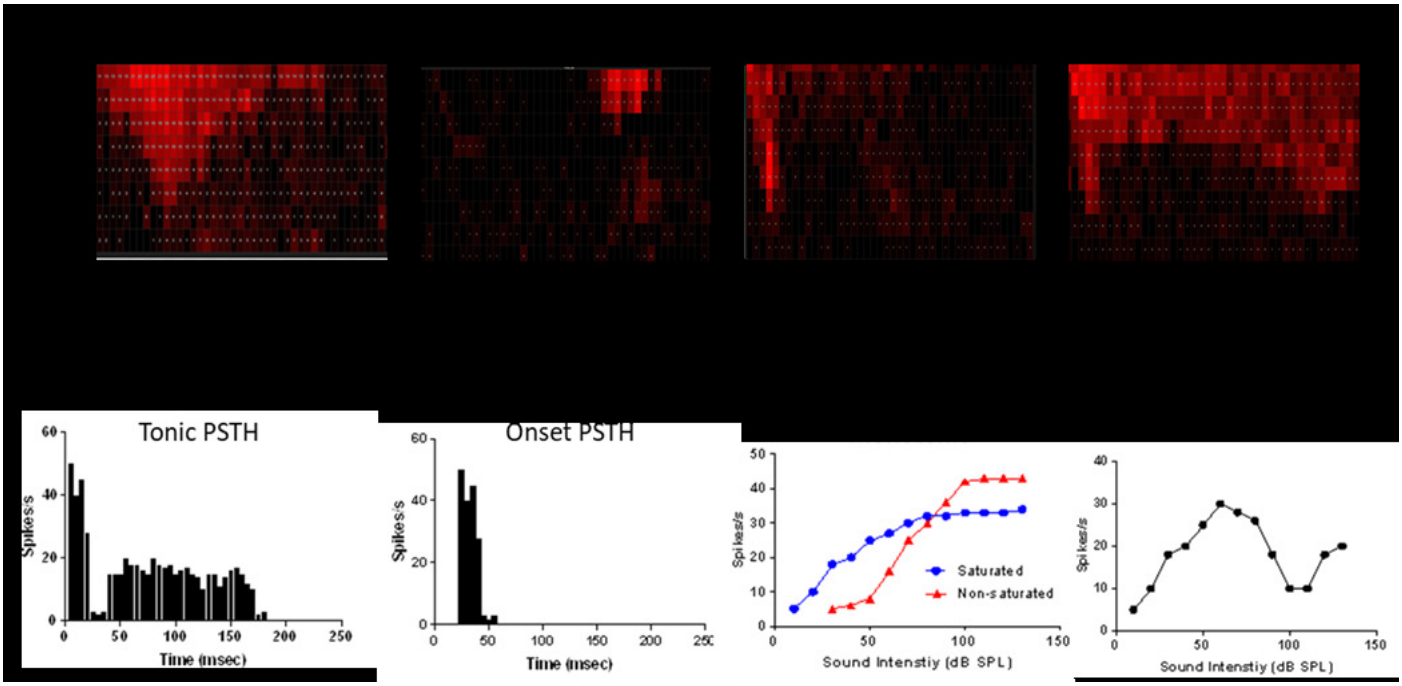


Figure 3.2 Representative response properties of IC neurons. The top panel are representaive FRAs, from left to right, examples are V-shaped, O-shaped, I- shaped and W-shaped FRAs. Of the bottom panel, from left to right, are representative examples of tonic and onset PSTHs and monotonic and non-monotonic IOFs at CFs.

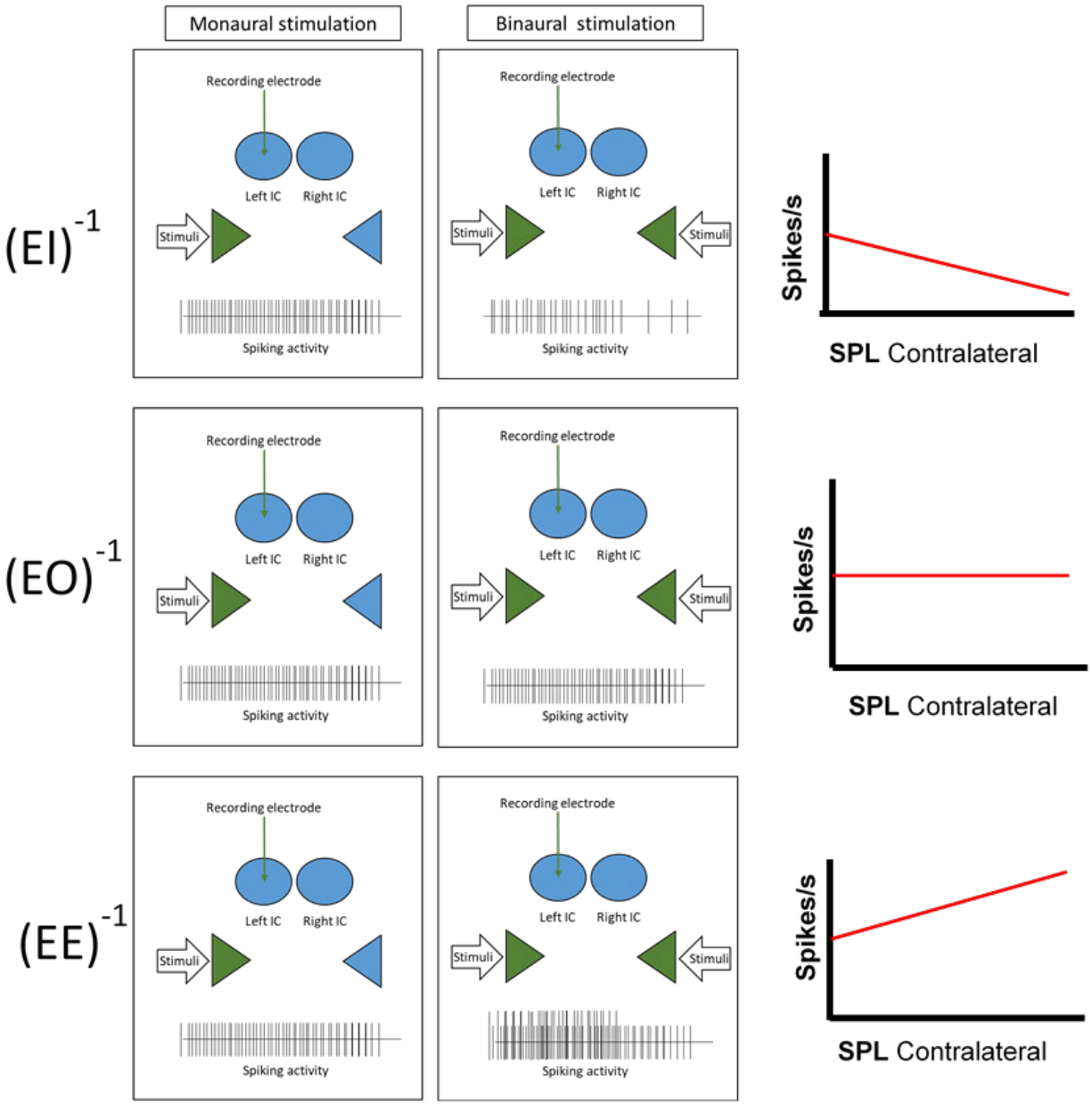


Figure 3.3. Schematic of binaural interactions. The convention of binaural classification prescribes the first letter as the monaural response to contralateral input and the second letter describes the binaural response when ipsilateral input is introduced. In this study, with the investigation of the binaural interaction of ipsilaterally excited neurons the above convention does not properly describe the event. To address this, the inverse '-1', describes the inverse parameters, the first letter as the monaural response to ipsilateral input. An $(EI)^{-1}$ response type, when there is a decrease of spikes/s as there is an increase of contralateral SPL intensities.

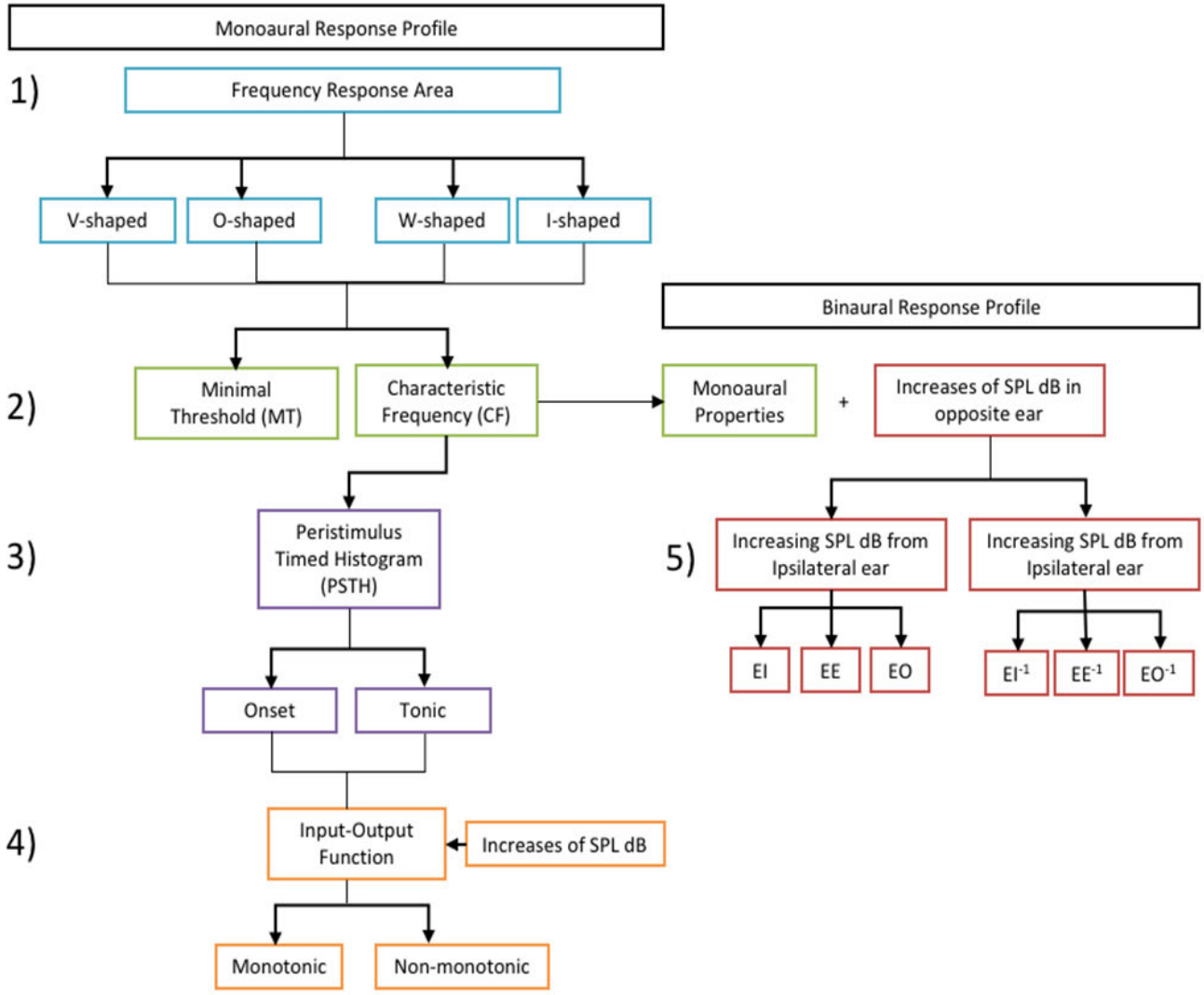


Figure 3.4. Schematic of the data analysis process. Offline spike sorting was done to remove artefacts and to sort the neuron clusters into single neuron events. In one recording channel, neurons clusters were typically separated into one to three neurons. Once sorted, characterising the single responses was completed using TDT Open Explorer. 1) Frequency Response Areas (FRA) was determined by plotting frequency, SPL dB presentations and number of spikes at those combinations. 2) Characteristic Frequency (CF) and Minimal thresholds (MT) were determined based on the minimal input needed to illicit an excitatory response. 3) At the CF/MT plot, a peri-stimulus timed histogram (PSTH) of the spikes showed the temporal relationship between the number spikes and the duration of the stimulus. 4) Increased SPL dB at the CF showed the input-output

Figure 3.4 Contiuned

of the neuron with respect to the number of spikes. 5) The CF/MT was presented monaurally and noise was introduced to the opposite ear in increasing 10 SPL dB increments to determine the binaural interaction of that monaurally excited neuron.

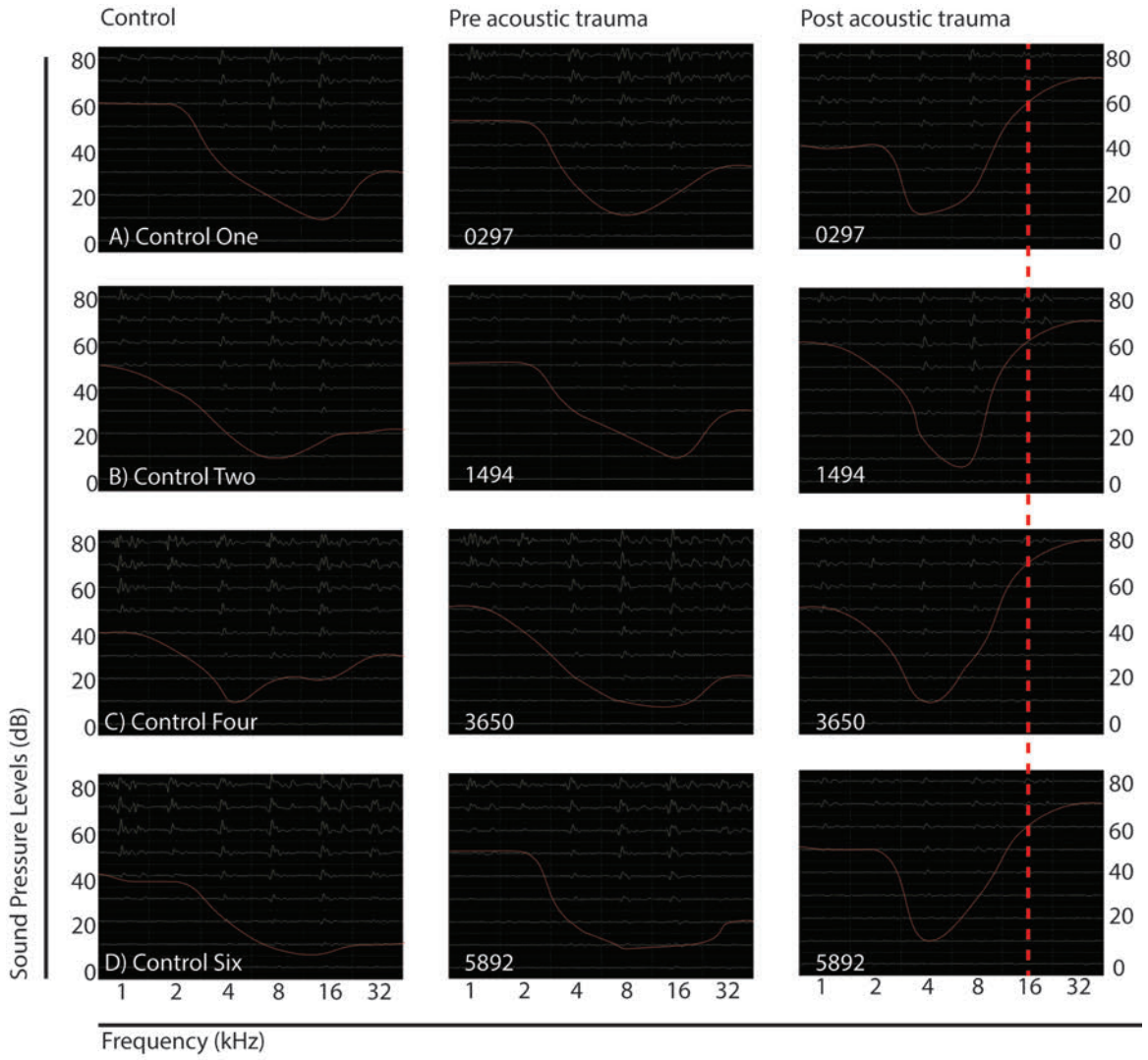


Figure 3.5. Represenative Auditory Brainstem Responses. Control and experimental animals were age-matched at 12 weeks. For example, (A) Control one was age matched with animal 0297. The red broken line indicates the 16kHz acoustic trauma tone.

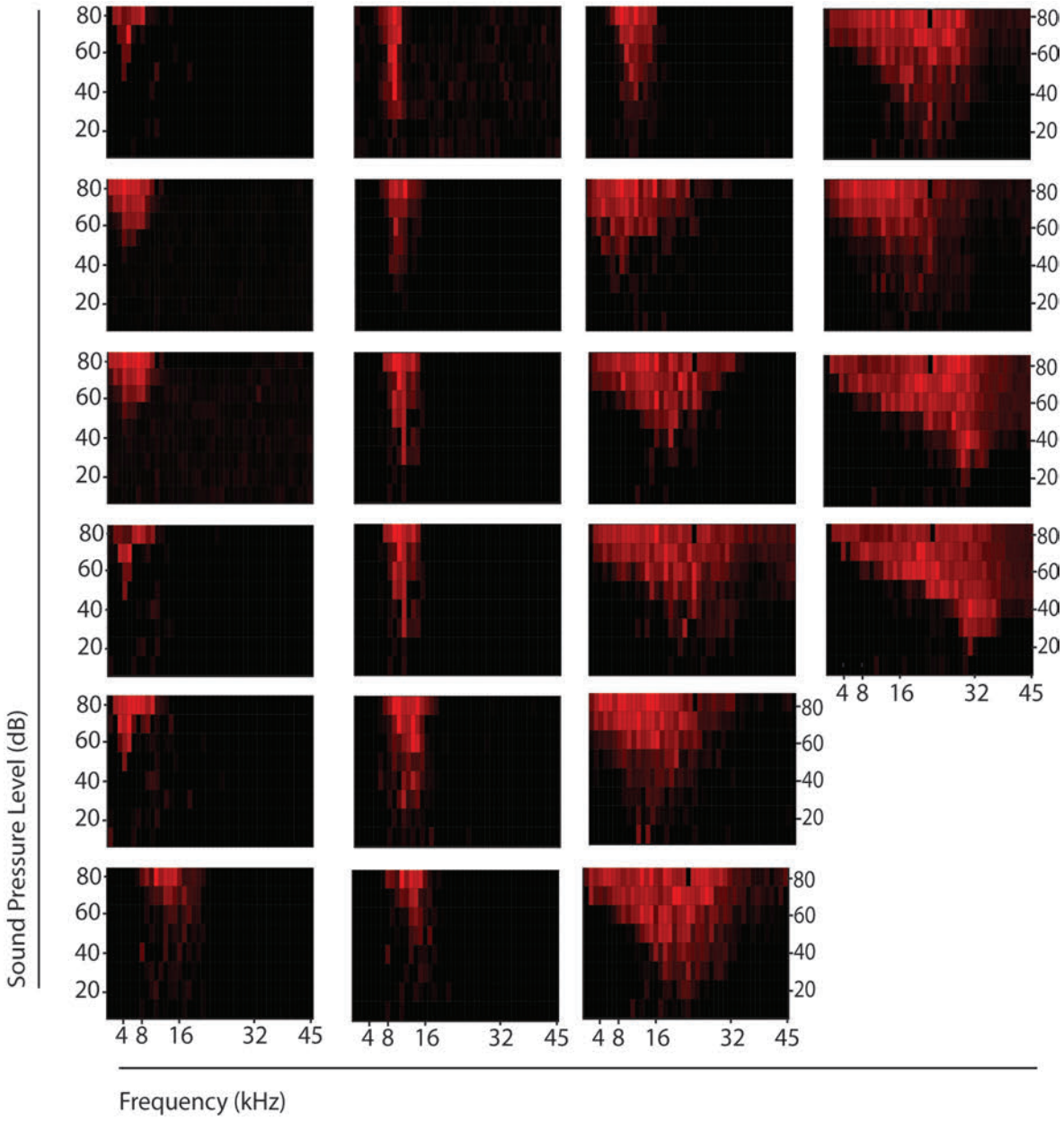


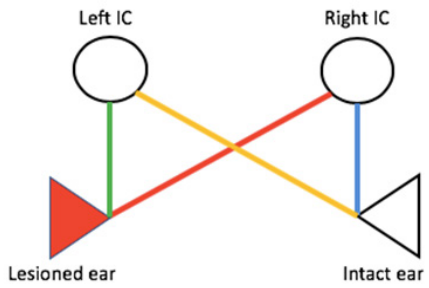
Figure 3.6. Represenative Frequency Response Areas of the Inferior Colliculus. Each neuronal response was defined by an increase of 60% over spontaneous activity within a spiking window as the minimum criteria. Selected FRAS with approximately similar CFs and shape, showing progessive changes plotted per column.

3.4 Results

3.4.1 Number of Recorded Neurons.

Excitatory neurons were recorded and categorised based on their experimental condition, either contralaterally or ipsilaterally excited and from the lesioned or the intact ear. Monaural and binaural responses were recorded from a total of 1,595 neurons in the IC. Of these neurons, 634 were from the control group and 961 neurons were from the UNIHL group (Tab.3.1). Further proportional analysis of the response characteristics was analysed based on the denominator of the total population of each experimental condition; contralaterally excited from (red) lesioned and (yellow) intact ear and ipsilaterally excited from (green) lesioned and (blue) intact ear (Fig.3.7).

(A)



(B)

Groups	Intact ear contralateral IC	Intact ear ipsilateral IC	Lesioned ear contralateral IC	Lesioned ear ipsilateral IC	Total
Control	439 (69%)	198(31%)			634
NIHL	394(41%)	192(20%)	211(22%)	166(17%)	961
					1595

Figure 3.7. (A) Schematic of experimental setup. Table 3.1. (B) Number and proportion of neurons recorded in the IC. Colours correspond with the stimulus and recording parameters. For example, yellow indicates the population of IC neurons recorded in the left IC that were contralaterally excited from the intact ear.

3.4.2 Thresholds: Characteristic Frequencies (CF) and Minimal Thresholds (MT)

3.4.2.1 Characteristic Frequencies

Characteristic frequencies were placed into 4kHz centred bins. The data was then plotted as cumulative frequencies. A statistical analysis was done using a two-way ANOVA and post hoc Tukey's multiple comparisons test.

In the contralaterally driven IC neurons (Fig.3.8A), there was a significant interaction between groups ($F(2,20) = 8.305$, $p = 0.0024$). When compared to normal hearing control animals (black), the CF distribution of IC neurons excited contralaterally from the lesioned ear (red), showed a left-sided shift, with the greatest degree of shift occurring below the 16 kHz lesion frequency indicating a loss of high-frequency CFs. There was a significant difference ($p = 0.0024$) in the CF distribution between these groups. However, no statistical difference was seen in the CF distribution between IC neurons excited from the intact ear (yellow) and control (black) ($p>0.05$).

In addition, there was no statistical difference in the CF distribution of IC neurons excited ipsilaterally (Fig.3.8B) either from the intact (orange) or lesioned (green) ear when compared to normal hearing control animals ($F(2,20) = 1.176$, $p>0.05$). When compared to normal hearing control animals (black), there was a small effect where there is a decrease in IC neurons with a 16kHz CF at the lesion frequency (red dotted line). Although not significantly different, this finding is of biological interest.

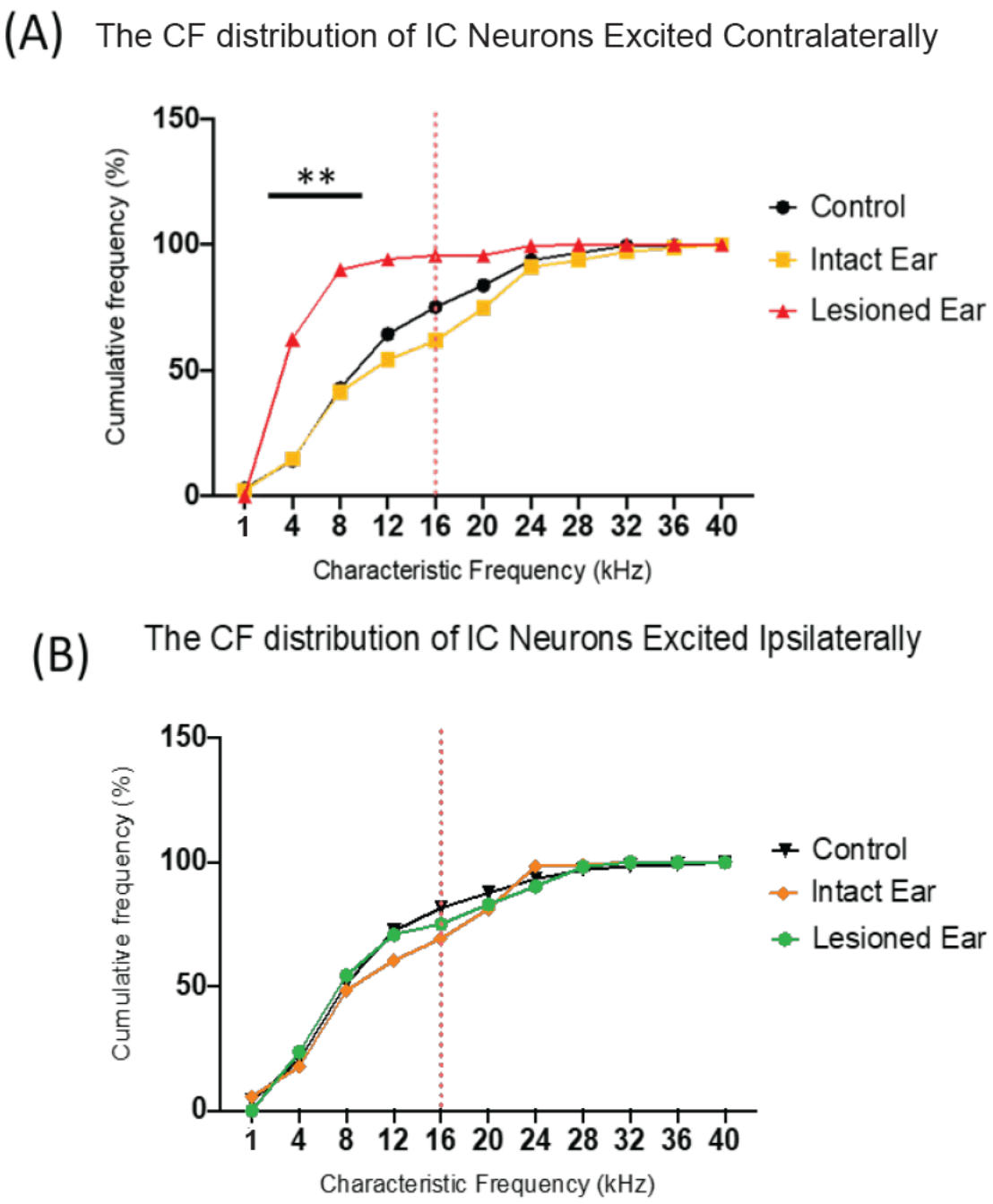


Figure 3.8. The cumulative frequency of CFs of IC neurons excited (A) contralaterally and (B) ipsilaterally, from the intact and the lesioned ears of experimental animals compared to normal hearing control animals. The red dotted line illustrates the lesion frequency of 16kHz. There was a significant left sided shift of the cumulative CF in IC neurons that were excited (A) contralaterally from the lesioned ear (red), when compared to control ($p=0.0024$).

3.4.2.2 Minimal Thresholds

The minimal thresholds of IC neurons were placed centered into 10 SPL dB bins. The data was then plotted as cumulative frequencies. A statistical analysis was done using a two-way ANOVA and post hoc Tukey's multiple comparisons test.

In contralaterally driven IC neurons (Fig.3.9A), there was a significant interaction between groups ($F(2,18) = 21.42$, $p<0.0001$). When compared to normal hearing control animals (black), the MT distribution of IC neurons excited contralaterally from the lesioned ear (red) showed a right-sided shift. A similar interaction between groups is seen in ipsilaterally driven IC neurons (Fig.3.9B) ($F(2,18)=18.77$, $p<0.0001$). When compared to normal hearing control animals (black), the MT distribution of IC neurons excited ipsilaterally from the lesioned ear (green), also showed a right-sided shift of the MT distribution. As a consequence of UNIHL, IC neurons that are excited from the lesioned ear showed a significant right-sided shift towards higher thresholds regardless of whether the IC neuron is ipsilaterally or contralaterally excited.

Compared to normal hearing control animals, there were no statistical differences in the MT distribution of IC neurons excited contralaterally (Fig.3.9A; yellow; $p>0.05$) or ipsilaterally (Fig.3.9B; orange; $p>0.05$) from the intact ear of treated animals.

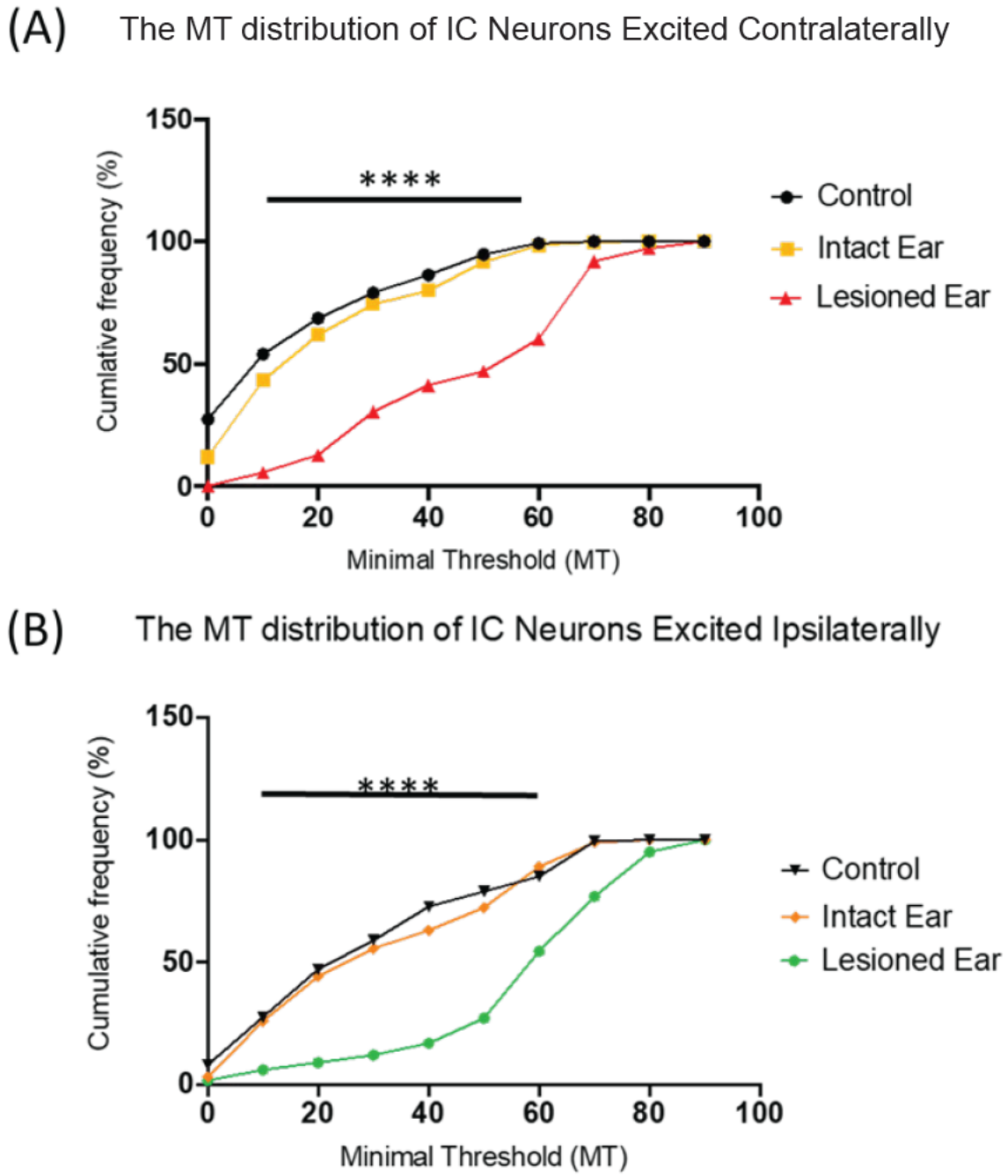


Figure 3.9. The cumulative frequency of the minimal thresholds (MT) of IC neurons excited (A) contralaterally and (B) ipsilaterally from the lesioned and intact ears of experimental animals compared to normal hearing control animals. There was a significant right sided shift in the MT distribution of IC neurons that are excited from the lesioned ear, both (A) contralaterally ($p<0.0001$) and (B) ipsilaterally ($p<0.0001$) when compared to the respective controls.

3.4.3 Binaural Profiles

3.4.3.1 Binaural Profiles: Control

The data has been analysed based on the relative proportion of IC neurons categorised into excitatory-excitatory (EE), excitatory-inhibitory (EI) and excitatory-null (EO) binaural response interactions. Convention has prescribed the first letter to be the contralateral input and the second to be the result of ipsilateral input. In line with convention, the additional '-1' is simply describing the inverse. For example, an $(EI)^{-1}$ is describing an ipsilaterally excited neuron that is inhibited when sound is introduced to the contralateral ear, evidenced by a decrease in spiking activity (Fig.3.3). Statistical analysis was done using a chi-squared test.

Comparing binaural proportions in the contralaterally driven neurons (Fig.3.10), there was a dominance of the binaural EI interaction (60%) and ipsilaterally there was a dominance of the EE^{-1} interaction (86%). A chi-squared analysis of the percentages of contralaterally and ipsilaterally excited neurons, showed a statistically significant difference within each of the binaural interaction categories ($p<0.0001$).

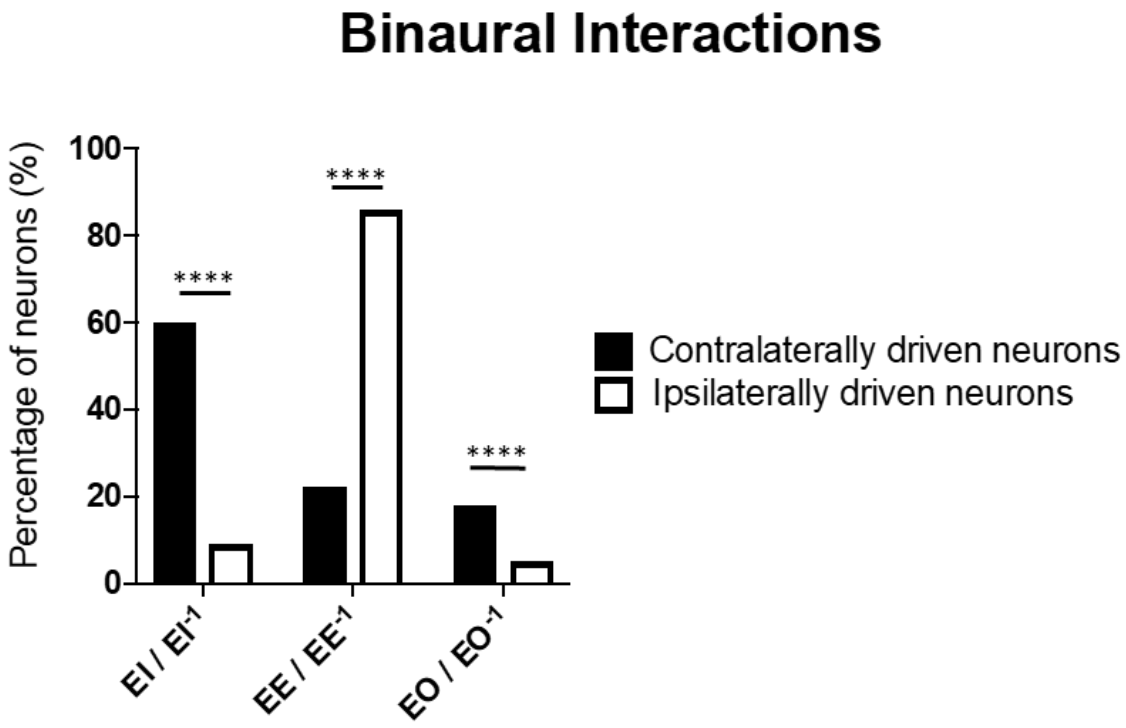


Figure 3.10. The binaural profile of contralaterally and ipsilaterally excited IC neurons of normal hearing control animals. Responses categories are excitatory-excitatory (EE), excitatory-inhibitory (EI) and excitatory-null (EO) binaural response interactions. There were significant differences in the proportions of all categorises ($p<0.0001$).

3.4.3.2 Binaural Profiles: Contralaterally Excited IC Neurons

The data has been anaylsed based on the relative proportion of IC neurons categorised into excitatory-excitatory (EE), excitatory-inhibitory (EI) and excitatory-null (EO) binaural response interactions. Statistical analysis was done using a chi-squared test.

All three groups maintained the dominant EI binaural interaction at similar proportions (60-63%)(Fig.3.11). There were differences within the EE and EO categories, however this was not statistically significant. The binaural profile of contralaterally excited neurons appeared to be unaffected by UNIHL.

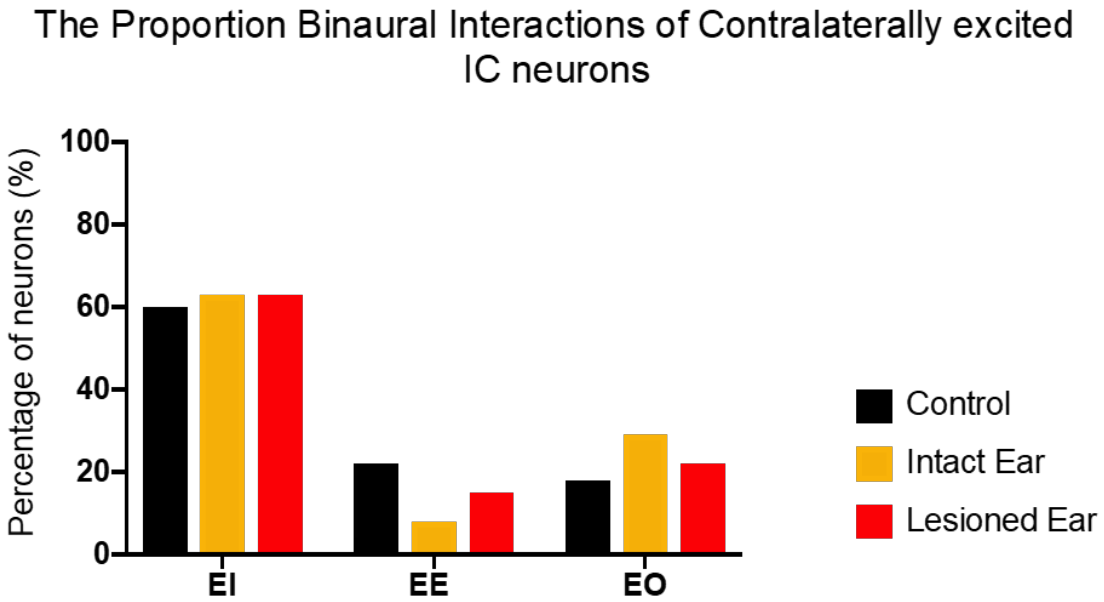


Figure 3.11. The binaural profile of contralaterally excited IC neurons from the intact and lesioned ear of treated animals and normal hearing control animals. Response categories of excitatory-excitatory (EE), excitatory-inhibitory (EI) and excitatory-null (EO) binaural response interactions showed no significant difference ($p>0.05$).

3.4.3.2 Binaural Profiles: Ipsilaterally Excited IC neurons

The data has been analysed based on the relative proportion of neurons categorised into excitatory-excitatory $(EE)^{-1}$, excitatory-inhibitory $(EI)^{-1}$ and excitatory-null $(EO)^{-1}$ binaural response interactions. Statistical analysis was done using a chi-squared test.

There was a significant difference in the binaural profile of ipsilaterally driven IC neurons of normal hearing control animals and ipsilaterally driven IC neurons excited of treated animals (Fig.3.12; $p < 0.0001$). As a result of UNIHL, ipsilaterally excited IC neurons from both the intact ear and lesioned ear, showed a significant decrease (58-60%), in the EE binaural response when compared to control. Interestingly, significant gain in EI binaural interactions was seen in treated animals. In IC neurons excited ipsilaterally from the intact ear, an increase of 31% was observed and from the lesioned ear, an increase of 45% was observed when compared to normal hearing animals. The binaural profile of ipsilaterally excited neurons appeared to be profoundly effected by UNIHL.

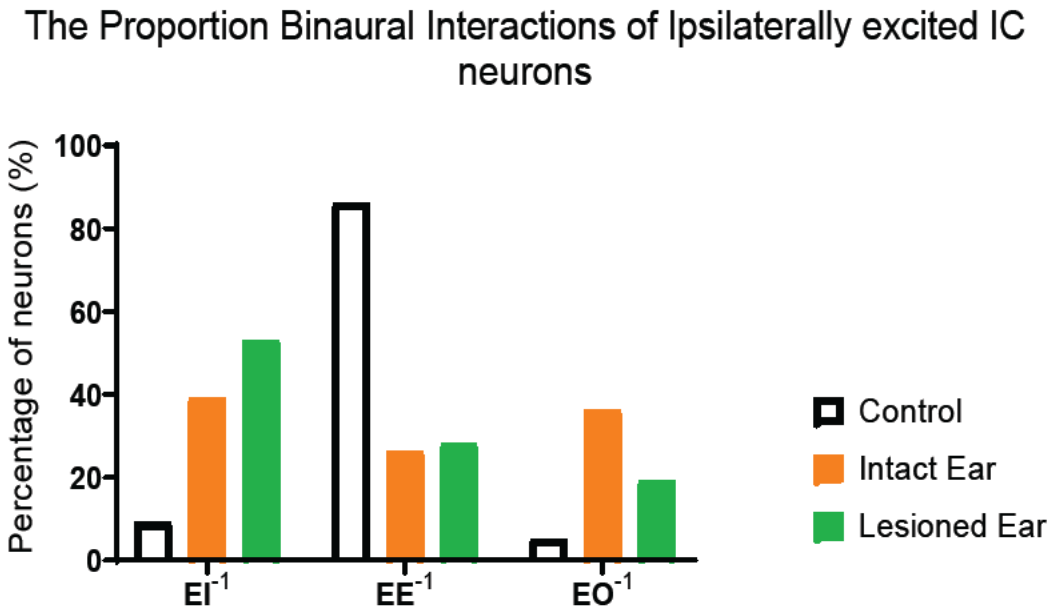


Figure 3.10. The binaural profile of ipsilaterally excited IC neurons from the intact and lesioned ear of treated animals and of normal hearing control animals. Response categories in excitatory-excitatory $(EE)^{-1}$, excitatory-inhibitory $(EI)^{-1}$ and excitatory-null $(EO)^{-1}$ binaural response interactions. There were significant differences in the proportions of all categories when compared to control ($p<0.0001$).

3.4.4 Frequency Response Areas

3.4.4.1 Frequency Response Areas: Control

Frequency response areas (FRA) of IC neurons were categorised into V-shaped, O-shaped, W-shaped and I-shaped FRAs. The data was then analysed as a proportion. A statistical analysis was done using a chi-squared test.

The proportion of V-shaped FRAs were similar between contralaterally (63%) and ipsilaterally (56%) excited IC neurons (Fig.3.13). Interestingly, W-shaped neurons were only seen in significant proportions (20%) in contralaterally excited neurons ($p<0.0001$). In ipsilaterally excited IC neurons, there is a higher proportion of O-shaped neurons ($p=0.014$).

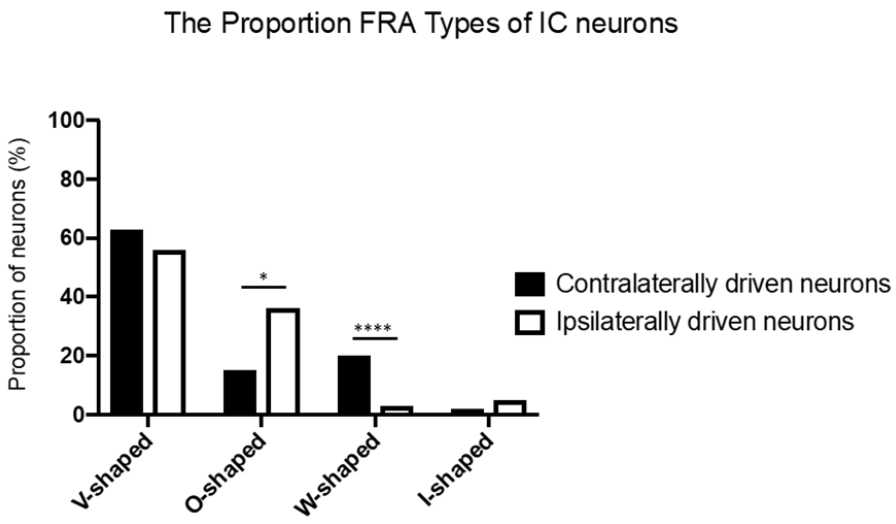


Figure 3.13. The Frequency Response Areas (FRAs) of contralaterally and ipsilaterally excited IC neurons of normal hearing control animals. Response categories are V-shaped, O-shaped, W-shaped and I-shaped. Between contralaterally and ipsilaterally excited IC neurons, there were significant differences in proportion of O-shaped ($p=0.014$) and W-shaped ($p<0.0001$) FRAs.

3.4.4.2 Frequency Response Areas: Contralateral and Ipsilaterally Driven Neurons

Frequency response areas (FRA) of IC neurons were categorised into V-shaped, O-shaped, W-shaped and I-shaped FRAs. The data was then analysed as a proportion. Statistical analysis was done using a chi-squared tests.

Compared to normal hearing control animal (black), IC neurons excited contralaterally (Fig.3.12A; red) and ipsilaterally (Fig.3.14B; green) from the lesioned ear, showed significant differences ($p < 0.0001$) in contralaterally excited neurons (Fig.3.14A).There was a 53% increase in the O-shaped FRAs. Similarly, in ipsilaterally excited IC neurons (Fig.312B), there was a 51% increase in the O-shaped FRA shape. In addition, there was a decrease in V-shaped FRAs, in contralaterally (37%; Fig.3.14A) and in ipsilaterally (52%; Fig3.12B) excited IC neurons when compared to normal hearing control animals.

There were small differences in the proportion of FRA types between normal hearing control and IC neurons excited from the intact ears of treated animals. A proportional 16% decrease of V-shaped FRAs was seen in ipsilaterally driven IC neurons that were stimulated from the intact ear, with the exception of V-shaped FRAs. In IC neurons excited from the intact ear, differences between non-V shaped FRAs were not statistically significant in both contralaterally excited (Fig.3.14A; yellow; $p>0.05$) and ipsilaterally excited IC neurons (Fig.3.14B; orange $p>0.05$).

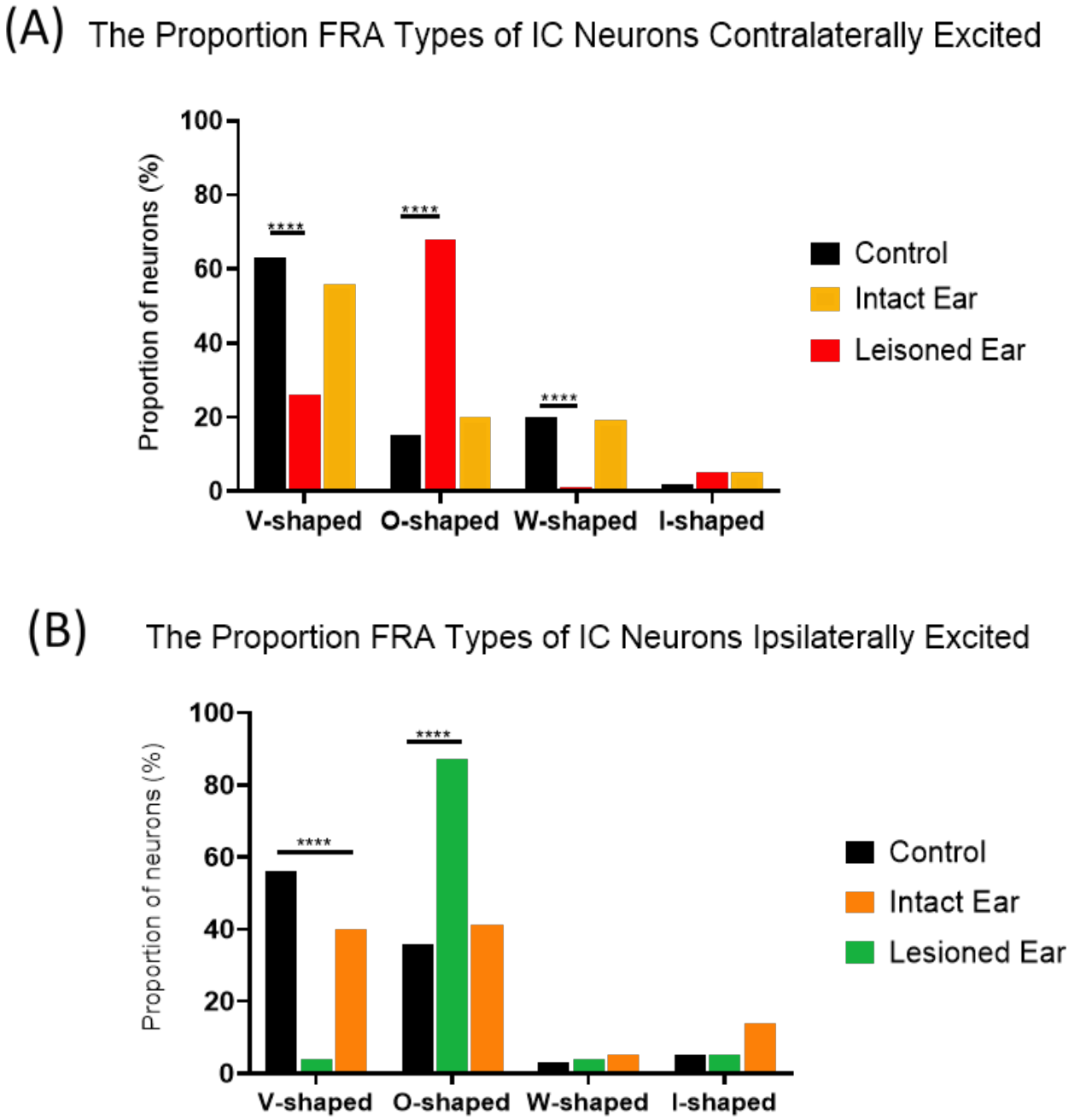


Figure 3.14. The frequency response area profiles of IC neurons excited from the (A) contralateral and (B) ipsilateral ears of treated animals and normal hearing control animals. The proportion of V-shaped and O-shaped FRAs differed between IC neurons of control animals and IC neurons excited from the lesioned ear. This was observed for both ipsilateral ($p<0.0001$) and contralateral conditions ($p<0.0001$).

3.4.4.3 Frequency Response Areas: Relative CF distribution of V-shaped neurons

Contralaterally and ipsilaterally excited IC neurons stimulated from the intact and lesioned ears that showed a V-shaped FRA, were further analysed for their CF. Characteristic frequencies were placed into 4kHz bins. The data was then plotted as relative proportions. Forsythe and Welch and Friedman tests were used to compare IC neurons excited from the intact and lesioned ears of treated animals to normal hearing control animals.

There was a significant difference in the CFs seen in IC neurons that were excited from the lesioned ear contralaterally (Fig.3.15A; red; $p=0.011$) compared to normal hearing control animals (black). These neurons (red) showed a higher relative proportion of CFs at the 4kHz frequency. In contralaterally driven IC neurons, a small difference between IC neurons excited from the intact ear (yellow) and control was observed, however these differences were not statistically significant ($p>0.05$).

There were significant differences in the CFs seen in ipsilaterally driven IC neurons (Fig.3.12B) that are excited from the lesioned ear (green; $p=0.007$) and intact ear (orange; $p=0.008$) when compared to normal hearing control animals (black). Neurons excited ipsilaterally from both the lesioned and intact ear, show a mirrored distribution when compared to control, with significant proportional increase of CFs above the 16kHz lesioned frequency.

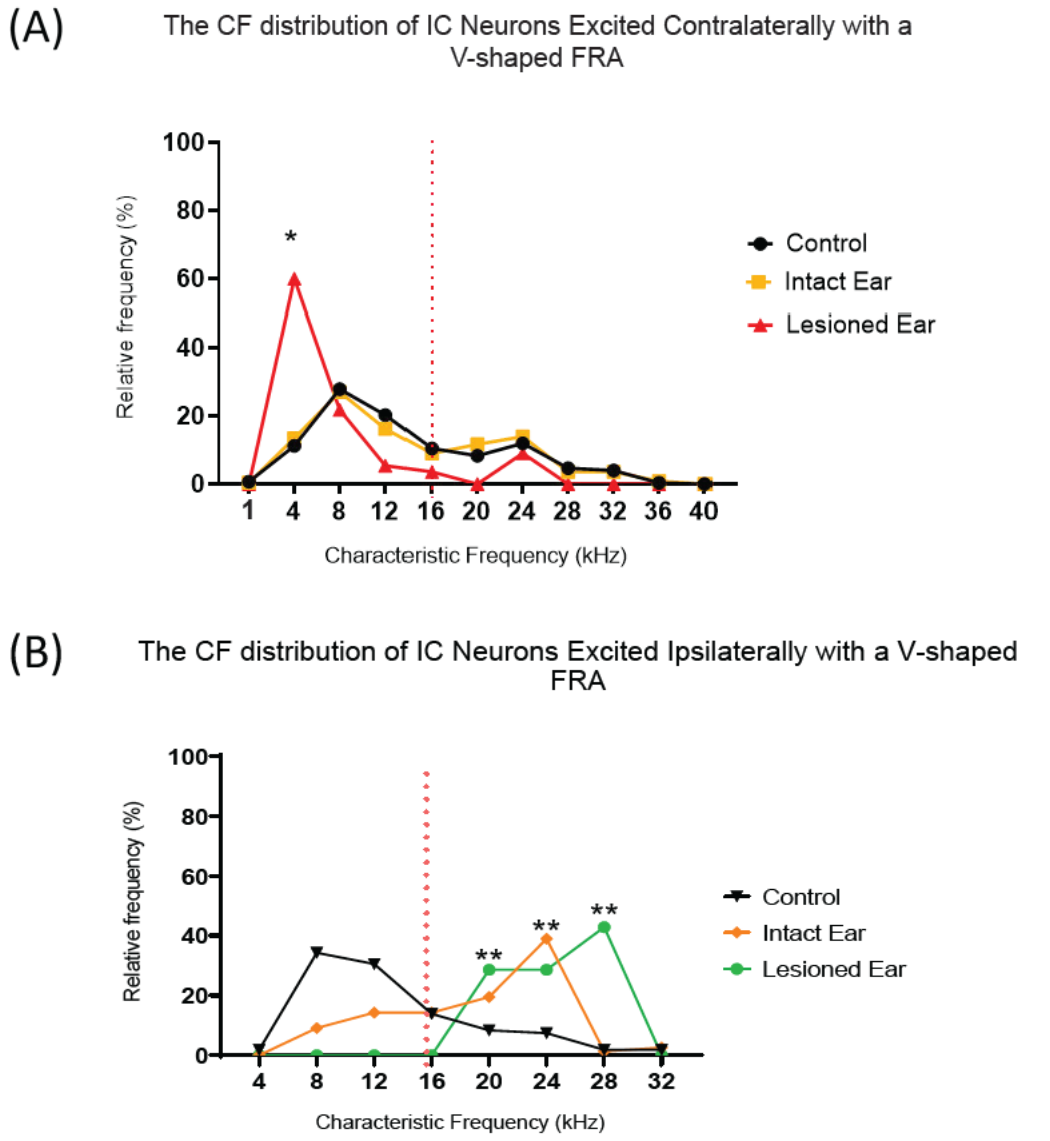


Figure 3.15. The CF distribution of V-shaped FRAs of IC neurons excited (A) contralaterally and (B) ipsilaterally from the intact and lesioned ear of treated animals and normal hearing control animals. In IC neurons excited contralaterally from the lesioned ear, there was an increase in relative frequency of CFs below the lesioned frequency of 16kHz (red vertical dotted line), when compared to the intact ear and control (p=0.011). There was an increase in the relative frequency of CFs above the lesioned frequency in IC neurons excited ipsilaterally from the lesioned ear (p = 0.007) and intact ear (p = 0.008), when compared to control.

3.4.4.4 Frequency Response Areas: Relative CF distribution of O-shaped neurons.

Contralaterally and ipsilaterally excited IC neurons stimulated from the intact and lesioned ears that showed an O-shaped FRA, were further analysed for their CF. Characteristic frequencies were placed into 4kHz bins (Fig.3.16). The data was then plotted as relative proportions. Forsythe and Welch and Friedman tests were used to compare IC neurons excited from the intact and lesioned ears of treated animals to normal hearing control animals.

There was a statistically significant difference in the CFs seen in IC neurons that were excited from the lesioned ear contralaterally (Fig.3.16A; red; $p=0.009$) compared to normal hearing control animals (black). These IC neurons (red) showed a higher relative proportion of CFs at the 4kHz frequency. In contralaterally driven neurons (A), a small difference between IC neurons excited from the intact ear and control was observed, however these differences were not statistically significant ($p>0.05$).

There was no statistical difference between the ipsilaterally excited neurons (Fig.3.16B; $p>0.05$) and normal hearing control animals. It appears UNIHL, does not effect the CF distribution of O-shaped FRAs in IC neurons excited ipsilaterally from either the intact or lesioned ear.

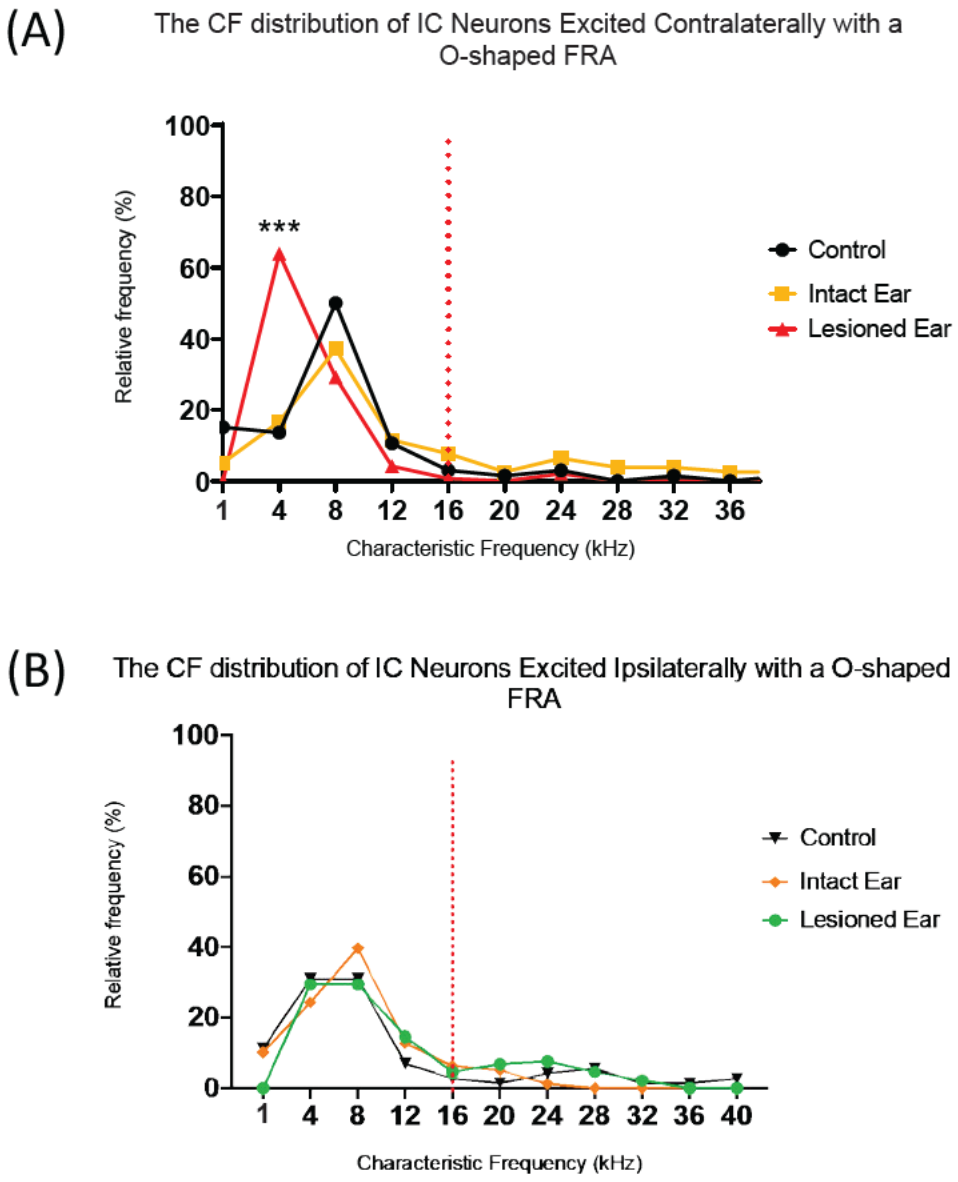


Figure 3.16. The CF distribution of O-shaped FRAs of IC neurons excited (A) contralaterally and (B) ipsilaterally from the intact and lesioned ear of treated animals and normal hearing control animals. An increase in relative frequency of CFs below the lesioned frequency of 16kHz (red vertical dotted line) is observed in IC neurons excited contralaterally (A) from the lesioned ear ($p=0.009$) when compared to the intact ear and control.

3.4.5 Input-Output Function

3.4.5.1 Input-Output Function: Contralaterally driven IC neurons

Contralaterally excited IC neurons stimulated from the intact (yellow) and lesioned (red) ears of treated animals and normal hearing control animals (black) had their input-output function (IOF) tested at CF (Fig.3.17). Responses were organised into monotonic and non-monotonic categories and proportions were compared. Statistical analysis was done using chi-squared and Fisher's exact tests.

In IC neurons excited contralaterally from the intact ($p=0.002$) and the lesioned ears ($p=0.007$) of treated animals, there was a statistical difference in the proportions of IOF at CF, when compared to normal hearing control animals (Fig.3.17). In IC neurons contralaterally excited from the intact ear (yellow) and lesioned ear (red), there were decreased proportions in the monotonic response and increased proportions in the non-monotonic response at CF.

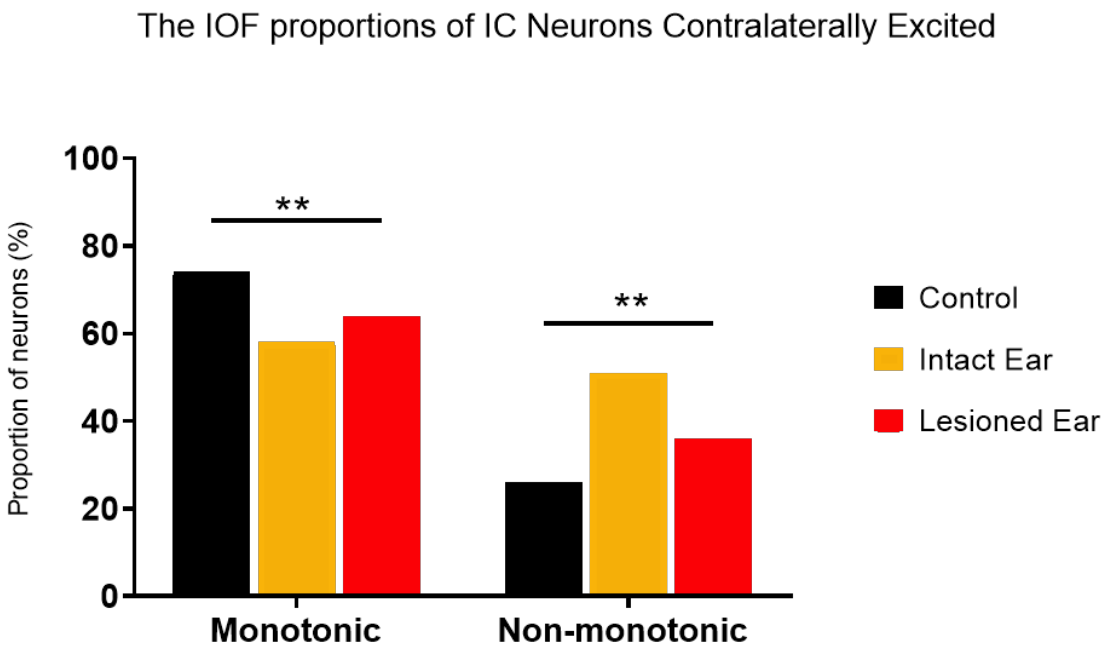


Figure 3.17. The input-output function profile of contralaterally excited IC neurons from the intact and lesioned ears of treated animals and normal hearing control animals. In IC neurons excited contralaterally from the intact (p=0.002) and the lesioned ears (p=0.007) of treated animals, there was a significant decreased in proportion of the monotonic response and increased proportion of the non-monotonic response at CF, when compared to normal hearing control animals.

3.4.5.2 Input-Output Function: Ipsilaterally Driven IC Neurons

Ipsilaterally excited IC neurons stimulated from the intact (orange) and lesioned (green) ears of treated animals and normal hearing control animals (black) had their input-output function tested at CF (Fig.3.18). Responses were organised into monotonic and non-monotonic categories and proportions were compared. Statistical analysis was done using chi-squared and Fisher's exact tests.

In IC neurons excited ipsilaterally from the intact ($p<0.0001$) and the lesioned ears ($p<0.0001$) of treated animals, there was a statistical difference seen in the proportions of IOF at CF, when compared to normal hearing control animals. In IC neurons ipsilaterally excited from the intact ear (orange) and lesioned ear (green), proportionally there was a decrease in the monotonic response and increase in the non-monotonic response at CF. This result indicates UNIHL profoundly effects the monotonicity of non-dominant ipsilaterally driven IC neurons.

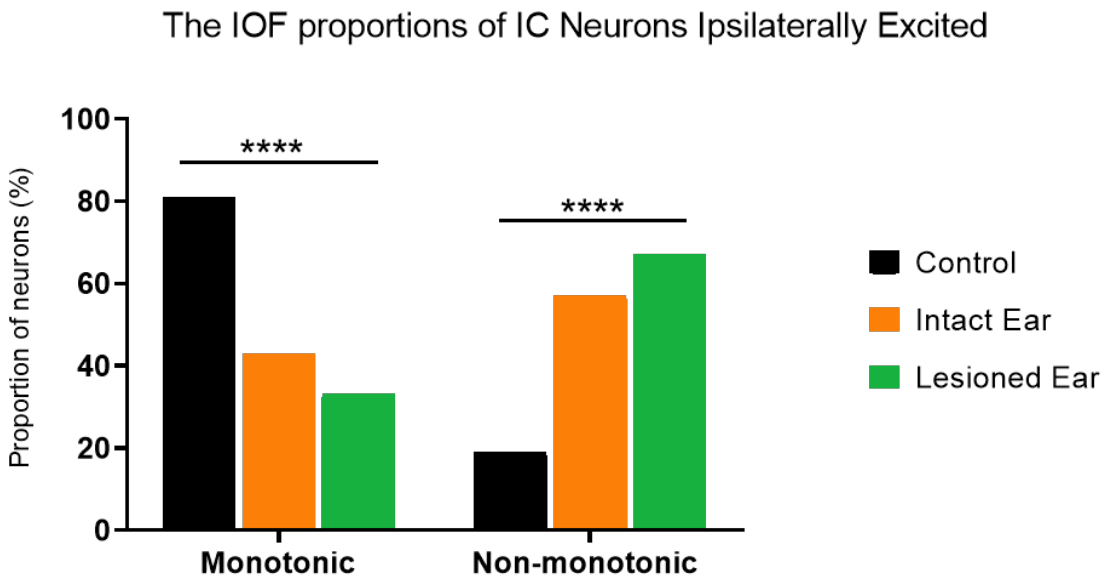


Figure 3.18. The input-output function profile of ipsilaterally excited IC neurons from the intact and lesioned ears of treated animals and normal hearing control animals. Ipsilaterally excited IC neurons from the intact ear and lesioned ear both showed a decreased proportion of the monotonic response ($p<0.0001$), and an increased proportion of the non-monotonic response ($p<0.0001$), when compared to control.

3.4.6 Proportions of Tonic and Onset Responses

3.4.6.1 Proportions Tonic and Onset Responses: Contralaterally Driven IC Neurons

Contralaterally excited IC neurons stimulated from the intact (yellow) and lesioned (red) ears of treated animals and normal hearing control animals (black) had their PSTH analysed at CF (Fig.3.19). Peri-stimulus timed histogram responses were organised into tonic and onset categories and their proportions were compared. Statistical analysis was done using chi-squared and Fisher's exact tests.

In IC neurons excited contralaterally from the intact ($p=0.016$) and the lesioned ($p=0.03$) ears of treated animals, there was a significant difference seen in the proportions of tonic and onset responses at CF, when compared to normal hearing control animals. In IC neurons contralaterally excited from the intact ear (yellow) and lesioned ear (red), proportionally there was decreases in the tonic response and increases in the onset response at CF. There were no statistical difference between IC neurons excited from the lesioned ear and the intact ear ($p>0.05$) in the experimental animal.

This result shows that in UNIHL animals, the proportion of the onset and tonic responses of contralaterally excited IC neurons was profoundly effected, irrespective of whether the stimulation is from the lesioned or intact ear.

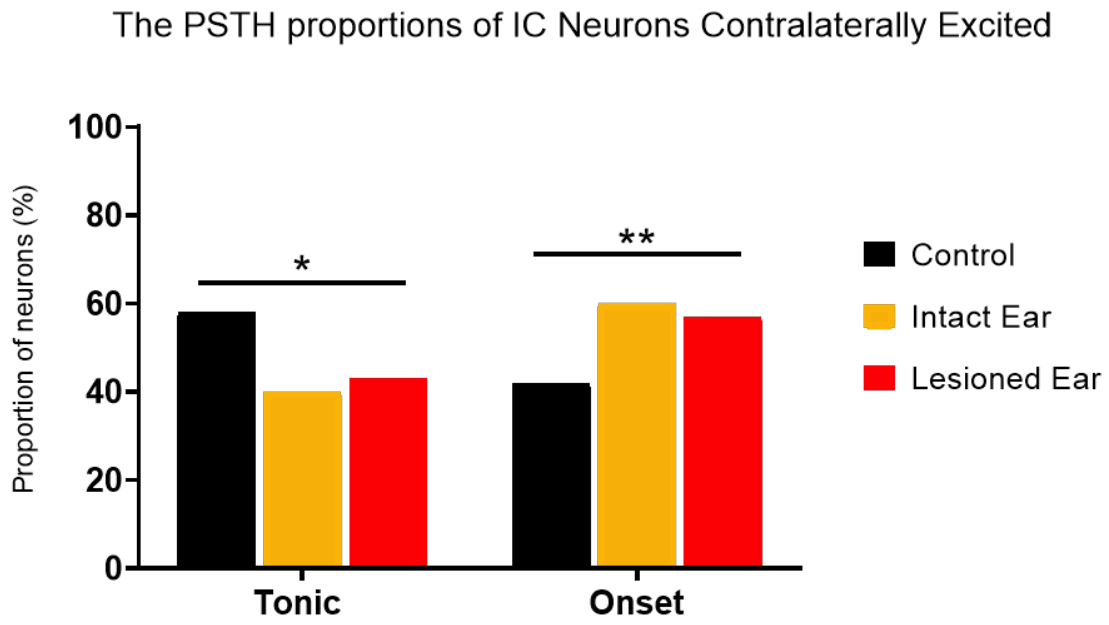


Figure 3.19. The peri-stimulus timed histogram profiles of IC neurons contralaterally excited from the intact and lesioned ears of experimental animals and normal hearing control animals. Contralaterally excited IC neurons from the intact ear and lesioned ear, both showed a significant decreased in proportion of the tonic response ($p=0.016$) and increased proportion of the onset response ($p=0.03$), when compared to control.

3.4.6.2 Proportions Peri-stimulus Timed Histograms: Ipsilaterally Driven IC Neurons

Ipsilaterally excited IC neurons stimulated from the intact (orange) and lesioned (green) ears of treated animals and normal hearing control animals (black) had their PSTH analysed at CF (Fig.3.20). Peri-stimulus timed histogram responses were organised into tonic and onset categories and their proportions were compared. Statistical analysis was done using chi-squared and Fisher's exact tests.

In IC neurons excited ipsilaterally from the lesioned ears of treated animals, there was a significant difference seen in the proportions of tonic ($p=0.001$) and onset ($p=0.02$) responses at CF, when compared to normal hearing control animals (Fig.3.20). In IC neurons ipsilaterally excited from the lesioned ear, proportionally there was an increase in the tonic response and a decrease in the onset response at CF. There was no statistical difference between IC neurons excited ipsilaterally from intact ear of the experimental animals and normal hearing control animals ($p>0.05$).

This result showed that UNIHL does not effect the proportion of onset and tonic responses at CF in IC neurons that are excited ipsilaterally from the intact ear only. All other excitatory IC neurons appeared to be profoundly effected.

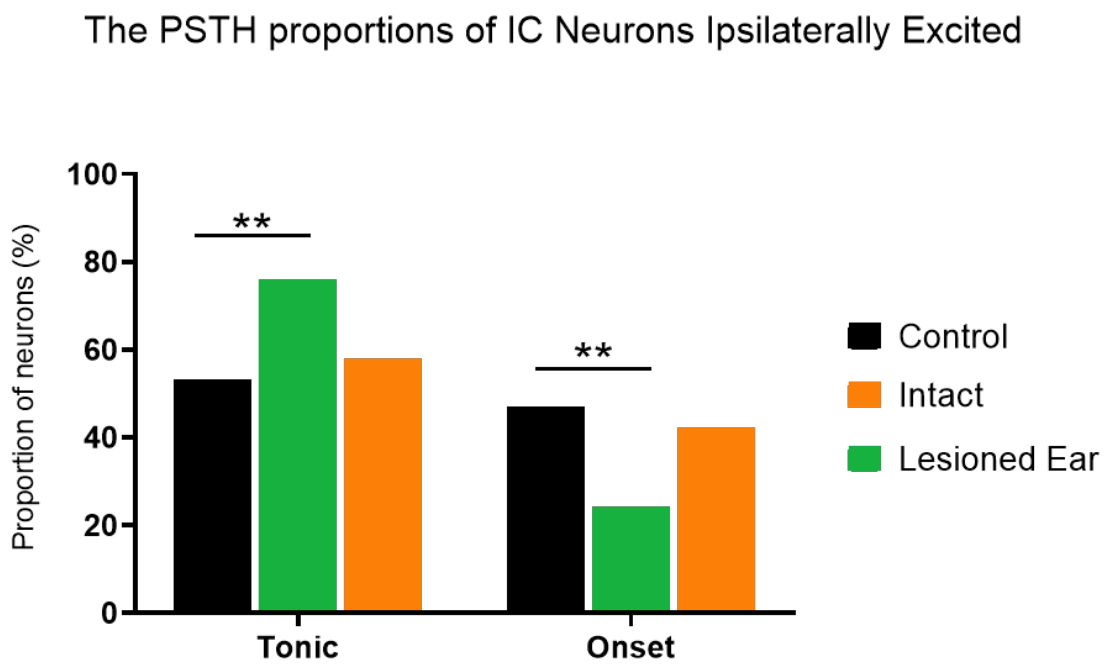


Figure 3.20. The peri-stimulus timed histogram profiles of IC neurons ipsilaterally excited from the intact and lesioned ears of experimental animals and normal hearing control animals. Ipsilaterally excited IC neurons from the lesioned ear, showed a significant increase in proportion of the tonic response (p=0.001) and a decreased proportion onset response (p=0.002), when compared to the intact ear and control.

3.5 Discussion

The three main consequences seen in this study are: 1) in normal hearing animals, contralaterally and ipsilaterally excited IC neurons have different response properties (Fig.3.21). As a result of UNIHL, 2) the lesion projection zone (LPZ) only effects the tonotopic organisation of IC neurons stimulated contralaterally from the lesioned ear, and 3) there was bilateral compensation occurring, evidenced by an increase in the population of ipsilaterally excited IC neurons stimulated from the intact ear (Fig.3.22).

3.5.1. Contralaterally Driven IC Neurons: Comparison with Published Studies

The characteristic response properties of IC neurons analysed in this study are not species specific. Similar response properties and characteristics have been observed in the IC of other animals including guinea pigs, cats, rats, mice, bats, gerbil, chinchilla and ferrets (Aitkin et al., 1975; Semple & Kitzes, 1985; McAlpine et al., 1997; Davis et al., 1999; Ramachandran et al., 1999; LeBeau et al., 2001; Lu & Jen, 2001; Biebel & Langner, 2002; Oliver, 2005; Ma et al., 2006; Mulders & Robertson, 2013; Palmer et al., 2013; Coomber et al., 2014). Across these different studies there were considerable disparities in proportions of IC neurons that displayed each of these response properties. Although there are differences, typically the reported results are of IC neurons that are contralaterally excited. In the following section I will consolidate the differences and similarities between the data of this present study and published results of previous authors of contralaterally driven IC neurons in normal hearing control animals.

The criteria applied when investigating monaural and binaural properties of IC neurons was as described by previous authors (Yin & Kuwada, 1983; Semple & Kitzes, 1985; Irvine

& Gago, 1990; Mulders & Robertson, 2013). In normal hearing control animals, previous authors have shown in IC neurons that are contralaterally excited, the dominant binaural interaction is of the EI response. In agreement with this, the current study reports the same result (Fig.3.10). The EI binaural response made up 60% of the binaural interactions in this population of IC neurons.

In contralaterally excited IC neurons, this study also showed that in normal hearing control animals, the IOF at CF is dominantly monotonic (Fig.3.19). This dominant monotonic IOF response is in agreement with other studies. In normal hearing animals, Mulders and Robertson (2013) reported 55% (n=50), Irvine and Gago (1990) reported 61% (n=188) and, Yin & Kuwada (1983) reported 60% (n=81) of IC neurons with this response. An explanation of this slight difference in percentages may be due to species, biological variance, experimental protocol or a varying combination of these three factors. However, the value here is even with these varying factors, the dominant IOF at CF of contralaterally excited IC neurons is monotonic.

Frequency response areas can be divided into two broad categories, namely V-shaped and non-V-shaped (Davis et al., 1999; Ramachandran et al., 1999; LeBeau et al., 2001; Palmer et al., 2013; Ropp et al., 2014). It is widely observed that the V-shaped FRAs is the most common type, and is considered the 'classic' response, as this tuning curve is seen at peripheral level in the cochlear nerve and centrally in the auditory cortex (Evans, 1972; Robertson & Irvine, 1989). Non-V shaped FRAs are reported to exist in smaller proportions and are considered specialised responses, as evident from the modification of central processing (Palmer et al., 2013). The names of non-V shaped FRAs may vary, however the shape of the receptive fields are the same. The O-shaped FRA has been described as 'closed' or 'C' type FRAs, because the receptive field is circumscribed within distinct frequency/dB SPL combinations. The I-shaped FRA has been described as 'Narrow'

or 'N', this FRA type share a frequency tuning curve similar to that of V-shaped FRAs. However, they are distinctly different as they show little to no expansion at higher dB SPL ranges. The W-shape FRA has been described as 'double-peaked' or 'D', because of the presence of two peaks at lower frequency/dB SPL combinations, and they are separated by an area of no excitatory activity (LeBeau et al., 2001; Palmer et al., 2013; Ropp et al., 2014). In agreement with previous studies, in contralaterally driven IC neurons of normal hearing control animals, the V-shaped FRA is the dominant FRA type and non-V shaped FRAs are observed in much lower proportions (Fig.3.15). Also, the control data of this study showed that the CF distribution of V-and O-shaped FRAs (Fig.3.15 & 3.16) are in agreement with that of Palmer et al. (2013) results, where they reported the CF of these two FRA types exists across the frequency axis.

Peri-stimulus time histograms in the IC can be classified as tonic or onset (Semple & Kitzes, 1985; Mulders & Robertson, 2013). Tonic or 'sustained' responses are defined by the presence of an onset response followed by a train of sustained spikes. Onset patterns showed a tighter distribution of spikes with shorter latencies, without a sustained train. In contralaterally driven IC neurons of normal hearing control animals, 58% of these neurons showed the tonic PSTH response pattern (Fig.3.19). This result is in agreement with Semple and Kitzes (1985)and Mulders and Robertson (2013), who showed that there is a significantly higher proportion of tonic responses at CF in IC neurons that are contralaterally excited.

Indeed, in this study, the characteristics contralaterally driven IC neurons of normal hearing control animals are in agreement with established literature. In this neuron population the expected characteristics seen in its highest proportions, include the dominance of the EI binaural interaction (60%)(Fig.3.13), a monotonic response at CF (74%)(Fig.3.17), a higher proportion of the 'classic' V-shaped FRAs (63%)(Fig.3.14) and the

'minimally transduced' PSTH pattern of the tonic response at CF (58%)(Fig.3.19). Taken together, these response properties provide further supporting evidence that the primary role of IC neurons in the contralateral pathway is excitatory (Schreiner & Winer, 2005). This would facilitate the faithful transmission of the signal to higher areas in the central auditory system. As the results in this study reflected the findings of previous authors, this gave confidence in the collection and analysis for subsequent investigation of ipsilaterally driven excitatory IC neurons. With these techniques and methodology, identical metrics were used in the same normal hearing control animals to investigate ipsilaterally excited IC neurons.

3.5.1.1 Comparison of Contralaterally and Ipsilaterally Excited IC neurons

Semple and Kitzes (1985) investigated the discharge properties of IC neurons and stated "these results reveal very different consequences of contralateral and ipsilateral monaural stimulation on the discharge properties of single units in the inferior colliculus" (Semple & Kitzes, 1985). The value of this earlier study, shows that ipsilaterally excited IC neurons are indeed different and yet there is limited literature on their response properties when compared to their contralateral counterparts. The following section compares contralaterally and ipsilaterally driven IC neurons in normal hearing control animals.

It has been estimated in mammalian IC, that the number of neurons from the CN projecting to IC contralaterally and ipsilaterally is approximately 50 to 1, respectively (Moore & Kowalchuk, 1988). This dramatic difference in input implies that the primary role of contralateral and ipsilateral projections is different, and therefore contribute to different roles in auditory processing. Binaural studies have shown that the dominant contralateral afferent projections are primarily excitatory, while the non-dominant ipsilateral afferent projections are primarily inhibitory (Semple & Kitzes, 1985; McAlpine et al., 1997). In agreement with these studies, the results reported here showed that in normal hearing animals, 69% of excitatory IC neurons were contralaterally stimulated while only 31% were ipsilaterally stimulated (Tab.3.1). Even though there was a lower density of ipsilaterally excited IC neurons, the CF distribution of these excitatory IC neurons followed the same tonotopic organisation as contralaterally excited IC neurons (Fig.3.8).

A further analysis of the inhibitory and excitatory characteristics of ipsilaterally excited IC neurons reveal properties where there were indeed differences between contralaterally and ipsilaterally excited IC neurons. The binaural profile of ipsilaterally excited IC neurons showed the EE interaction as the dominant proportion, observed in 86% of these neurons (Fig.3.11) and at CF, 81% of these neurons showed a monotonic IOF response (Fig.3.18).

Together, these response properties indicated that, in the majority of ipsilaterally driven excitatory IC neurons, there is an absence of inhibitory drive. These results indicate that ipsilaterally excited IC neurons showed characteristics of central gains of excitatory activity. Potentially, these excitatory IC neurons in this primarily inhibitory ipsilateral pathway may function to amplify neural gains.

In normal hearing animals, the differences between contralaterally and ipsilaterally excitatory IC neurons may be functionally significant. In contralaterally excitatory IC neurons, response properties are associated with the faithful transmission of sound, while in contrast, ipsilaterally excitatory IC neurons showed characteristics indicative of central excitatory gains (Fig.3.21). The importance of this result is that, the characterisation of this smaller population of ipsilaterally excitatory IC neurons is significant as central gains or hyperactivity has been traditionally associated with dysfunction (Section 1.1.4.1). The understanding of 'normal' is valuable as the effects of UNIHL on this neuron population is further explored.

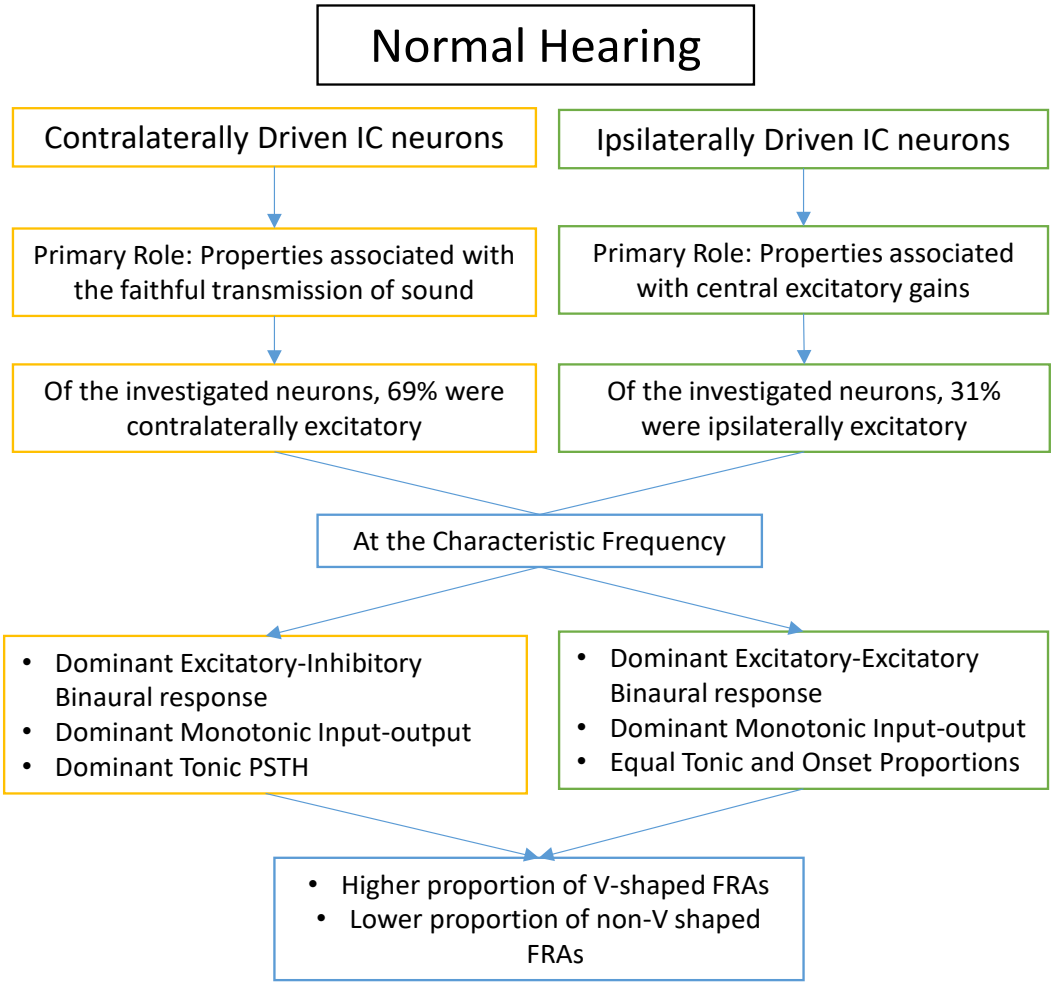


Figure 3.21. A schematic flowchart describing the differences and similarities between contralaterally and ipsilaterally excitatory IC neurons in normal hearing control animals. In normal hearing animals, response properties of contralaterally excitatory IC neurons (yellow boxes), displayed characteristics associated with the faithful transmission of sound. Response properties of ipsilaterally excitatory IC neurons (green boxes) showed characteristics indicative of central excitatory gains. Both neuron populations showed similar proportions of V-shaped and non V-shaped FRAs (blue box).

3.5.2 Tonotopic re-organisation and Lost Central Gains

The lesion projection zone (LPZ) has been extensively described in the auditory cortex (Seki & Eggermont, 2003; Eggermont & Roberts, 2004; Roberts et al., 2010). Analogous to other sensory deficits, there is a congruous loss between central organisation and the loss of the associated peripheral input. Similarly, this study showed that, in contralaterally excited IC neurons stimulated from the lesioned ear (Fig.3.8A), a profound deficit in the CF distribution was centred around the acoustic trauma tone. Interestingly, this profound deficit was not seen in IC neurons excited ipsilaterally from the lesioned ear. In fact, the tonotopic map remained largely unaffected (Fig.3.8B). This may be due to two factors: a) a smaller number of ipsilaterally excitatory neurons are effected and thus comparatively less afferent input are impacted by the acoustic assault and b) the effect of UNIHL on ipsilaterally driven IC neurons is likely to impact on the inhibitory characteristics – as it is the dominant role of the ipsilateral pathway.

Changes in inhibitory activity can be inferred by investigating the response properties of excitatory neurons in this pathway. In the previous section it was argued that ipsilaterally excited IC neurons showed response properties indicative of central gains of excitatory activity. Following UNIHL, these functions indicative of excitatory gains are diminished, and in fact as a consequence, an increase in inhibitory characteristics were observed. Binaurally, there was a 60% loss of the binaural EE interactions accompanied by a 30% increase in EI interactions (Fig.3.12). Ipsilaterally excited IC neurons were no longer dominantly monotonic at CF and instead showed a 38-48% increase of the non-monotonic IOF (Fig.3.18). This inferred an inhibitory drive, when neurons were subjected to higher SPLs. Also, a significant increase in the proportion of O-shaped FRAs was seen (Fig.3.14B). An increased proportion of O-shaped FRAs was observed in both contralaterally (53%) and ipsilaterally (51%) driven IC neurons excited from the lesioned ear, however a disruption of CF distribution in these FRAs is only seen in contralaterally excited IC neurons (Fig.3.16A).

This suggests, as a result of UNIHL there was an increase of inhibitory interactions globally that were producing a higher proportion of restricted FRAs, however a disruption to the tonotopic organisation of those FRAs was only observed in contralaterally driven IC neurons.

Turning to molecular studies, there is evidence of altered inhibitory interactions within the IC due to peripheral dysfunction (Mossop et al., 2000; Argence et al., 2006). Mossop et al. (2000) demonstrated that in the adult gerbil, unilateral deafening causes a reduction of inhibition within the IC contralateral to the deafened ear. Glutamic acid decarboxylase (GAD) is the synthesizing enzyme for GABA and western blotting for GAD protein levels showed significant decreases in the IC contralateral to cochlear ablation, relative to those in the ipsilateral IC, 24 hrs and 7 days post survival after the ablation. Furthermore, after cochlear ablation, there was a 60% increase in proportion at which ipsilateral stimulation resulted in neural activity. Unilateral cochlear ablation and UNIHL are two different hearing loss interventions. It can be rationalised that the mechanistic reasoning of this study's reported electrophysiological results may be due to the down regulation of GABAergic systems. It is possible, in the ipsilaterally excited IC neurons of this study, the normal inhibitory GABA-ergic interactions mediating central gains of excitatory activity are now dysfunctional. Further immunohistological investigations are required in order to support the theory that dysfunctional GABA-ergic mediated interactions are responsible for this increase in inhibitory activity seen in ipsilaterally driven excitatory IC neurons.

In summary, contralaterally excited IC neurons are tonotopically effected with a loss congruous with the lesion frequency whilst ipsilaterally excitatory IC neurons are tonotopically unaffected by the acoustic trauma. Instead characteristics associated with normal central gains of excitatory activity were diminished and increased inhibitory response characteristics were instead observed (Fig.3.22).

3.5.3 Bilateral Compensation

In acutely unilaterally cochlear ablated ferrets, it was reported that there were increases in stimulus evoked activity in ipsilaterally excited IC neurons from the intact ear (Mossop et al., 2000). The authors proposed a rapid unmasking of excitatory responses due to the consequential loss of inhibition that is normally driven by the presence of stimulation from the contralateral ear. That is, as a result of damage to one side, excitatory activity is unmasked in the intact unaffected side. The 12-week recovery timeframe of this present study may incorporate the residual-response hypothesis (Irvine et al., 2003) and associated central plasticity. The acute consequences are likely to be physiologically significant 12 weeks after the hearing loss intervention.

In normal hearing control animals, of the 637 excitatory IC neurons analysed, 69% were contralaterally stimulated whilst 31% were ipsilaterally stimulated (Tab.3.1). In line with the Mossop et al (2000) study, there was a 16% increase of ipsilaterally excited IC neurons stimulated from the intact ear. Indeed, as a percentage, these neurons made 47% of excitatory neurons found in the right ICs of treated animals (Fig,3.20). The rationale for this result is in line with other authors, that the removal of input from the dominant, excitatory, contralateral ear produces a marked increase in the level of excitation in response to stimulation of the remaining ear (McAlpine et al., 1997; Mossop et al., 2000). This upregulation of excitatory activity is interesting and potentially this effect is due to a residual activity rather than intrinsic central manifestations. As previously mentioned, an 8-week recovery period will likely capture events that are intrinsically associated with central manifestations occurring as a consequence of UNIHL (Mulders & Robertson, 2011). Further studies investigating the time course of these changes would be needed to resolve the question of how much influence central intrinsic events vs residual events are contributing to this result. This is the first time that an increase in the population of excitatory IC neurons ipsilaterally stimulated from the intact ear has been reported in an UNIHL animal model.

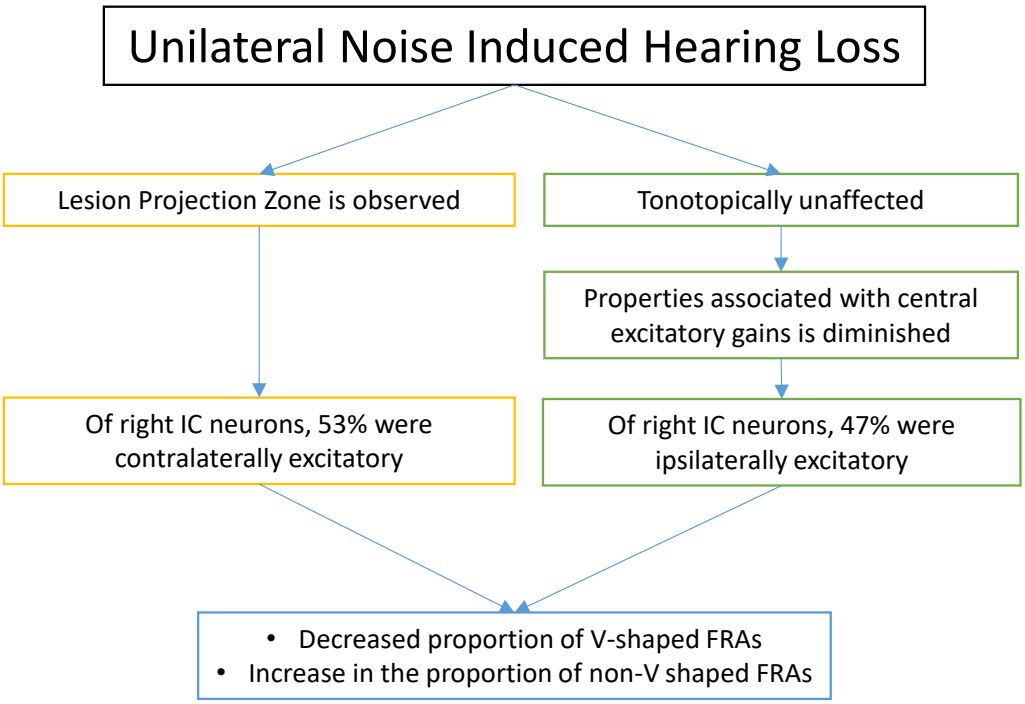


Figure 3.20. A schematic flowchart describing the differences and similarities between contralaterally and ipsilaterally excitatory IC neurons in as a consquence of UNIHL. As a consquence of UNIHL, contralaterally excited IC neurons (yellow boxes) are tonotopically effected with a loss congruous with the lesion frequency indicating a lesion projection zone. Whilst ipsilaterally excitatory IC neurons (green boxes) are tonotopically unaffected by the acoustic trauma. Changes in the characteristics associated with normal central gains of excitatory activity were diminished and instead increased inhibitory response characteristics were observed. Both neuron populations showed similar consequentical proportions of V-shaped and non V-shaped FRAs (blue box).

3.5.4 Are these IC response properties inherent or plastic?

The results of this study showed that excitatory IC neurons ipsilaterally stimulated displayed characteristics of central gains of excitatory activity and as a result of UNIHL, this function is diminished with an accompanying increase in inhibitory characteristics. Furthermore, as a result of UNIHL, a LPZ appeared only in IC neurons contralaterally stimulated from the lesioned ear (Fig.3.8). Of these response characteristics and the subsequent consequences observed – are theses properties dynamic and is it possible to exploit mechanisms of plasticity to alter theses consequential outcomes?

Frequency responses areas of the IC have been previously suggested to be dependent and inherent on projections from lower auditory nuclei. Ramachandran et al (1999) reported that, in decerebrated cat, each FRA type exists within distinct frequency distributions and has distinct binaural characteristics. The authors proposed three sources of input for each FRA type. The V-shaped FRAs were restricted to low frequency areas and therefore originated from the medial olive, I-shaped FRAs originated from the lateral olive and O-shaped FRA, from the dorsal cochlear nucleus (Davis et al., 1999; Ramachandran et al., 1999). Although the authors reasoning is compelling, more recent studies investigating the receptive field of the IC suggests a higher degree of underlying complexity. Palmer et al. (2013) analysed 2826 IC FRAs, and reported that all the FRA types observed were represented over a range of CFs. In agreement with other authors, they found V-shaped FRAs over the entire frequency range and not just within low frequency ranges (Egorova et al., 2001; Hernández et al., 2005; Alkhatib et al., 2006; Palmer et al., 2013) Indeed taken together, FRA types are observed to exist on a continuum.

The distinct types of FRAs in this current study are broad categorisation of the distinct types described by Palmer et al. (2013). However, the properties of those categorised

FRA are consistent. The study by Palmer et al (2013), provides evidence that FRAs appear not to be based on the source of its projections from lower nuclei. It is unknown if the results between Ramachandra (1999) and Palmer et al (2013) are at a variance due to the experimental methods or a real difference between cats and other species.

LeBeau et al. (2001) reported that V shaped and non-V shaped FRAs can be generated de novo. The authors paired electrophysiological recordings with microiontophoresis of bicuculline and strychnine into the IC. When bicuculline, a $GABA_A$ receptor antagonist and strychnine, a glycine receptor antagonist were injected, non-V shaped FRAs showed marked expansion where the FTC resembled a V-shaped FRA. Indeed, this effect was reversible when the injections ceased. These results emphasise the critical role of inhibitory interactions on signal processing within the IC.

Palmer et al. (2013)and LeBeau et al. (2001) showed that response properties of the IC are dynamic and consistent with the principle that these response characteristics are dependent on different inhibitory inputs of different strengths. The result of this present study showed that there are proportional differences of FRA shapes seen in ipsilaterally and contralaterally driven IC neurons (Fig.3.14) and that these FRA types exists along the tonotopic axis (Fig 3.15 & 3.16). Furthermore, as a result of UNIHL, there was difference in those proportions (Fig.3.14) and there were a shift in the FRA concentration along in the lesioned sides (Fig 3.15 & 3.16) which suggested the influence of inhibitory interactions. The results of this present study as well as the results of previously mentioned studies, provide evidence that FRAs shapes in the IC are unlikely to replicate their downstream origin. Indeed, it is likely FRA shapes and the receptive field of IC neurons are governed by different strengths of different inhibitory inputs that are inherently dynamic.

To date most UNIHL studies have primarily characterised the consequences that have occurred in contralaterally driven IC neurons (Szczepaniak & Moller, 1996; Browne et al., 2012; Papesh & Hurley, 2012; Manzoor et al., 2013; Wang et al., 2013; Coomber et al., 2014). As a result there is very scant literature investigating the consequence that occur in ipsilaterally driven IC neurons. The results of this study showed that there are significant differences in the consequences of UNIHL in ipsilaterally driven IC neurons and contralaterally driven IC neurons. A widely agreed principle that underlie the tinnitus disorder, is the loss of normal excitatory and inhibitory interactions in the central auditory system (Eggermont & Roberts, 2004; Kaltenbach, 2011). The value of this study adds to the vast literature supporting this principle.

The ability to locate sound in space is dependent on normal excitatory and inhibitory interactions and the central integration of information arriving at the two ears (Schreiner & Winer, 2005). As a result of UNIHL, the binaural results showed at the level of the IC, inhibitory and excitatory interactions vastly differ from the expected normal physiology in ipsilaterally driven IC neurons. Furthermore, in IC neurons excited ipsilaterally, which is primarily inhibitory, central gains of excitatory activity is diminished - this novel result could possibly contribute to the complexity underlying the pathology of tinnitus.

More importantly, this present study captures the expected response properties of both ipsilaterally and contralaterally excited IC neurons, in a commonly used physiological animal model of tinnitus. Indeed, using this criterion and utilising different means to mediate or change these expected bilateral consequences in the IC may give insights to future studies aimed at understanding the maladaptive consequences of tinnitus.

Chapter 4
The Inferior Colliculus: Cholinergic Enhancement and Auditory Training after Unilateral Noise Induced Hearing Loss

4.1 Introduction

4.1.1 Cholinergic Modulation in the Auditory System

Acetylcholine is a critical neuromodulator, that permits long lasting experience-dependent cortical plasticity. Interventions that specifically cause dysfunction in the central cholinergic system antagonise training induced plasticity in the motor and sensory cortices and by consequence, inhibit the recovery from brain damage (Metherate & Weinberger, 1990; Conner et al., 2005; Ramanathan et al., 2009; Gawel et al., 2014). On the other hand, cholinergic promotion combined with sensory stimuli directly affects the receptive field of cortical auditory neurons and thus enhances an animal's discriminatory ability (Kilgard & Merzenich, 2001; Kilgard et al., 2007; Shepard et al., 2013; Voss et al., 2016). There is a growing body of evidence to suggest that, cholinergic modulation generally acts to optimise the processing of signals in attention demanding contexts (Hasselmo & McGaughy, 2004; Sarter et al., 2005; Jääskeläinen et al., 2007; Ayala & Malmierca, 2015).

Voss et al. (2016) used two-tone frequency discrimination training combined with cholinergic promotion that resulted in rats learning the task more quickly, compared to their age-matched training-saline cohort. The authors also reported a reduction in the overlapping of the two tones in the auditory cortex (A1), suggesting an enhancement of cortical segregation of the two tones. This in turn infers an enhanced discriminability of the two tones as reflected behaviourally in both young and aged rats (Voss et al., 2016). In vivo studies that combine cholinergic promotion and electrophysiological techniques have shown that the receptive field of the auditory neurons are indeed malleable (Rasmusson, 2000; Kilgard et al., 2001; Kilgard et al., 2007; Shepard et al., 2013). An area of interest for studies investigating cholinergic activity is the nucleus basalis (NB). The nucleus basalis is in the basal forebrain that contains the largest population of cholinergic neurons with axons that project to the entire cortical mantle (Wenk, 1997). Paired electrical stimulation of the NB with auditory stimuli, cause cortical neurons to shift their receptive field towards the auditory stimulus that the animal had been exposed to and this was accompanied by the release of acetylcholine. The authors reported an increase of approximately 60% in the size of the receptive field of those A1 neurons. Interestingly, modulated and varied sounds produced a smaller degree of expansion (35%) in the receptive field (Kilgard & Merzenich, 1998; Kilgard et al., 2001).

Acetylcholine has also been implicated in stimulus-specific adaptions (SSA) in the inferior colliculus (IC) (Ayala & Malmierca, 2015). Stimulus-specific adaptation (SSA) describes the decrease in a neuron's response to a specific repeated sound. This is due to the repeated sound no longer being considered as novel, and therefore the neuron becomes adapted and in response, decrease their firing rate. When the same neuron is present with a deviant or novel stimuli, it resumes the higher rate of firing. This is a representative of repeating sounds being behaviourally inconsequential and can therefore be presumed as unimportant, whilst novel and rare sounds warrant the adaptive behaviour of attention (Ayala et al., 2016). Neurons that display SSA are found throughout the central

auditory system, including the auditory cortex (Ulanovsky et al., 2003; von der Behrens et al., 2009), auditory thalamus (Antunes et al., 2010) and in the IC (Pérez-González et al., 2005; Malmierca et al., 2009). Paired microiontophoretic application with in vivo electrophysiology in the IC, showed that adaptive modulation is influenced by acetylcholine (Ayala & Malmierca, 2015). Scopolamine is a muscarinic receptor antagonist and injections into the IC, decrease the SSA of IC neurons –that is, there is maintenance of the higher firing rate in response to the repetitive tone input. In the IC, by antagonising acetylcholine receptors, there is an increase in the extracellular availability of acetylcholine resulting in the persistence of neural excitability for the repetitive tone. Interestingly, enhanced sensory coding was not seen in response to the novel deviant tone, suggesting the maintained firing is not due to the overall system being hyperactive but specifically in response to the repetitive sound. This study showed, in the IC, that an increase in extracellular acetylcholine resulted in response properties that were suggestive of a lack of adaptability.

Taken together, these studies suggest that acetylcholine plays a modulatory role in the central auditory system, potentially affecting the auditory system in ways that primes the system to consider certain sounds to be behaviourally or physiologically relevant. These studies also highlighted the great potential for exploiting cholinergic modulation in central maladaptive conditions that manifests as a result of peripheral sensory deficits.

4.1.2 Cholinergic Projections to the IC

Several studies that combined microiontophoretic injections of acetylcholine receptor antagonists or agonists with in vivo electrophysiological recordings in the IC, have shown that over 80% of IC neurons are responsive to acetylcholine, in that there is a change the response property of these neurons (Watanabe & Simada, 1973; Farley et al., 1983; Habbicht & Vater, 1996). Supporting these studies, are molecular investigations that have confirmed muscarinic and nicotinic receptors and acetylcholinesterase (the enzyme that breakdowns acetylcholine) are present throughout the IC (Shute & Lewis, 1967; Schwartz, 1986; Glendenning & Baker, 1988; Morley & Happe, 2000). The authors proposed that acetylcholine in the IC is involved in processes aimed to enhance specific response properties to certain stimuli, possibly affecting the signal to noise ratio of the stimulus input (Farley et al., 1983). Together, these studies inferred that acetylcholine is implicated as a modulator, however there is no evidence of cholinergic neurons located within the IC, suggesting that the cholinergic modulatory effects seen electrophysiologically are extrinsically sourced.

Paired tracer studies with immunohistochemistry for choline acetyltransferase (ChAT) have shown that cholinergic input to the IC primarily originate from descending auditory nuclei (Motts & Schofield, 2009). Indeed, Motts and Schofield (2009) reported that, although there are cholingeric neurons in lower auditory structures, it is intresting that no cholinergic neurons directly project into the IC that are of the 'traditional' ascending auditory pathway. Cholinergic neurons projecting into the IC have been reported to originate from two large tegmental nuclei – the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT)(Schofield & Motts, 2009). Their widespread cholinergic projections innervate several brain regions associated with arousal, the sleep-wake cycle, stimulus–reward learning and pre-pulse inhibition of the acoustic startle (Koch et al., 1993; Reese et al., 1995; Dormont et al., 1998; Steriade, 2004). Furthermore, Schofield

(2010) paired anterograde and retrograde tracer injections in A1 and IC with ChAT immunohistochemistry staining to determine if A1-IC acetylcholine interactions occurred via PPT and LDT. The authors showed that ChAT-positive PPT and LDT neurons had anterograde FluoroRuby-labelled cortical axons in close contact with retrograde Fast Blue-labelled inferior colliculi boutons. Furthermore, more contacts were found ipsilaterally and less so contralaterally to the injected cortex. In concert, these studies showed that it is highly likely that A1 projections elicit cholinergic effects in the auditory midbrain.

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4.1.3 Unilateral Lesions in the Auditory System and Neuroplasticity

To date, only a single study has reported the effect of unilateral noise induced hearing loss (UNIHL) on cholinergic receptor expression in the central auditory system (Forrest et al., 2019). In their study, Forrest et al. (2019) reported a downregulation of the muscarinic acetylcholine receptor (mAChR) in the A1 region contralateral to the acoustic trauma. Furthermore, the authors showed that, ipsilaterally to the acoustic trauma, there was an upregulation of nicotinic acetylcholine receptor (nAChR) in layer two of the adjacent dorsal rostral belt. Although these two receptors are found throughout the IC, it is speculative whether a similar consequence of cholinergic receptor expression occurs within the IC. Clarkson et al. (2012) investigated the gene expression of neurons within the cortico-colliculi pathway ninety days after unilateral cortical lesions. The cortico-colliculi pathway is made-up of bilateral projections from A1 to the IC, however the dominant pathway is ipsilateral. The authors reported that IC neurons ipsilateral to the cortical lesion showed gene expression associated with apoptosis and axonal regeneration. In IC neurons contralateral to the lesion, an upregulation of gene expression related to neurotransmission, cell proliferation and synaptic growth was reported (Clarkson et al., 2012). In the dorsal cochlear nucleus (DCN), as a result of intense noise exposure, all superficial neurons in the DCN, except for the fusiform cell layer, showed an increase in hyperactivity (Kaltenbach & Zhang, 2007). The application of a cholinergic agonist, carbachol, resulted in suppression

of hyperactivity in the fusiform cell layer, with this suppression observed more profoundly in the sound exposed rats. The authors inferred that sound exposure resulted in an increased sensitivity to the inhibitory influence of cholinergic inputs (Kaltenbach & Zhang, 2007). These three studies highlighted the capacity in which neuroplasticity associated events could possibly occur that involve the cholinegric system, illustrating a dynamic consequence that occurs within each hemisphere, with respect to the side of the lesion.

Sudden sensorineural hearing loss (SSNHL) is characterised by the specific audiometric criteria of a 30 SPL dB decrease of hearing unilaterally when compared to thresholds on the opposite ear, and if the hearing loss is of at least three adjacent frequencies on a standard pure tone audiogram. By definition, SSNHL occurs spontaneously and develops over the course of three days (Okamoto et al., 2014). Constraint induced sound therapy (CIST) is based on a well-known neuro-rehabilitation approach (Liepert et al., 1998; Miltner et al., 1999; Taub et al., 1999; Okamoto et al., 2014). It is characterised by the plugging of the unaffected ear, whilst extensive sound stimulation is presented to the affected ear (Okamoto et al., 2014). To date, two studies have investigated the effect of CIST as a treatment for SSNHL. Okamoto et al. (2014) reported standard corticosteroid treatment (SCT) paired with CIST, showed a significantly improved hearing recovery compared to those who only received SCT. In contrast, Kuo (2019) reported paired SCT + CIST treatment showed no statistical difference in the hearing thresholds in patients that had received SCT-only treatment. However, these patients did report a decrease in anxiety and distress levels. It is difficult to conclude, based on only two studies, if SCT + CIST therapy is effective for patients with SSNHL. It is possible that more sensitive and sophisticated tests are required to understand the underlying mechanistic physiology exploited centrally by this treatment. Indeed, animal studies that investigate unilateral ear plugging of the unaffected ear and acoustic enrichment may provide some insight.

Animal studies that investigated unilateral ear plugging, have shown that there are

hemisphere specific consequences of unilateral ear plugging in neonatal ferrets. (Nordeen et al., 1983; Moore & Kowalchuk, 1988; Moore et al., 1989; McAlpine et al., 1997). Unilateral conductive hearing loss, that resulted from ear plugging, led to the rearrangement of auditory brainstem connectivity as evidenced by a retrograde injection from the contralateral IC (Moore et al., 1989). It was reported that, there was a significant increase in the number of retrograde labelled neurons in the contralateral cochlear nucleus (CN) of the plugged ear, when compared to age matched hearing animals. In addition, Nordeen et al. (1983) investigated unilateral cochlear ablation in gerbils, by injecting horseradish peroxidase (HRP) into the IC contralateral to the cochlear ablation. The authors were able to observe that there was a significant decrease in the number of labelled neurons in the IC as a result of the de-afferented CN. Furthermore, in gerbils that had their cochlear ablated neonatally, the authors reported that, in addition to the decreased number of labelled neurons in the IC contralateral to the cochlear ablation, there was a significant increase of labelled neurons in the IC ipsilateral to the unoperated side (Nordeen et al., 1983). The authors proposed neural competition to be the underlying cause of the differences seen in the adult and neonatal animal. The authors speculated that, the immature neurons of the CN having not received its full complement of afferent terminals that the ipsilateral CN neurons were therefore opportunistically terminating on the IC of the intact unoperated side.

Addressing the music therapy component of CIST treatment, the effects of passive noise exposure in cats, after noise induced hearing loss was investigated (Noreña & Eggermont, 2005; 2006; Pienkowski & Eggermont, 2009; 2012). Noreña and Eggermont (2006) demonstrated that immediately after the acoustic trauma, the central auditory system is in a malleable state, particularly primed to experience-dependent plasticity. The sensory environment an animal is exposed to immediately after the acoustic trauma is critical in determining the tonotopic reorganisation of A1 neurons (Noreña & Eggermont, 2005; 2006). Indeed, cats placed in a quiet environment after an acoustic trauma and left

to recover for three weeks, showed that within the reorganised areas of A1, there were increases in SFR and neural synchrony. In contrast, cats placed in an enriched acoustic environment (EAE) and passively exposed to content representative of a wide range of frequencies and level combinations, showed that the re-organised tonotopic map and the accompanied response characteristics that are typically seen as a consequent of acoustic trauma, was prevented (Noreña & Eggermont, 2005; 2006). Taken together, these studies indicated that after a sudden loss of peripheral input recovery is critically dependent on the type of exposure immediately introduced after the intervention.

Taken together, these studies provide evidence that the loss of function on one side has dynamic consequences with respect to both hemispheres. As a result of UNIHL, cortical lesions and cochlear ablations, there are different characteristics and degrees of central reorganisation in neurons ipsilateral to the lesion compared to neurons contralateral to the lesion, in both the cortex and the midbrain.

In the previous chapter, we characterised the response properties of IC neurons following UNIHL. As a result of UNIHL, the binaural results showed that at the level of the IC, inhibitory and excitatory interactions vastly differ from the expected normal physiology in ipsilaterally driven IC neurons. Furthermore, in IC neurons excited ipsilaterally, central gains of excitatory activity were diminished. Taken together, the results of the aforementioned studies and the results from Chapter 3, strongly suggest that any attempt to remediate the neural consequences of UNIHL should also take into consideration the interactions between ipsilaterally and contralaterally driven neurons.

4.2 Hypothesis and Aims

In this chapter, pharmacological and environmental interventions were used to alter and guide the neural plastic processes that occur as a consequence of UNIHL. Acetylcholine promotion, the neural competition between the two ICs and the sensory environment immediately after the acoustic trauma were considered and incorporated within the experimental design.

To date, there have been no studies investigating the response properties of IC neurons in a UNIHL animal model following acetylcholine promotion paired with auditory training (AT). The hypothesis is that after UNIHL the animals' central auditory system is in a malleable state. This malleable state can be enhanced by increasing acetylcholine levels and guided by manipulating the animal's acoustic environment.

It was hypothesised UNHL would induce favourable conditions where AT paired with acetylcholine promotion would result in,

- A shift in the tonotopic organisation towards the training tones would occur.
- Significant changes in the proportion of binaural interactions.
- Significant changes in the proportions of FRA types would be observed.

4.3 Research Methods

The animal preparation, anaesthesia, unilateral noise exposure and the auditory brainstem response tests were completed as described in Chapter 2. The outline of the experimental procedures for the four groups, normal hearing control, UNIHL control, UNIHL: Rivastigmine + AT and UNIHL: saline + AT is as shown in Figure 4.1.

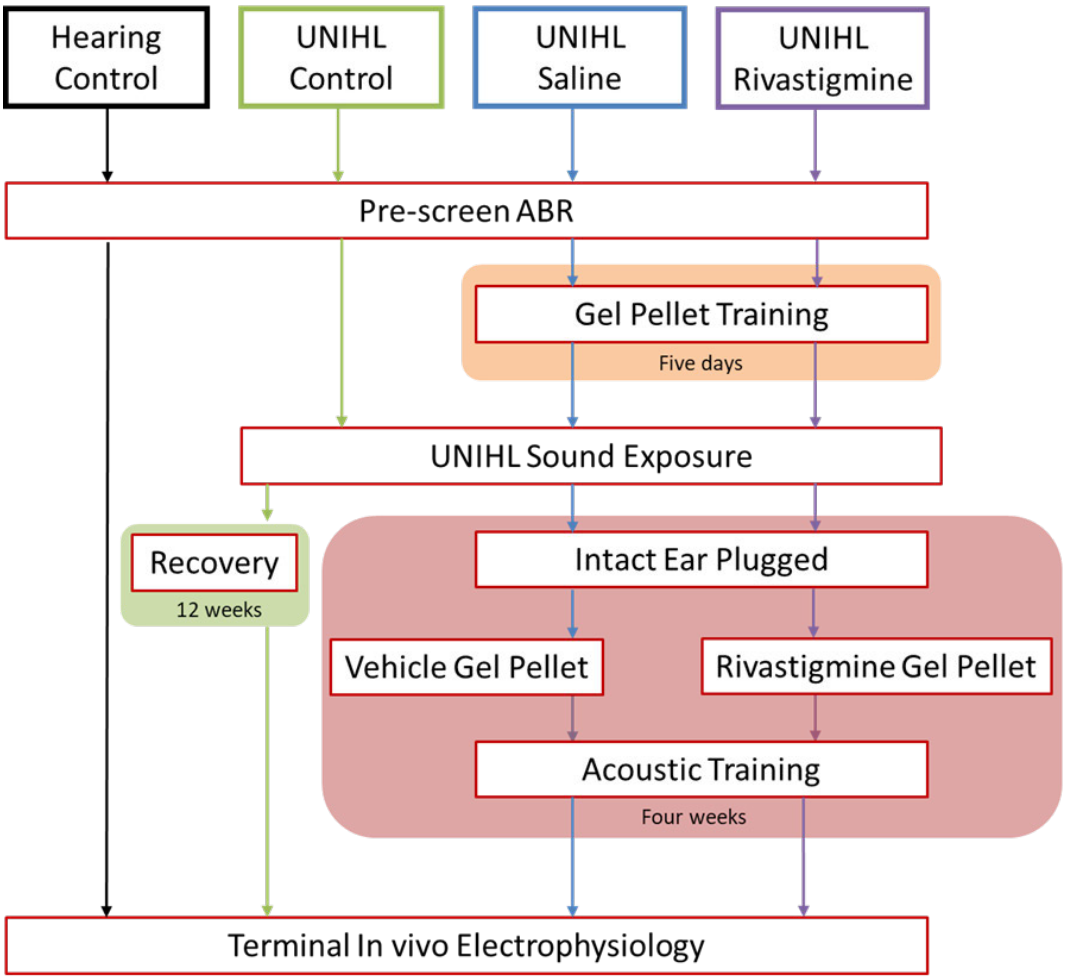

Figure 4.1. Flowchart of experimental timeline

4.3.1 Animals and Ethics

The experiments described in this study complied with the Australian code for the care and use of animals for scientific purposes, the New South Wales Animal Research Act and were approved by the Western Sydney University Animal Care and Ethics Committee (A12671). Twelve three-month old male Wistar rats, were obtained from Animal Resources Centre (ARC) Canning Vale, Western Australia. The animals were subjected to the quarantine procedures of the Animal Facility, School of Medicine, Western Sydney University and subsequently housed and cared for in the facility. The animals were housed and cared for as described in the general methods (Section 2.1.1). Rats were subjected to a pre-screen ABR to quantify normal hearing (Section 2.1.2) prior their allocation into Saline (n=6) or Rivastigmine treated (n=6) cohorts. Once the gel pellet habituation and training were achieved, animals were subjected to UNIHL (Section 2.1.3) (Fig.4.1). Animals were housed in pairs and subjected to the same daily monitoring, handling and auditory training. The experimenter was a aware of the group allocation subjected to the housing of pairs. Of each pair, one animal received the gel pellets containing the Rivastigmine (n=6) and the other a saline vehicle (n=6). Individual rats were identified using AVID microchips that was place subcutaneously on the dorsal aspect of the neck whilst under anaesthesia after the ABR. Unit anaylsis was done based in AVID identification chips blind to the group allocation of the animals. All experiments described were completed in the Auditory Neuroscience Laboratory (30.2.69) at the School of Medicine, Western Sydney University.

4.3.2 Drug Delivery Habituation and Auditory Training

Prior to the UNIHL, the animals underwent training with a gel pellet. The stock of the gel pellet consisted of Aeroplane™ Original Strawberry Flavour Jelly (Aeroplane Jelly, Clayton South, VIC, AU) (7gm), McKenzie's™ Gelatine Powder (Altona, VIC, AU) (4gm) and Equal® sweetener (Equal® sweetener, Yarraville, VIC, AU) (0.17gm) mixed in 30 mL of hot water of 40°C. When the solution reached room temperature, the gel pellet solution was transferred into each well of a 24 well plate. The plate was left to set at 4°C. Training was done using a final volume of 1.5mL (as this was the largest volume an animal would take in four weeks). Training was considered successful when the animal would voluntarily and reliably consume the gel pellets consecutively over three days. Typically, this would take a week to achieve, requiring two-three days of habituation. Following successful training, the rats were fed gel pellets containing Rivastigmine (0.2mg/kg) (Sigma-Aldrich Pty Ltd, North Ryde, NSW, AU) in MilliQ water or an equivalent volume of saline.

Whilst the animals were under anaesthesia after acoustic trauma, the right (unaffected) ear was plugged using moulded Otoform Kc™ (Dreve Otoplastik GmbH, Unna, DEU) and secured using 3M Vetbond™ (St Paul, MN, USA). This plugging procedure is similar to the procedure used by previous authors (Nodal et al., 2010). After a 24-hour recovery period, the animals were placed in separate feeding enclosures. The right (unaffected) ear was inspected to ensure the silicone plug had not been removed. In the event that the plug had been removed, a replacement plug was inserted. A visual inspection was also performed to ensure no redness; inflammation or damage was done to the intact ear as a result of ear plugging. Each animal was given a gel pellet containing either clinically relevant dose of 0.2mg/kg of Rivastigmine or a saline equivalent (Voss et al., 2016). Once both animals had consumed their gel pellets, they were transferred into the same sound enclosure and provided with enrichment and sound was delivered using Tucker Davis Technologies (TDT)™ system 3 Software (OpenEx) and hardware (Tucker-Davis Technologies, Alachua,

FL, USA) and a CTS power line speaker (KSN-1188; Piezo Source, San Jose, CA, USA). The speakers were placed within a modified lid, covering their enclosure. The training tones consisted of tones outside of the predicted LPZ. The stimulation consisted of 4 & 8kHz tones presented at 10ms apart, respectively and repeated every 71ms at 50 dB for 2 hours. The exposure time coincided with the bioavailability of the drug, which has a half-life of 1.5 hours (Tayebati et al., 2004; Voss et al., 2016). Drug treatment and auditory training were performed daily, Monday to Friday, for a period of four weeks.

4.3.3 Electrophysiology and Histology

After four weeks of the gel pellets and AT, the animals underwent an in vivo electrophysiology study at four week to observes any acute influences as a result of the interventions. At the end of the experiment the animals were euthanased by means of exsanguination using cardiac perfusion with 150 mL of sodium lactate (Hartmann's) solution (Fresenius Kabi Australia Pty Limited, Mount Kuring-gai, NSW, AU) followed by 150mL of 4% paraformaldehyde in phosphate buffer saline. The brain was removed and processed for histological analysis of electrode tracking and confocal imaging. The in vivo electrophysiology experiment, data collection, histology and confocal imaging were performed as described in in Chapter 2.

4.4 Results

4.4.1 Number of neurons recorded

Recordings were made from excitatory IC neurons (i.e. those that responded to an auditory stimulus). Responses were categorised based on their experimental condition, either contralaterally or ipsilaterally excited and from a lesioned or intact ear. In this study, monaural and binaural responses were recorded from a total of 1,823 neurons in the IC. Of those recordings, 1,045 were from the UNIHL saline + auditory training (AT) group and 778 neurons were from the UNIHL Rivastigmine + AT group (Tab.4.1). A proportional analysis of the response characteristics was calculated based on the denominator of the total population of each experimental condition; contralaterally excited from (a) the lesioned ear and (b) the intact ear and ipsilaterally excited from (c) the lesioned and (d) the intact ear. Together with the results from Chapter 3, a total of 3,423 excitatory IC neurons were analysed for this chapter.

Table 4.1. Number of recorded IC neurons from each experimental group.

	Intact Ear Contralateral IC	Intact Ear Ipsilateral IC	Lesioned Ear Contralateral IC	Lesioned Ear Ipsilateral IC	Total
Hearing Control	439 (69%)	198 (31%)			637
UNIHL Control	394 (41%)	192 (20%)	211 (22%)	166 (17%)	963
UNIHL Saline + AT	400 (38%)	217(21%)	216 (20%)	212 (20%)	1045
UNIHL Rivastigmine + AT	305 (39%)	158 (20%)	177 (22%)	138 (17%)	778
					3423

4.4.2 Characteristic Frequency (CF) and Minimal Thresholds (MT)

4.4.2.1 Characteristic Frequency (CF)

Characteristic frequencies were placed into 4 kHz bins. The data was then plotted as cumulative frequencies (Fig.4.2). A statistical analysis was done using the Friedman test and a Dunn's multiple comparisons test.

In contralaterally driven IC neurons stimulated from the lesioned ear (Fig.4.2B), when compared to normal hearing control animals (black), the UNIHL cohorts (green, purple & blue) showed a left-sided shift, with the greatest degree of shift occurring below the 16 kHz lesion frequency ($p=0.0003$). As a result of UNIHL, there was an increased proportion of IC neurons presenting with a CF below 16 kHz. However, there were no statistical differences between the UNIHL groups ($p>0.05$). In IC neurons excited ipsilaterally from the intact ear (Fig.4.2D), there was a significant difference centred at the 16 kHz lesion frequency between normal hearing control animals (black) and animals treated with AT paired with Rivastigmine (purple; $p=0.0317$).

There were no statistical differences between the four cohorts in IC neurons ipsilaterally excited from the lesioned ear (Fig.4.2A, $p>0.05$) and in IC neurons contralaterally excited from the intact ear (Fig.4.2C, $p>0.05$).

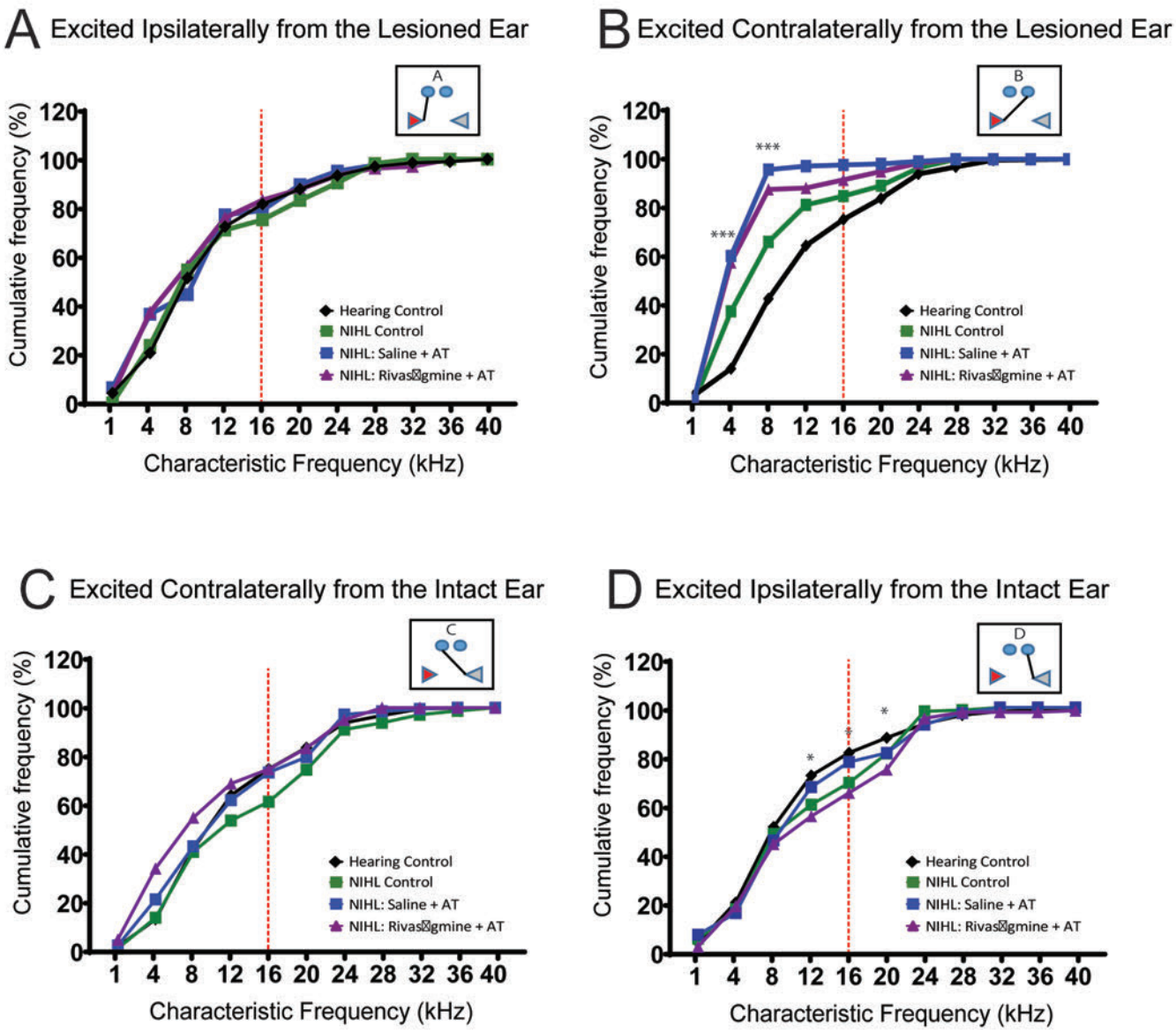

Figure 4.2. The cumulative frequency of CFs of IC neurons excited from (A&B) the lesioned ear (>) and from (C&D) the intact ear (<). The red dotted line illustrates the lesion frequency of 16 kHz. There was a significant left sided shift in IC neurons that are excited contralaterally (B) from the lesioned ear, when compared to control ($p=0.0003$), however there were no significant differences between AT groups and NIHL control ($p>0.05$).

4.4.2.2 Minimal Threshold (MT)

The minimal thresholds of IC neurons were categorically organised into bins of 20 dB SPL. The data were then plotted as cumulative frequencies. A statistical analysis was done using a Friedman test and a Dunn's multiple comparisons test.

There was a decrease in the hearing sensitivity in the UNIHL groups in IC neurons excited from the lesioned ear (Fig.4.3A&B, green, purple & blue; $p=0.005$). Hearing sensitivity was unaffected in IC neurons stimulated from the intact ear (Fig.4.3C&D, $p>0.05$). When compared to normal hearing animals (black), the UNIHL cohorts (green, purple & blue), a right sided shift in the cumulative frequencies of MTs between 20 and 60 dB SPL was seen. However, between the UNIHL cohorts (Fig.4.3A&B, green, purple & blue), there were no statistical differences in the MT of IC neurons excited from the lesioned ear ($p>0.05$).

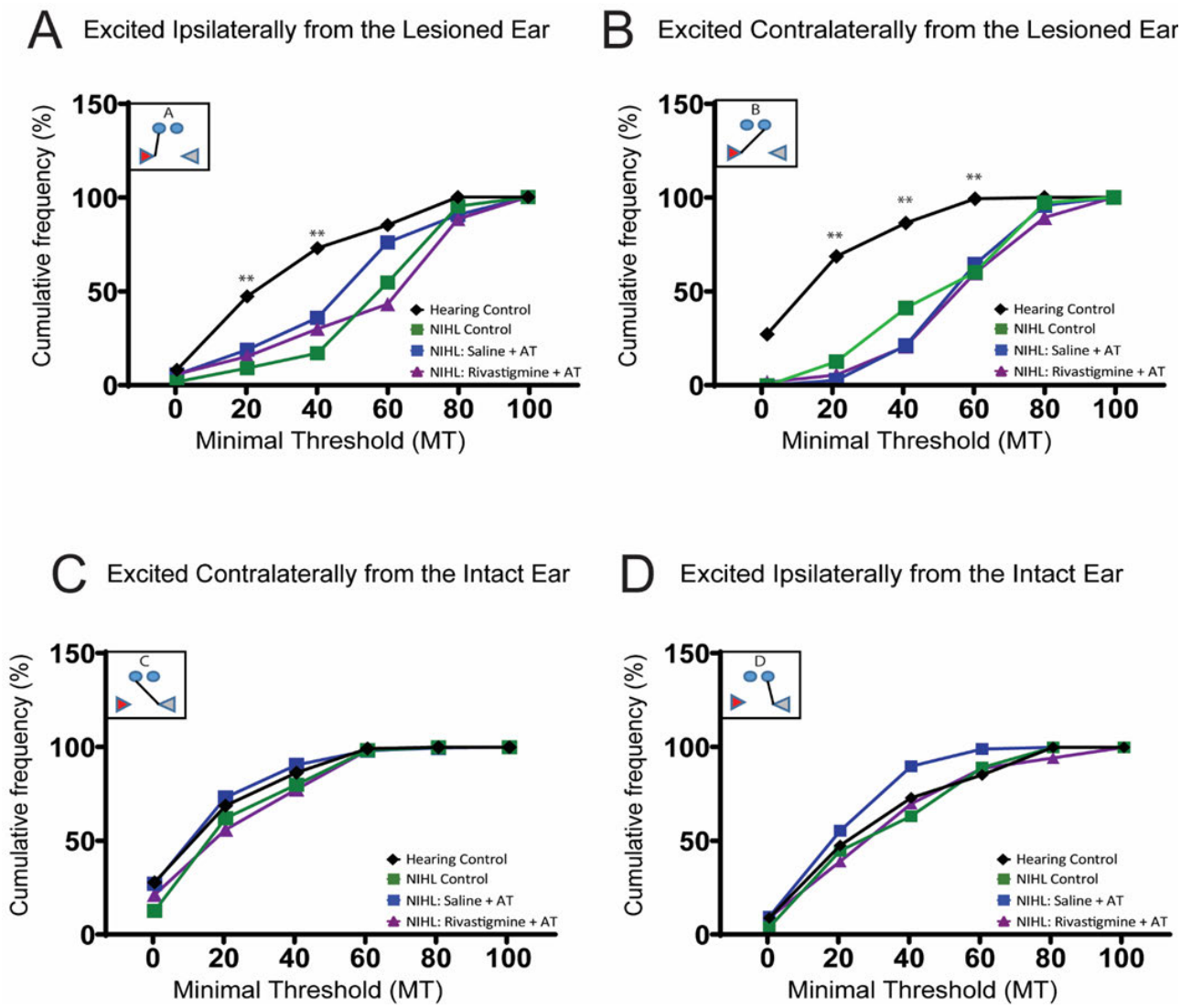

Figure 4.3. The cumulative frequency of MTs of IC neurons excited from (A&B) the lesioned ear (>) and from (C&D) the intact ear (<). There was a significant right sided shift in IC neurons that were excited from the lesioned ear (A&B), when compared to control (A&B, p=0.005). However, there were no significant differences between UNIHL cohorts (green, purple & blue; p>0.05).

4.4.3 Frequency Response Areas (FRA)

The percentage of IC neurons of a specific FRA type was analysed based on the total number of neurons recorded under one stimulus condition. Statistical analysis was done using chi-squared tests.

In IC neurons excited ipsilaterally from the lesioned ear (Fig.4.4A), there were significant differences in the FRA proportions when AT groups (purple & blue) were compared to UNIHL control (green) ($p<0.0001$). When treated with AT + Saline (blue), these IC neurons showed a proportional increase of the V-shaped FRA when compared to UNIHL control proportions. When treated with AT + Rivastigmine (purple), these IC neurons showed a proportional increase of the narrower, I-shaped FRA when compared to UNIHL control proportions. Overall, in these two AT groups (blue & purple) there was a decrease of the O-shaped FRA, when compared to UNIHL control (green) ($p <0.001$).

In IC neurons excited contralaterally from the lesioned ear (Fig.4.4B), similar to graph A, there were proportional decreases of O-shaped FRAs in the AT groups (purple & blue), when compared to proportions seen in the UNIHL control cohort (green)($p=0.0049$). As a result of AT (purple & blue), a significant increase of the V-shaped FRA was observed, when compared to UNIHL control (green)($p=0.004$). There were no differences between the AT groups (purple & blue)($p>0.05$).

In IC neurons excited contralaterally (Fig.4.4C) and ipsilaterally (Fig.4.4D) from the intact ear, there were no differences in FRAs between the three UNIHL groups and normal hearing animals ($p>0.05$).

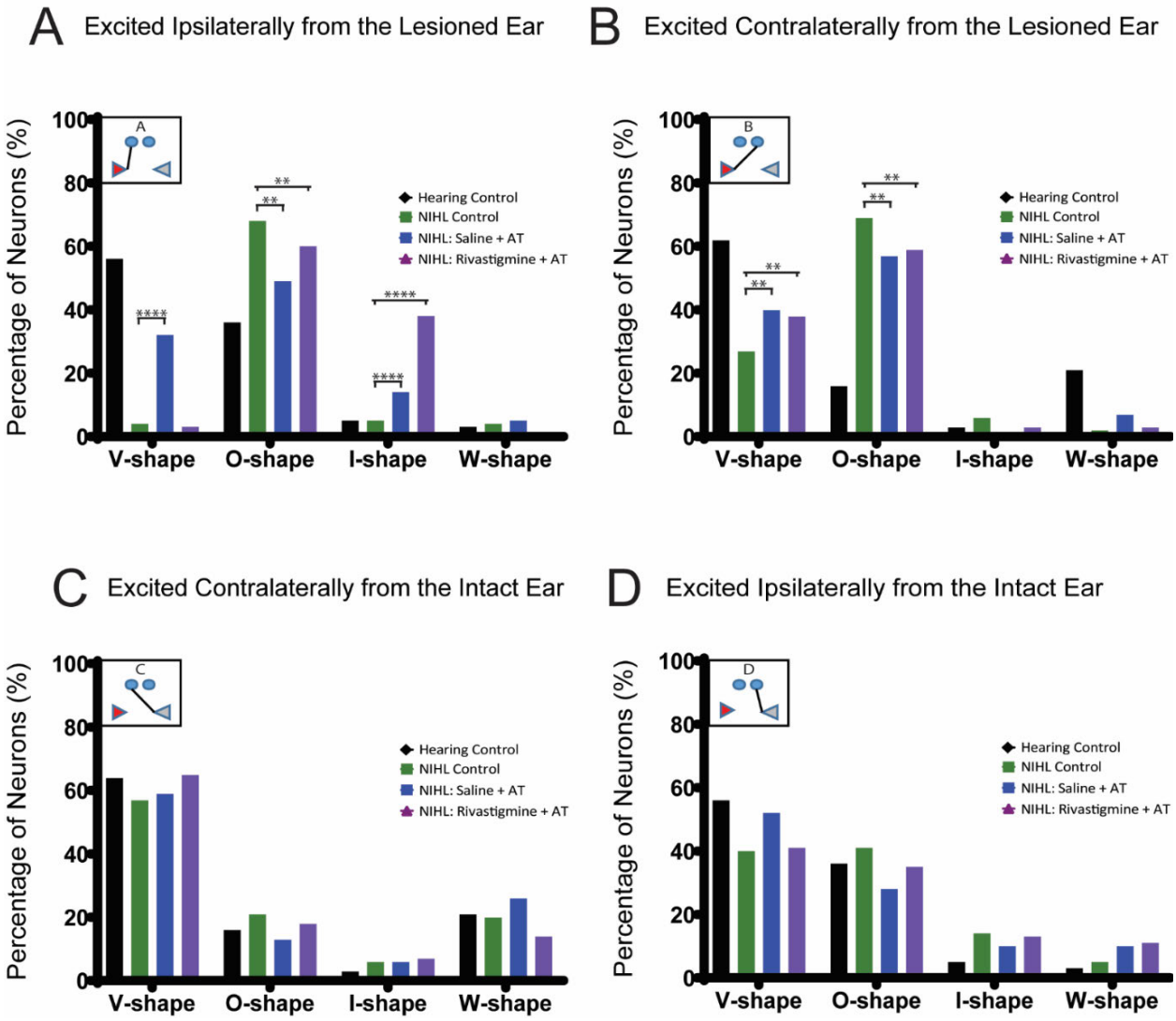

Figure 4.4. The percentage of IC neurons with a type of FRA profile under different stimulus conditions. (A) In IC neurons excited ipsilaterally from the lesion ear, AT+ saline treatment (blue) increases the percentage of IC neurons with a V-shaped FRA and AT + Rivastigmine treatment (purple) increase the percentage of neurons with an I-shaped FRA, when compared to UNIHL control (green)($p < 0.0001$). (B) In IC neurons excited contralaterally from the lesioned ear, as a result of AT (blue & purple) there was a proportional increase of V-shaped FRAs, when compared to UNIHL control (green)($p = 0.004$).

4.4.3.1 Cumulative Frequency of CF of V-shaped FRAs

The cumulative frequency of the CFs of V-shaped FRAs of IC neurons were placed into 4kHz bins. The data were then plotted as cumulative frequencies. A statistical analysis was done using a Friedman test and a Dunn's multiple comparisons test.

In IC neurons excited ipsilaterally from the intact ear (Fig.4.5D), there were significant differences in the CFs of V-shaped FRAs of IC neurons, in AT cohorts (purple & blue) when compared to UNIHL control (green)($p=0.0007$). As a result of AT, there was a left sided shift in the CF distribution which followed a very similar trend line to that of the normal hearing cohort (black). There were no statistical differences in the CFs of V-shaped FRAs between the AT cohorts (purple & blue) and the hearing control cohort (black) ($p>0.05$).

In IC neurons excited contralaterally from the lesioned ear (Fig.4.5B), there were no statistical differences between the UNIHL groups (blue, purple & green), however compared to hearing control (black) there was a significant left side shift in the CFs of V-shaped FRAs ($p=0.0029$).

In IC neurons excited ipsilaterally from the lesioned ear (Fig.4.5A), there were insufficient V-shaped FRAs within the UNIHL control and UNIHL: Rivastigmine + AT cohorts to make a statistical comparison. In IC neurons excited ipsilaterally from the intact ear (Fig.4.5C), there was no significant differences between the three UNIHL groups and normal hearing animals ($p>0.05$).

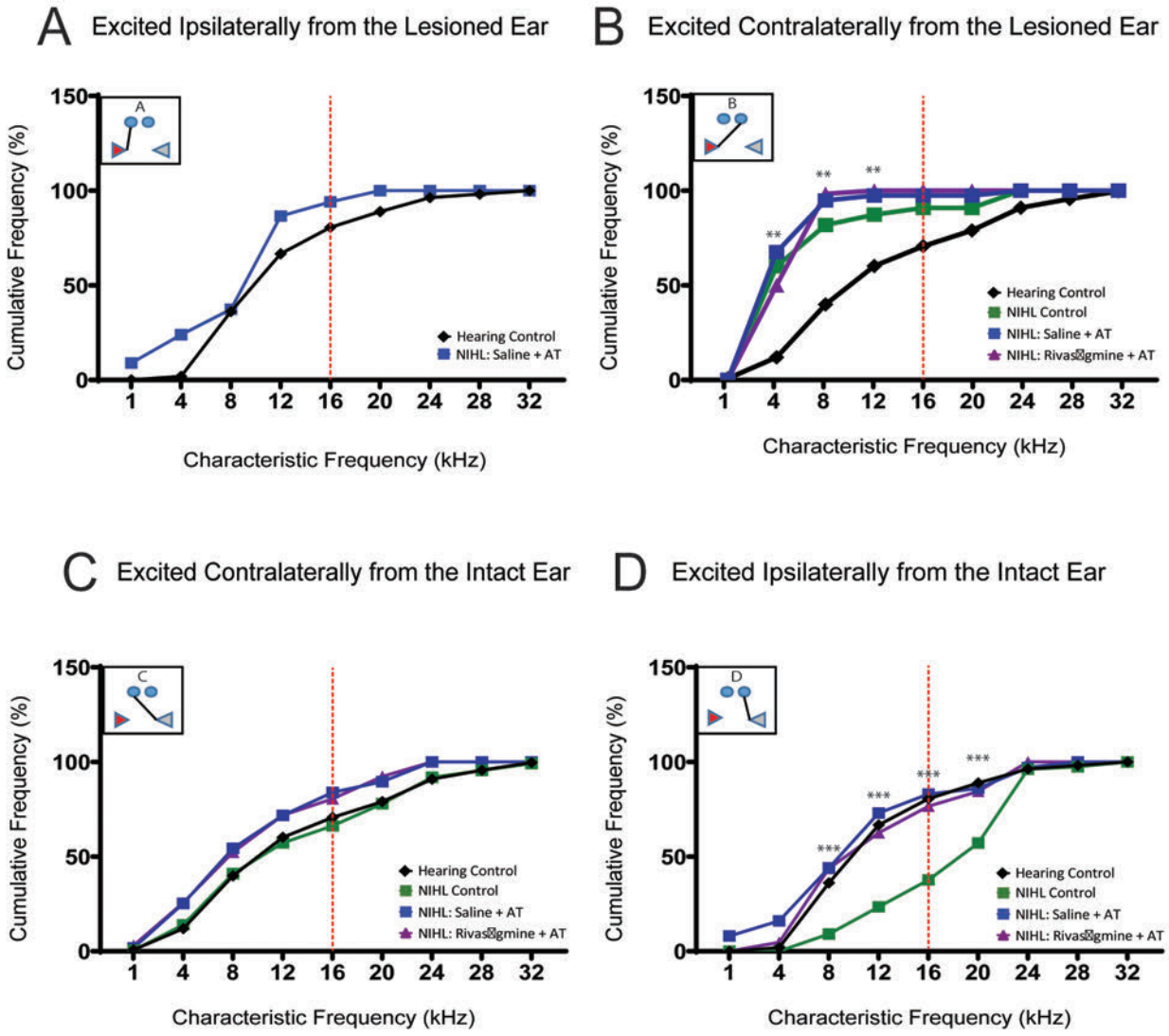

Figure 4.5. The cumulative frequency of CFs of IC neurons with a V-shaped FRA, excited by (A&B) the lesioned ear (>) and from (C&D) the intact ear (<). The red dotted line illustrates the lesion frequency of 16kHz. (D) In IC neurons that were excited ipsilaterally from the intact ear, there were significant left sided shifts of the AT groups (blue & purple) when compared to UN-HIL control (green)(p=0.0007) and there were no differences between AT groups (blue & purple) and hearing control (black) (p>0.05).

4.4.3.2 Cumulative Frequency of CF of O-shaped FRAs

The cumulative frequency of the CFs of O-shaped FRAs of IC neurons were placed into 4kHz bins. The data were then plotted as cumulative frequencies. A statistical analysis was done using a Friedman test and a Dunn's multiple comparisons test.

In IC neurons excited contralaterally from the intact ear (Fig.4.6C), there were significant differences in the CFs of the O-shaped FRAs of IC neurons, when the AT + Rivastigmine treated animals (purple) were compared to UNIHL control animals (green) ($p=0.0013$). There was significant left sided shift, which followed a similar trend line to that of normal hearing control animals (black). Between the AT + Rivastigmine treated cohort (purple) and the hearing control cohort (black), there were no statistical differences in the trend lines above the 8kHz category ($p>0.05$).

In IC neurons excited contralaterally from the lesioned ear (Fig.4.6B), there were no statistical differences in the cumulative frequency of CF of O-shaped FRAs between the UNIHL groups (blue, purple & green), however when compared to normal hearing control animals (black) there is a significant left sided shift ($p=0.0065$). In IC neurons excited ipsilaterally from the lesioned ear (Fig.4.6A) and the intact ear (Fig.4.6D), there were no differences in the cumulative frequency of CF of O-shaped FRAs between the UNIHL cohorts when compared to normal hearing control groups ($p>0.05$).

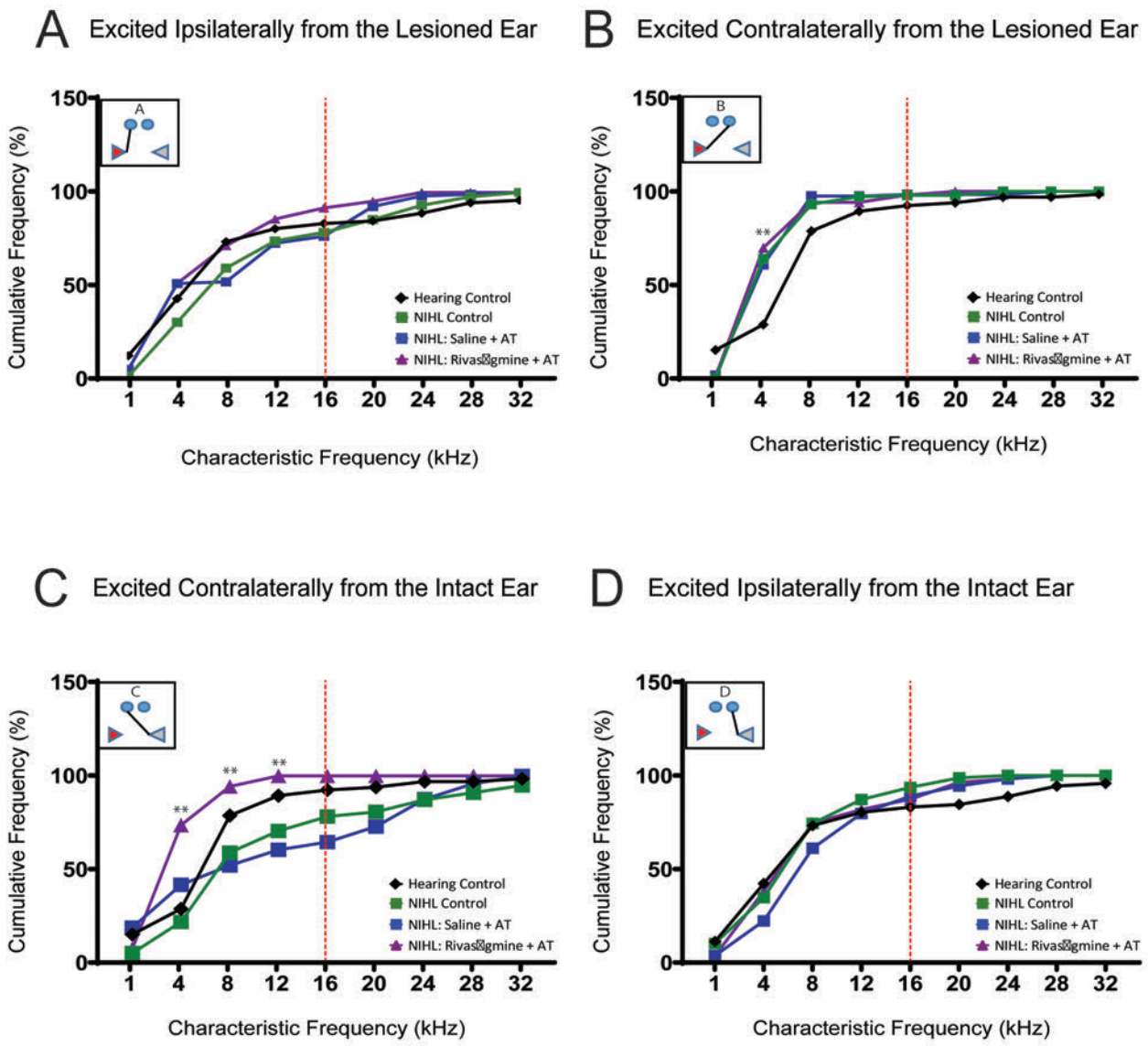

Figure 4.6 The cumulative frequency of CFs of IC neurons with a O-shaped FRA, excited from (A&B) the lesioned ear (>) and from (C&D) the intact ear (<). The red dotted line illustrates the lesion frequency of 16kHz. (C) In IC neurons that were excited contralaterally from the intact ear, a significant left-sided shift was seen in the AT + Rivastigmine cohort (purple), when compared to AT+ saline and UNIHL control (p=0.0013).

4.4.4 Binaural Proportions Profile

The percentage of IC neurons with a binaural interaction was calculated based on the number of IC neurons showing the type of binaural interaction, over the total number of IC neurons recorded under each stimulus condition. Response categories were organised into excitatory-excitatory (EE/$(EE)^{-1}$), excitatory-inhibitory (EI/$(EI)^{-1}$) and excitatory-null (EO/$(EO)^{-1}$) binaural response interactions. Statistical analysis was done using chi-squared tests.

In IC neurons excited ipsilaterally from the lesioned ear (Fig.4.7A), there were significant differences in the proportion of binaural interactions when AT groups (blue & purple) were compared to UNIHL control (green)($p<0.0001$). When compared to UNIHL control, AT cohorts showed there was a 20-23% decrease of IC neurons with an EE^{-1} response. This result was accompanied with a 9% increase of the EI^{-1} interactions in AT groups. There was no statistical differences between the AT groups.

In IC neurons excited ipsilaterally from the intact ear (Fig.4.7D), there were significant differences when AT groups (blue & purple) were compared to UNIHL control (green) ($p <0.0001$). Overall when compared to the UNIHL control, as a result of AT + saline or Rivastigmine treatment, there was a 34-36% increase in the $(EI)^{-1}$ interactions. There were no statistical differences between the AT groups ($p>0.05$).

In IC neurons excited contralaterally from the lesioned ear (Fig.4.7B) and the intact ear (Fig.4.7C), there were significant differences when AT groups (purple & blue) where compared to UNIHL control (green). As a result of AT, an increase in EI interactions ($p<0.0001$) were seen. In Rivastigmine treated animals (purple), in contralaterally excited IC neurons, 80-81% of these neurons showed an EI interaction, whether it was stimulated from the intact (Fig.4.7C) or lesion ear (Fig.4.7B). In contrast, saline treated animals

(blue) showed 96% of IC neurons with an EI interaction, when excited from the intact ear (Fig.39C) and 74% of IC neurons showed this interaction, when stimulated from the lesioned ear (Fig.39B). This suggests Rivastigmine results in a more even percentage of EI interactions between the left and the right ICs.

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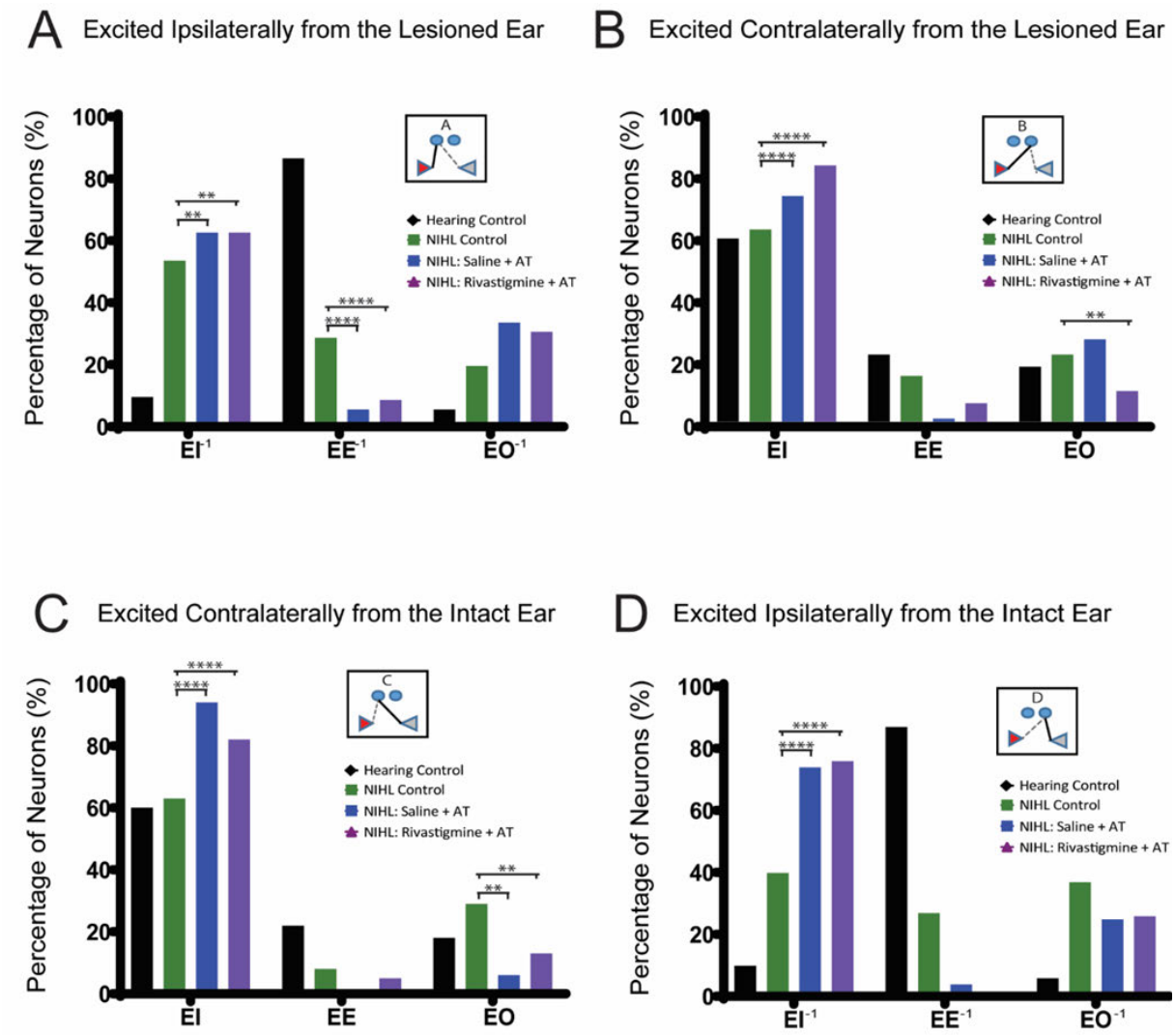


Figure 4.7. The binaural response profile of IC neurons excited (A&B) by the lesioned ear (>) and (C&D) by the intact ear (<). There were significant proportional increases of the excitatry-inhibitory (EI/ $(EI)^{-1}$) binaural interaction under all stimulus conditions (A,B,C &D), when compared to UNIHL control. The greatest increase was seen in (D) IC neurons excited ipsilaterally from the intact ear ($p<0.0001$).

4.4.5 Percentage of Monotonic and Non-monotonic Responses

The data were analysed based on the number of IC neurons with a monotonic or non-monotonic response, over the total number of neurons recorded under each stimulus condition. Statistical analysis was done using chi-squared tests.

In IC neurons excited ipsilaterally from the lesioned ear (Fig.4.8A, $p=0.0249$) and in IC neurons excited contralaterally (Fig.4.8C, $p=0.013$) and ipsilaterally from the intact ear (Fig.40D, $p<0.0001$), there were significant differences in the monotonicity proportions seen at CF between the UNIHL control (green) and AT + saline treated cohorts (blue). As a result of AT + Saline treatment (blue), there were increases of the non-monotonic response at CF. This affect was not seen in the AT + Rivastigmine treated cohort (purple).

In IC neurons excited contralaterally from the lesioned ear (Fig.4.8B), there were significant differences between UNIHL control (green) and AT cohorts (purple and blue) ($p=0.0009$). In these IC neurons, in the AT cohorts, monotonic responses at CF was the dominant response.

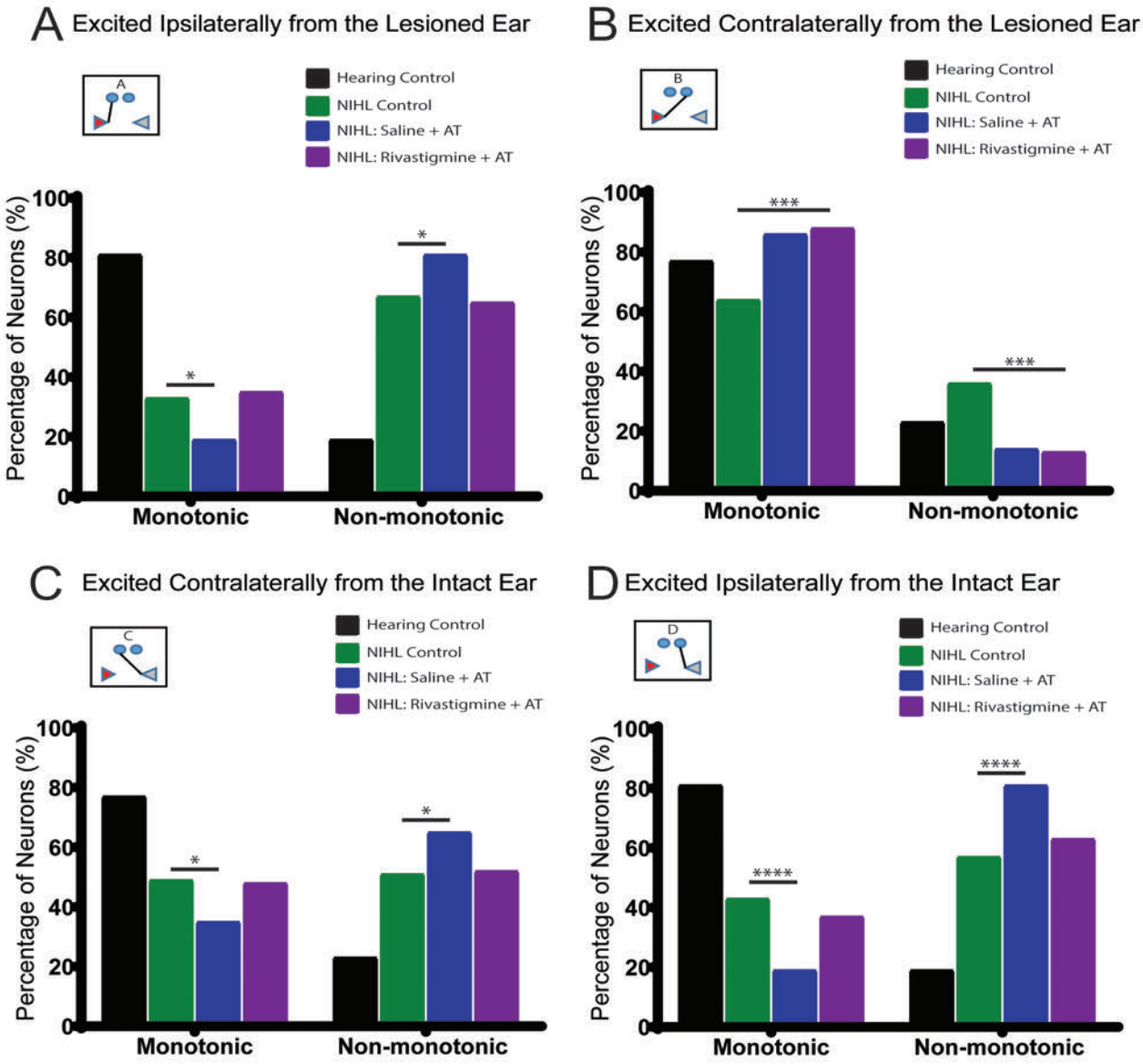


Figure 4.8. The percentage of IC neurons with a monotonic or non-monotonic response at CF, excited from (A&B) the lesioned ear (>) and from (C&D) the intact ear (<). There were significant increases of the non-monotonic response at CF, in AT + saline treated cohorts (blue) when compared to UNIHL control (green) (A, C&D, $p<0.05$). This effect was not seen in (B) IC neurons contralaterally excited from the lesioned ear.

4.4.6 Percentage of Tonic and Onset Responses

The data was analysed based on the number of IC neurons with an onset or tonic response, over the total number of neurons recorded under each stimulus condition for each group. Statistical analysis was done using chi-squared tests.

In IC neurons excited contralaterally from the lesioned ear (Fig.4.9B; $p<0.0001$) and the intact ear (Fig.4.9C; $p=0.002$), there were significant differences in the PSTH response at CF, when AT treated cohorts (blue and purple) were compared to NIHL control cohort (green). When compared to UNHL control, AT resulted in a significant increase of IC neurons with a tonic response at CF. In IC neurons excited contralaterally (Fig.4.9B&C), there are no statistical differences between the AT cohorts and normal hearing control animals ($p>0.05$).

In IC neurons excited ipsilaterally from the lesioned (Fig.4.9A) and the intact (Fig.4.9D) ears, there were no statistical differences between the UNIHL control (green) and AT groups (blue & purple) ($p>0.05$).

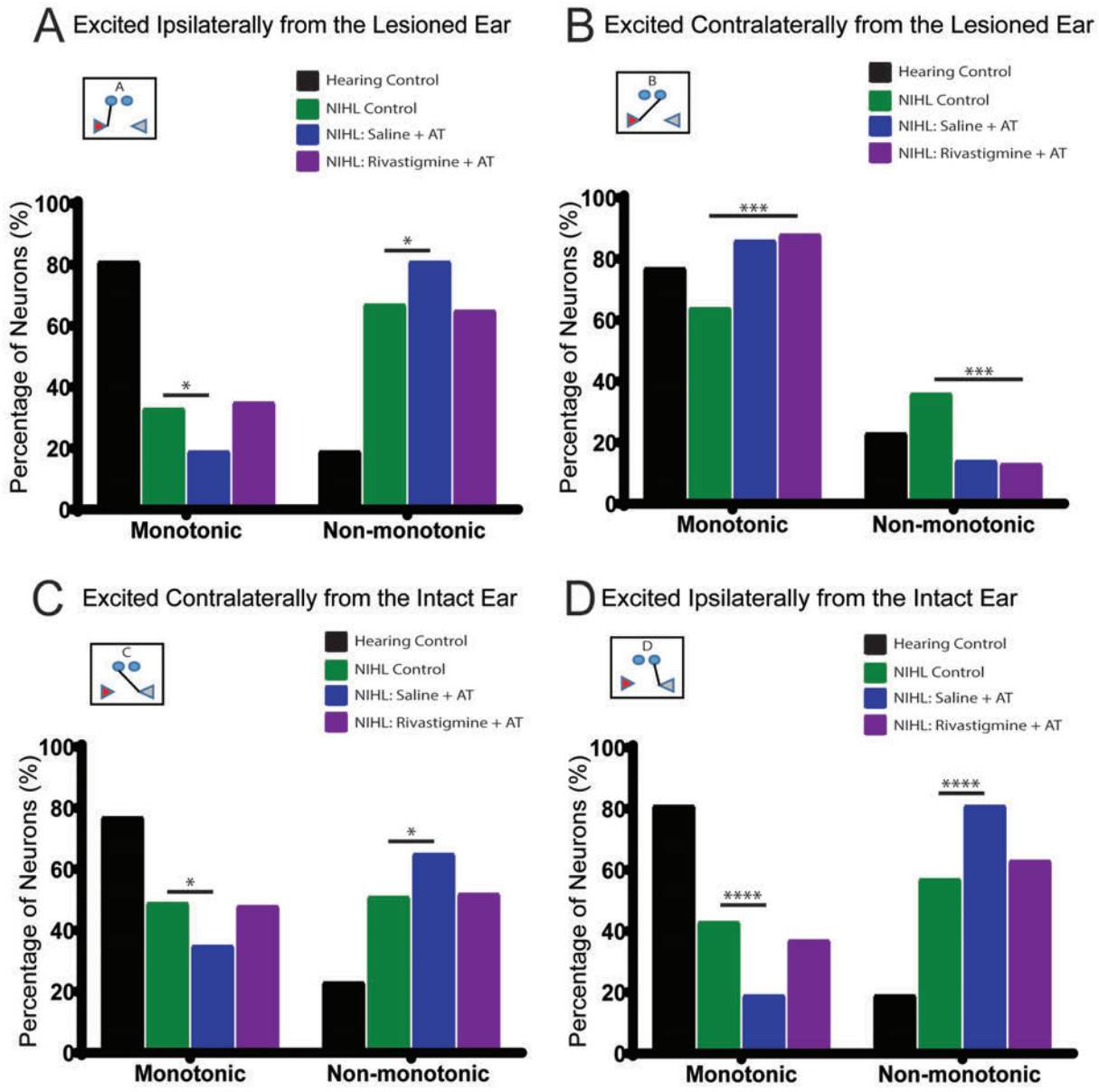


Figure 4.9. The percentage of IC neurons with an onset or tonic PSTH response at CF, excited from (A&B) the lesioned ear (>) and from (C&D) the intact ear (<). In IC neurons excited contralaterally from the lesioned (B; p <0.0001) and intact (C; p=0.0021) ears, there were significant proportional increases of the tonic response at CF, when AT treated cohorts (blue and purple) when compared to UNIHL control (green).

4.5 Discussion

In this chapter, the hypothesis tested that increasing acetylcholine levels will increase the malleability of IC neurons and in turn this mallability can be influenced by controlling auditory stimuli. In addition, this can be guided by manipulating the animal's acoustic environment. In this study, three major consequences were observed; 1) In the response properties analysed, AT profoundly affect contralaterally excited IC neurons, 2) Paired AT and cholinergic enhancement appeared to only affect selected populations of ipsilaterally excited IC neurons.; and 3) AT and Rivastigmine resulted in an even percentage of binaural EI interactions within the left and right ICs (Fig.4.10).

4.5.1. The Effect of Auditory Training

Consistent with other studies, the results of this study showed that the auditory environment an animal is exposed to immediately after an intervention, can influence response properties of auditory neurons (Noreña & Eggermont, 2005; 2006; Pienkowski & Eggermont, 2009; 2012; Voss et al., 2016). Indeed, in IC neurons contralaterally excited from the lesioned ear (exposed), a significantly higher proportion of CFs between 4 and 8 kHz, was found in the AT cohort when compared to UNIHL control animals (Fig.4.2B). However, between the Rivastigmine treated and saline treated cohorts, there were no statistical differences.

A defining characteristic of UNIHL is a significant loss of IC neurons displaying V-shaped FRAs accompanied by a significant increase in O-shaped FRAs (Fig.4.4). It was hypothesised, that significant changes in the proportions of FRA types would be observed with AT and cholinergic enhancement. In IC neurons contralaterally excited from the lesioned ear (exposed), the AT cohorts showed a significantly higher proportion of V-shaped FRAs, accompanied with a significantly lower proportion of O-shaped FRAs when compared to UNHIL control animals (Fig.4.4B). Similarly, to the CF distribution

results, between the Rivastigmine treated and saline treated cohorts, there were no statistical differences.

In IC neurons excited contralaterally, as a result of AT there was a significant recovery of the tonic response at CF (Fig.4.9B&C). In IC neurons excited from the lesioned ear (exposed) and the intact ear (plugged), the proportions were indeed comparable to that of normal hearing control animals. In these neurons between normal hearing animals and AT cohorts, there were no statistical differences in this response property. This suggests the influence of plugging the intact ear and stimulating the lesioned ear resulted in a PSTH response profile closer to that of normal hearing animals. Similarly, in the CF distribution results and consequential FRA proportions described previously, between the Rivastigmine treated and saline treated cohorts, there were also no statistical differences.

Several studies that combined microiontophoretic injections of acetylcholine antagonists or agonists with in vivo electrophysiological recordings in the IC, have shown that over 80% of IC neurons were responsive to acetylcholine (Watanabe & Simada, 1973; Farley et al., 1983; Habbicht & Vater, 1996). In this study, it was observed that IC neurons excited contralaterally, were not sensitive to cholinergic enhancement and the effects seen were primarily due to AT. A difference seen in the effectiveness of acetylcholine, may be due to the different means of cholinergic enhancement, the extended duration of the Rivastigmine treatment or the response properties measured were not sensitive enough to capture differences between the saline and Rivastigmine treated cohorts. Indeed, previous authors have only reported their findings based on an acute response to acetylcholine. The results of this present study suggest that in IC neurons that were excited contralaterally, a clinically relevant dose of an acetylcholinesterase inhibitor given for four weeks did not influence the response properties analysed. They were however dramatically influenced by four weeks of AT, and this influence is possibly mediated by a different neurotransmitter. To date, this is the first study to show in an UNIHL animal model, AT resulted in a significant change in the response properties of contralaterally excited IC neurons.

4.5.2. Effect of Rivastigmine Treatment

Peripheral hearing loss manifests due to cochlear damage and this damage is irreversible. Cochlear cells that are damaged or have underlying neuropathy do not regenerate (Liberman & Dodds, 1984; Hickox & Liberman, 2014; Roberts, 2018). As a result of UNIHL, IC neurons excited from the lesioned ear showed an increase in threshold (Fig.4.3A&B) and the CF distribution resulted in a left-sided shift towards lower frequencies (Fig.4.2A&B). These central characteristics indicate hearing loss to one side. Motts and Schofield (2009) reported that there is no cholinergic input to the IC that originate from nuclei that are from the 'traditional' ascending auditory pathway. Therefore, in line with these molecular results, it is unlikely that Rivastigmine treatment would affect ascending signal processing like hearing sensitivity and rescue normal hearing thresholds on the effect side.

The IC receives most of its cholinergic input from the auditory cortex via PPT and PPD (Motts & Schofield, 2009; Schofield, 2010). It is likely that Rivastigmine would influence this cholinergic descending pathway and therefore a difference would likely occur in response properties that are sensitive to descending modulations. In Saline and Rivastigmine treated animals in IC neurons excited ipsilaterally from the lesioned ear, a differential in the resultant proportions of FRA types was observed (Fig.4.4A). Both groups showed a significant decrease in the O-shaped FRA when compared to UNIHL control, an effect possibly due to AT. However, saline treatment resulted in a significant proportional increase in the V-shaped FRAs and Rivastigmine treatment resulted in a significant proportional increase of the I-shaped FRAs. In IC neurons excited contralaterally (Fig.4.4B), the differential effect is not seen and instead both Saline and Rivastigmine groups show a significant increase in the V-shaped FRA. A rationale for this ipsilateral and contralateral difference may be due to the density of cholinergic projections. Schofield's study (2010) reported more A1/ (PPD/PPT)/ IC contacts were found ipsilaterally when compared to contralateral contacts, suggesting that physiologically there is possibly more

'scaffolding' present in the descending ipsilateral pathway to pharmacologically exploit.

Palmer et al. (2013) in their influential paper described FRA shapes form a continuum with many IC neurons having receptive fields that are intermediate to categorical extremes. In consideration to the continuum, V-shaped FRAs (V) and I-shaped FRAs (N) are similar in shape and a significant number of VN-like FRAs fall between the two classes of FRAs. Briefly, V-shaped FRAs are considered the 'classical' response and non-V-shaped FRAs are responsible for more complex aspects of sound processing. Palmer et al. (2013) report that FRAs correspond to different inputs of different strengths and frequency disposition. FRAs that are I-shaped can be interpret as 'tighter' V-shapes, only responding to a narrow range of frequency-SPL combinations. The results in this present study showed as a differential of Rivastigmine treatment, there is a higher population of IC neurons ipsilaterally excited from the lesion ear that are tighter in the tuning. It can be interpreted that this affect is not seen in IC neurons that are contralaterally driven due to a lower density of descending cholinergic contacts (Schofield, 2010).

Interestingly, instead IC neurons that were excited contralaterally from the intact ear, O-shaped FRA showed a significant left-sided shift in the AT + Rivastigmine cohort (purple), when compared to AT+ saline and UNIHL control (Fig 4.6). This suggests increase sensitivity to lower frequencies due to Rivastigmine treatment. This effect in O-shaped neurons is perhaps only seen in the intact ear, due to fact there are undamaged cholingeric inputs in this pathway and that O-shaped FRAs are normally cholinergically influenced. Using this animal model, further in vivo molecular techniques that can quantify levels of cholinergic activity paired with simultaneous in vivo multi-electrode array recordings of A1 and IC, will need to be performed in order to confirm if this contralateral and ipsilateral differential is indeed occurring due to acetylcholine.

In an animal model of UNIHL, cholinergic enhancement paired with AT does not 'rescue' hearing sensitivity. Of the traditional ascending auditory nuclei that have projections that converge into the IC, none have been reported to be cholinergic (Motts & Schofield, 2009; Schofield & Motts, 2009; Schofield, 2010). Therefore, cholinergic enhancement will likely only affect response properties sensitive to descending cholinergic projections. In IC neurons that were excited ipsilaterally from the lesion ear, there was a higher proportion of I-shaped FRAs as a result of paired cholinergic enhancement and AT. This was not achieved from AT alone. It is possible, that descending projections from the A1 via cholinergic projections from PDT/PPT nuclei are driving the tighter and more specific tuning properties of ipsilaterally excited IC neurons. This result shows in an UNIHL animal model, AT paired with cholinergic enhancement can significantly influence ipsilaterally excited IC neurons.

4.5.3. Binaural Interactions

It is well documented that hearing loss disrupts normal processing of excitatory and inhibitory interactions. The disruption is due to a loss of peripheral input which results in central manifestations, associated with central compensatory patterns (Eggermont & Roberts, 2004; Saunders, 2007; Roberts et al., 2010; Roberts, 2018). It is well known that the dominant contralateral afferent projections are primarily excitatory and the non-dominant ipsilateral afferent projections are primarily inhibitory (Nordeen et al., 1983; Semple & Kitzes, 1985; Hutson, 1997; McAlpine et al., 1997). As a consequence of AT, there were increased proportions of IC neurons exhibiting EI interactions (Fig.4.7). The dominant EI binaural interaction was seen in IC neurons excited from either the intact or lesioned ear, contralaterally or ipsilaterally. A possible explanation of this increased response is due to the timeframe in which these results were captured. Four weeks post the acoustic trauma is considered acute. Indeed, previous authors have shown an eight- week time point in the IC, is most the appropriate time point to record the long term chronic consequences of an

acoustic trauma where the resultant activity is more likely due to central manifestations (Salvi et al., 1979; Mulders & Robertson, 2009; Dong et al., 2010; Wang et al., 2013).

The results of this present study suggest, where compared at the 12-week time point, there are significant increases of the EI interaction in IC neurons. It is not possible with the result of this present study, to attribute the increased EI binaural response property to AT or the temporal changes that occur at four weeks. However, what can be inferred is that Rivastigmine differentially affected the binaural interactions of contralaterally driven IC neurons at the four-week time point (Fig.4.7B&C). Rivastigmine treatment paired with auditory training resulted in IC neurons that were contralaterally excited from the lesioned and intact ear to have almost equal proportions of EI binaural interactions, 81% and 80%, respectively (Fig.4.7B&C). In contrast, the same training but with saline, 94% and 74% of IC neurons that are contralaterally excited from the lesioned and intact ears showed an EI binaural interaction, respectively. That is a 20% difference of EI interactions in the left and right IC, that occurred in these animals when sound was introduced to both ears. From the results, AT without a cholinergic enhancement resulted in an unbalanced proportion of the dominant EI binaural interaction of IC neurons excited contralaterally.

The equal population of IC neurons that responded with EI interactions contralaterally from lesioned and the intact ears may be physiologically significant. It may indicate that the system processes inhibitory interactions equally whether sound predominantly arrives to the lesioned or intact ears. This is particularly significant as there is indeed, profound hearing loss to the left side of these animals.

4.5.4. Conclusion

By measuring a battery of response characteristics of IC neurons, it was observed that AT profoundly affects contralaterally driven IC neurons. Specifically, in these neurons there was a significant shift in the CF towards the 4 and 8 kHz training tones (Fig.4.2B), a higher proportion of V-shaped FRAs (Fig.4.4B) and higher proportion of tonic responses at CF (Fig.4.9B&C). However, between, the Rivastigmine treated and saline treated cohorts there were no statistically significant differences suggesting that these response properties in contralaterally driven IC neurons are unlikely to be modulated by descending cholinergic neurons. It is possible the response properties analysed were not sensitive enough to capture the differences in this neuron population and a more acute measure is required.

In contrast, cholinergic enhancement has its greatest effect on IC neurons ipsilaterally excited, possibly owing to the higher density of cholinergic scaffolding from descending nuclei (Schofield, 2010). In ipsilaterally excited IC neurons, as a consequence of paired AT and cholinergic enhancement there were significant decreases of the O-shaped FRAs accompanied with a higher proportion of the narrower and tighter I shaped FRAs (Fig.4.4A). Furthermore, AT with cholinergic enhancement resulted in equal proportions of the dominant EI binaural interaction in both left and right ICs (Fig.4.7B&C). This equal proportion of EI interactions is particularly interesting, as it is within the context of profound hearing loss to the left side (Fig.4.2A&B; Fig.4.3A&B). It is possible that increasing cholinergic activity promotes ipsilateral inhibitory pressure to be equal in both the left and right IC, irrespective if the intact or lesioned ear were being stimulated. These results add to the growing body of evidence that suggests cholinergic modulation generally acts to optimise the processing of signals in attention demanding contexts (Hasselmo & McGaughy, 2004; Sarter et al., 2005; Jääskeläinen et al., 2007; Ayala & Malmierca, 2015).

The results of this present study may have implications for the development of therapies for tinnitus. The finding that cholinergic enhancement paired with AT results in 'favourable' response characteristics suggests a potential descending pathway to exploit, even within the context of irreversible and profound peripheral hearing loss. Future studies investigating the neuroplastic capacity of IC neurons in the context of cholinergic enhancement may provide better insight to potential therapeutic targets.

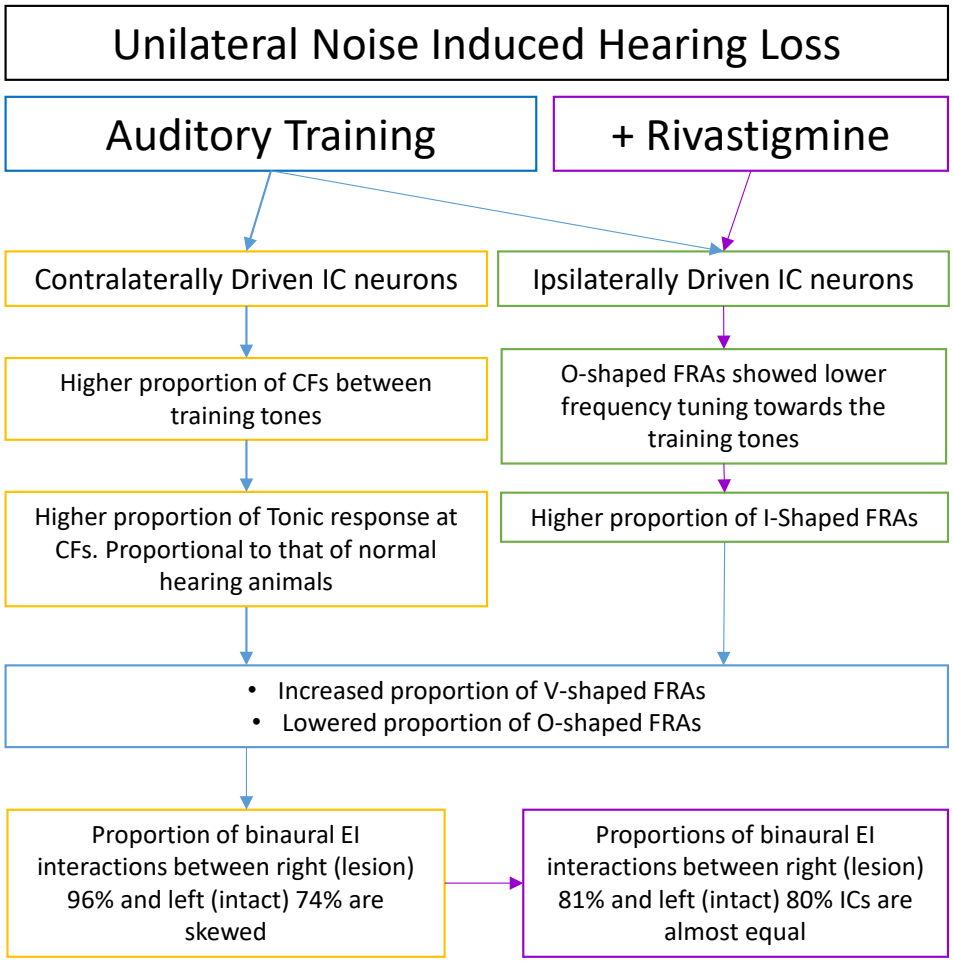


Figure 4.10. A schematic flowchart describing the influence of Auditory Training and Rivastigmine treatment in the response properties of IC neurons within an animal model of unilateral noise induced hearing loss. In an animal of UNHIL, the response properties of contralaterally excited IC neurons (yellow boxes) are greatly influenced by auditory training (blue & arrows box). When AT is paired with Rivastigmine (purple & arrows boxes), the binaural profile contralaterally excited IC neurons of resulted in a more equal proportion of the excitatory-inhibitory response. Whilst response properties of ipsilaterally excitatory IC neurons (green boxes) are observed to be more influenced by AT paired with Rivastigmine. Both neuron populations showed similar consequentical proportions of V-shaped and non V-shaped FRAs as a result the AT intervention.

Chapter 5
Stimulus-Timing Dependent Plasticity in the Inferior Colliculus

5.1. Introduction

The adult central auditory system has an extraordinary capacity to be manipulated, which can be controlled by regulating the input of a stimulus (Rauschecker, 1999; Saunders, 2007; Shepard et al., 2013; Roberts, 2018). Unilateral noise-induced hearing loss (UNIHL) is one means to induced malleability and to investigate the central plastic consequences due to the damage of the peripheral sensors. It has been shown that UNIHL can alter the response properties of the central auditory neurons (Szczepaniak & Moller, 1996; Meltser & Canlon, 2010; Browne et al., 2012; Furman et al., 2013; Manzoor et al., 2013; Wang et al., 2013; Coomber et al., 2014; Hickox & Liberman, 2014). These changes may be due to the types of plasticity associated with the formation and elimination of synapses, synaptic strengthening and weakening or both, but at different locations within the central auditory system.

One means to control synaptic capacity is by regulating the timing of pre-synaptic and post-synaptic activity. In associative plasticity, rapid sequential stimulation of two synaptically connected neurons leads to a change in the strength between the synapse (Wigström et al., 1986; Lisman, 1989; Bröcher et al., 1992). When pre-synaptic spikes precede the post-synaptic spike by up to 10 milliseconds synaptic transmission is strengthened, and the inverse activity weakens the synaptic strength. This phenomenon has been termed spike-timing dependent plasticity and has been demonstrated in numerous species, in different neuronal systems and within different neuronal cell types (Markram et al., 1997; Zhang et al., 1998; Caporale & Dan, 2008; Feldman, 2012).

Spike-timing-dependent plasticity was initially investigated in vitro with acute brain slices and cell cultures (Markram et al., 1997; Zhang et al., 1998). In this closed system, current injections precisely controlled the timing and order of pre-synaptic and post-synaptic stimulations. Efforts to control neuronal activity by regulating sensory input in vivo have shown that stimulus-timing dependent plasticity (STDP) alters response properties in a similar manner that is seen in vitro with spiked timed dependent plasticity. Although the manner of changes are similar in these two systems, the expression of these changes seen in vivo are different (Dahmen et al., 2008).

Stimulus-timing dependent plasticity has been implicated as an attractive mechanism underlying the plastic changes undergone by neurons in a deprived system. In a deprived system, STDP in vivo has been investigated in the rat somatosensory (Celikel et al., 2004) and cat visual cortices(Young et al., 2007). In line with the Hebbian principle of neuroplasticity, these studies showed precisely timed sensory stimuli can drive receptive field plasticity and changes in response properties that involve single neurons as well as networks of neurons via STDP-like mechanisms (Celikel et al., 2004; Young et al., 2007; Caporale & Dan, 2008).

In the auditory system, STDP has been demonstrated using paired frequency tones and paired somatosensory and auditory stimulation (Dahmen et al., 2008; Basura et al., 2015). Specifically, areas that have been studied include the auditory cortex (A1) (Dahmen et al., 2008) and dorsal cochlear nucleus (DCN)(Tzounopoulos et al., 2004a). To date, there are no studies investigating STDP in the inferior colliculus (IC) in an animal model of UNIHL.

In the previous chapter, the results indicated that immediately after UNIHL, subsequent auditory training (AT) resulted in significant changes in the response properties of IC neurons that were excited contralaterally from the lesioned ear. In this population of neurons, cholinergic enhancement did not further promote this effect. However, several studies that combined microiontophoretic injections of acetylcholine antagonists or agonists with in vivo electrophysiological recordings in the IC, have shown that over 80% of IC neurons are responsive to acetylcholine (Watanabe & Simada, 1973; Farley et al., 1983; Habbicht & Vater, 1996). A difference seen in the effectiveness of acetylcholine may be due to the means by which the results were captured. It is possible that the response properties analysed in the previous chapter were not sensitive enough to capture the differences and a more acute test is required. Indeed, past studies have reported findings within an acute context. With a consideration to an acute measure, an exploration of the STDP capacity of this system may give insight to the ability of the synapses to adapt acutely and whether or not this adaptation is sensitive to cholinergic enhancement.

5.2 Hypothesis

The physiologically realistic induction requirements of STDP meant that further testing could easily be done in addition to the previous chapter's experimental design. The acute capacity of STDP was opportunistically explored using paired frequency tones in the UNIHL animal model delivered to the intact ear. The hypothesis was that auditory training paired with Rivastigmine treatment would result in a cholinergic primed system that could acutely adapt to STDP conditioning. To determine if there is indeed an interaction between STDP conditioning and the cholinegric system within the IC, the intact dominate pathway was opportunistically used.

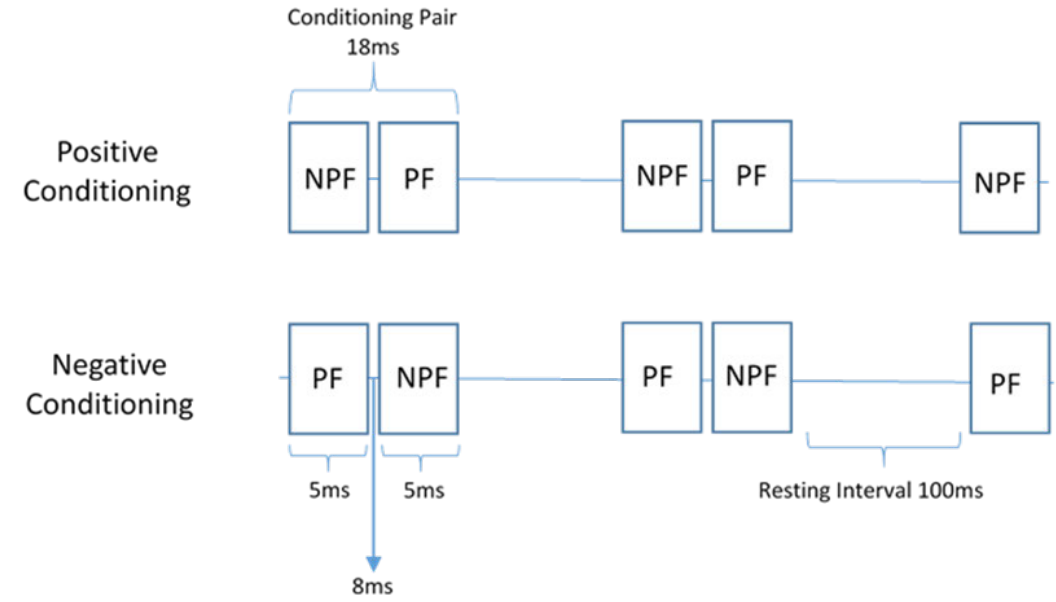
5.3. Methods

Animal Preparation: A total of six adult male Wistar rats were used in this study, three were treated with Rivastigmine and three with saline. The Rivastigmine and saline delivery procedures were performed as previously described in Section 4.3.2. The auditory brainstem response (ABR) test, surgery, in vivo electrophysiology experimental set-up, data collection, histology for electrode tracking and confocal imaging were done as described in Chapter Two. An ABR determined that hearing thresholds were normal in the intact ear of all the animals. The intact ear was stimulated and the contralateral IC was recorded and analysed.

5.3.1. Stimulus presentation and Electrophysiological recording

The conditioning tones were determined based on the greatest number of characteristic frequencies (CF) that fell within a particular frequency range. From the results of Chapter Four, it was determined that the greatest concentration of CFs were between 6 and 14kHz. A pre-conditioning map recording confirmed the number of IC neurons clustered with CFs between those two frequencies. This was followed with the conditioning stimulus. The conditioning stimulus protocol is adapted from Dahmen et al. (2008) investigating STDP in the A1 of ferrets. The conditioning stimulus consisted of an 8 and a 12 kHz pure tone presentation for 5 ms, respectively, spaced by 8 ms, with a resting interval of 100ms for 71 secs, that is, a total of 600 pairs of pure tones presented at 50 dB SPL (Fig.5.1A). Three mapping recordings were done post-stimulus. The first two were 15 minutes apart and the third map was completed one-hour post stimulus condition (Fig.5.2). A positive or negative condition for a neuron was categorised based on the relationship of the CF to the two tones. If, the first 8 kHz tone was a non-preferred tone and the second 12kHz tone was the preferred tone, this neuron cluster was categorised as having been subjected to positive conditioning. If, the first 8 kHz tone was a preferred tone and the second 12kHz tone was a non-preferred tone this neuron cluster was categorised as having been subjected to negative conditioning (Fig.5.1B).

A



B

CF	Fq of 1st Stimulus (8kHz)	Fq of 2nd Stimulus (12kHz)	Conditioning
8	Preferred	Non-Preferred	Negative
12	Non-Preferred	Preferred	Positive

Figure 5.1. Conditioning stimuli and conditioning categories. (A) Schematic of conditioning stimuli. Pairs of conditioning stimuli consisted of two pure tones of 8 and 12 kHz, respectively for 5 ms and 8 ms apart. Each conditioning pair where separated by a resting interval of 100ms. Each conditioning block consisted of 600 conditioning pairs. (B) A positive or negative condition for a neuron was categorised based on the relationship of the CF to the two tones.

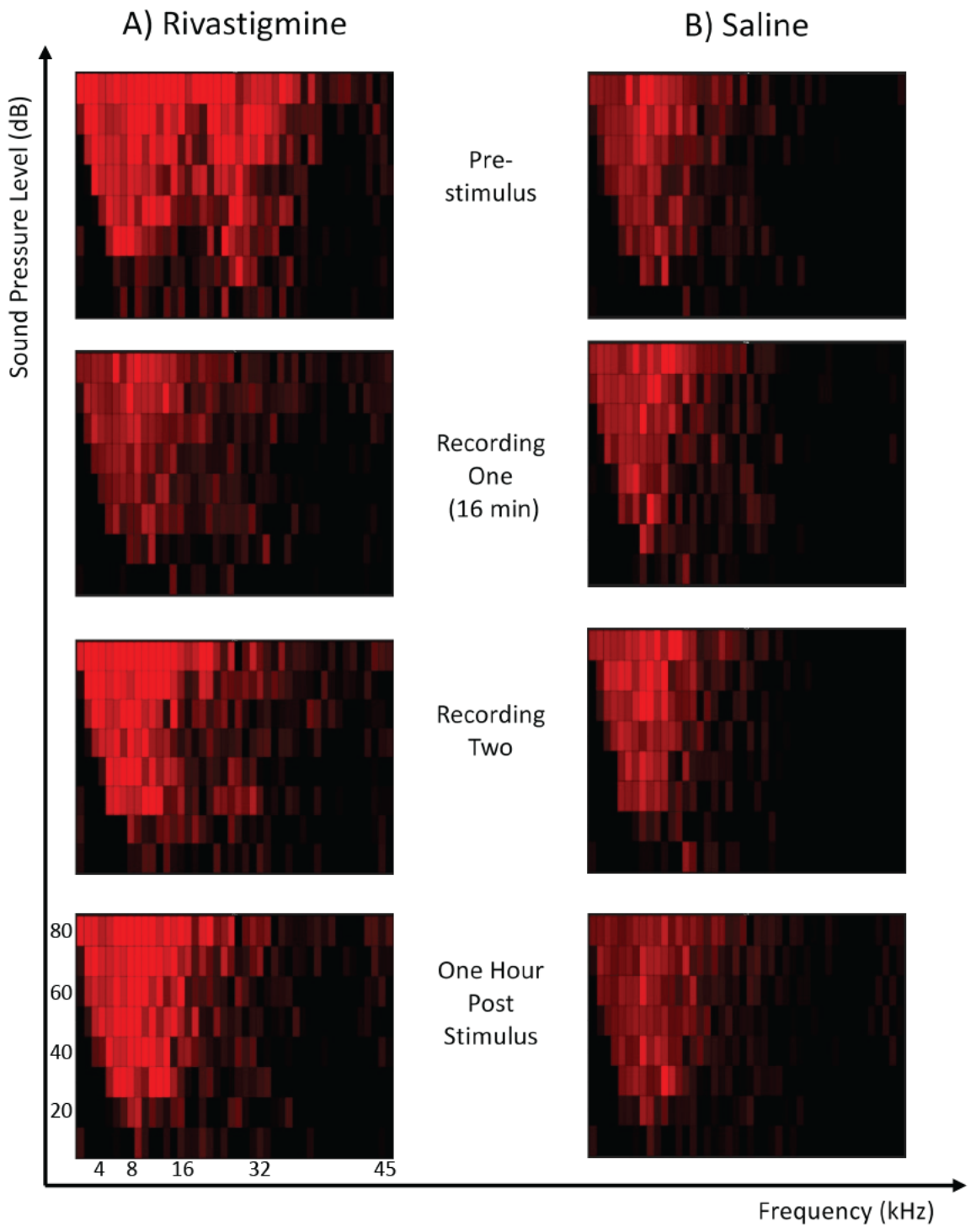


Figure 5.2. Representative examples of Frequency Response Maps of two IC neurons. Representative FRAs of an IC neuron in a (A) Rivastigmine and (B) Saline treated animal. Pre-stimulus recording was done prior to the conditioning block, followed by two mapping blocks, 15 minutes apart. The fourth mapping block was done one hour post the conditioning block. Both condtions were subjected to negative conditioning. The x-axis describes the frequency (KHz) and y-axis describes sound pressure levels (dB).

5.3.2. Data analysis

Unit anaylsis was done based in AVID identification chips blind to the group allocation of the animals. Spike sorting was done offline to isolate single neurons. From the frequency response areas (FRA) (Fig.5.3), the characteristic frequency (CF) (Fig.5.3B) and minimal threshold (MT)(Fig.5.3A) were determined based on the minimal input required to drive stimulus-driven spiking activity. The Q value of an FRA indicates the sharpness or broadness of the receptive field of a neuron. The higher the Q value, the sharper the tuning and the inverse is observed for broader tuning. A Q10 is calculated from the CF over the frequency bandwidth (BW)10dB above the CF (Fig.5.3C) and Q30 calculated with the frequency bandwidth 30dB above the CF (Fig.5.3D). The equation is as follows:

$$\text{BW of QdB} = \text{High Fq} - \text{Low Fq}$$

$$\text{Q dB} = \text{CF/BW}$$

A Rate Level Function (RLF) analysis was done at 8 and 12 kHz from 0 to 70 dB. This measured the density of spiking activity at 8 and 12kHz prior to and after the conditioning blocks (Fig.5.3E).

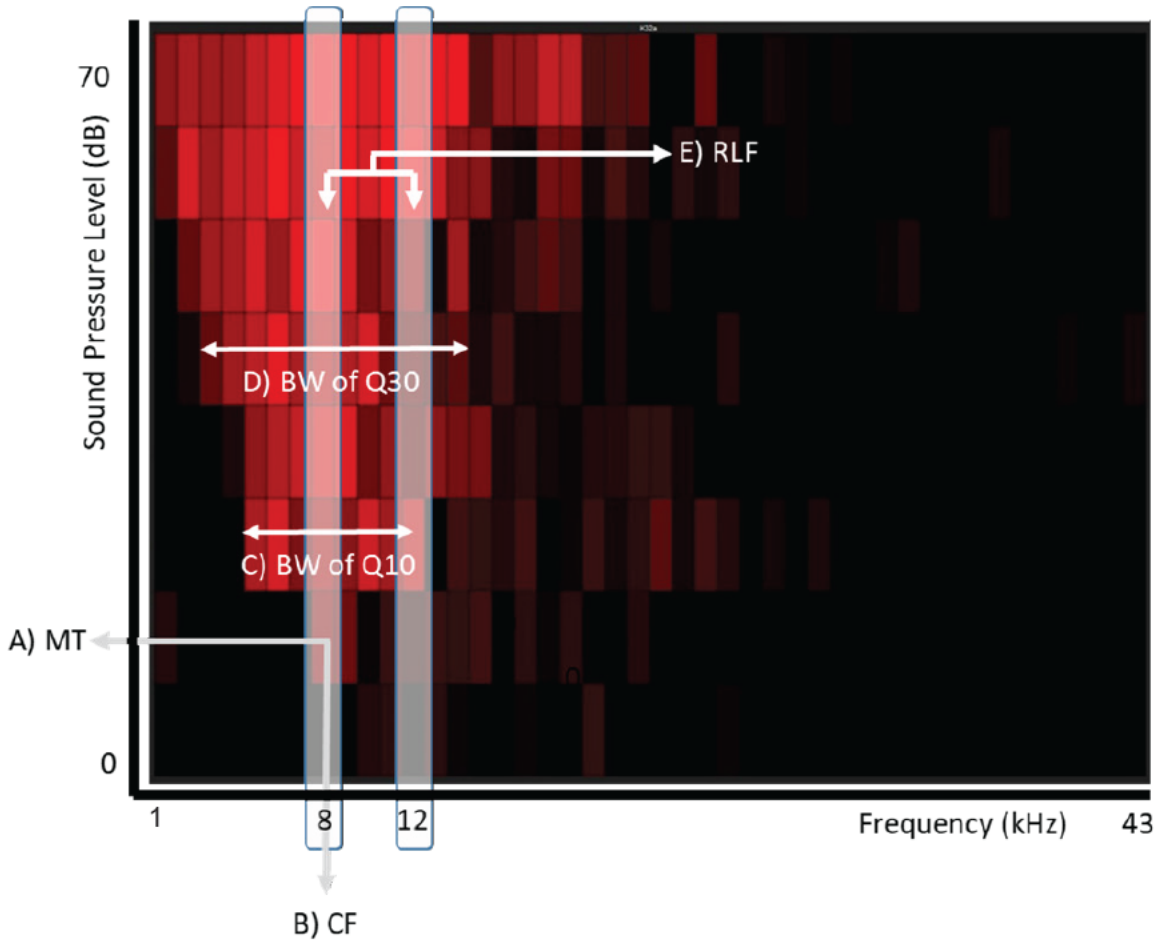


Figure 5.3. Representative example of an FRA analysis. The x-axis represents frequency (kHz), the y-axis, sound pressure level (dB). The red intensity represents the density of spikes at the frequency-sound pressure level combinations. (A) Minimal Threshold (MT) and (B) Characteristic Frequency (CF) is the minimal input required to produce stimulus-driven spiking activity. (C) Represents the frequency bandwidth (High Fq value- Low Fq value) 10dB above the CF, to calculate the Q10. (D) Represents the frequency bandwidth (High Fq value- Low Fq value) 30dB above the CF, to calculate the Q30. (E) Highlights the Rate Level Function at 8 and 12 kHz taken from 0 to 70 dB.

5.4. Results

A total of 52 neurons were recorded over four mapping blocks (208 FRAs anaylsed), 29 were from Rivastigmine treated animals and 23 were from saline treated animals. Of the 29 neurons recorded from the Rivastigmine treated animals, 14 were subjected to a negative conditioning and 15 were subjected to a positive conditioning. Of the 23 neurons recorded from the saline treated animals, 16 were subjected to a positive conditioning and 7 were subjected to a negative conditioning (Tab.5.1). Of the 52 neurons tested, 37 responded to the stimulus conditioning in a STDP compliant trend. STDP compliance is defined by the expected directionaility of the shift. Specifically, positive conditioning resulting in a relative shift towards the non-preferred tone, and negative conditioning resulting in a relative shift away from the non-preferred tone.

Table 5.1. Results summary of the total number of neurons recorded.

	Total	Positive Conditioning	Negative Conditioning	STDP Compliant
Rivastigmine	29	14	15	22 (76%)
Saline	23	16	7	15 (65%)

5.4.1. Shift in Characteristic Frequencies

The data were taken from individual neurons across the three different time points (pre STDP stimulus, the greatest shift post the STDP stimulus and one hour post the STDP stimulus). Statistical analysis was done using a two-tailed Wilcoxon test for of each event of each unit (22), three times. Overall, the data represents an anaylsis of 66 events.

In the Rivastigmine treated animals, in both the positive (Fig.5.4A, $p=0.002$) and negative STDP conditions (Fig.5.4C, $p=0.0005$), IC neurons showed a significant shift in CF in response to the STDP stimulus. A paired analysis of pre and post time points showed no statistical differences (Fig.5.4A, $p>0.05$) (Fig.5.4C, $p>0.05$), indicating the shift was temporary and the neurons resumed their normal CF an hour after the stimulus.

The positive STDP conditioning resulted in a significant CF shift in saline treated animals (Fig.5.4B, $p=0.03$). There were no statistical differences in the pre and post time points (Fig.5.4B, $p>0.05$), indicating that the shift was temporary and the neurons resumed their normal CF an hour after the stimulus. Although IC neurons showed a small shift in response to the negative STDP conditioning, the CF shift was statistically not significant (Fig.5.4D, $p>0.05$).

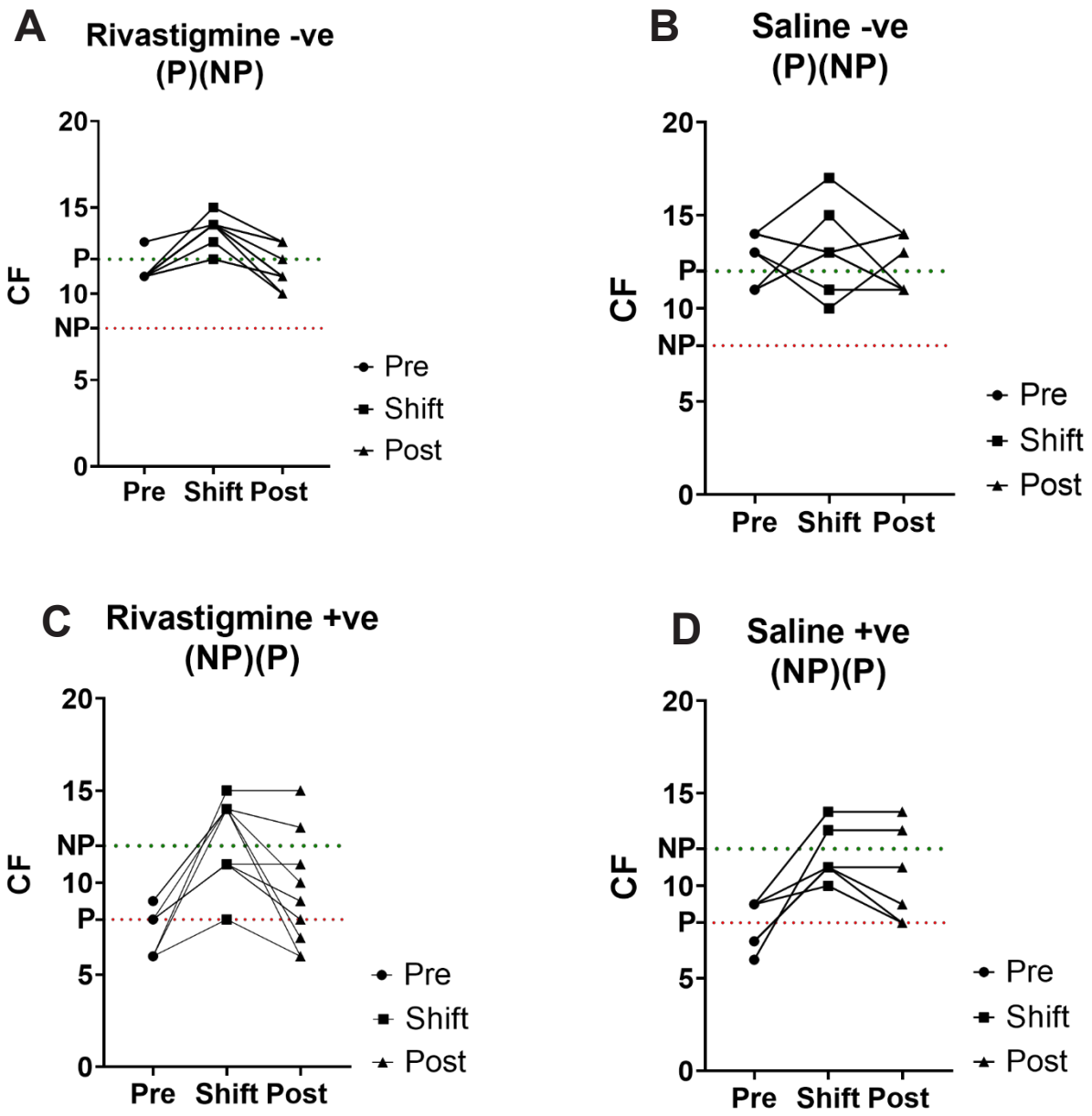


Figure 5.4. The CF of individual neurons pre STDP stimulus, the greatest shift post the STDP stimulus and 1-hour post the STDP stimulus. Rivastigmine treated animals showed a significant shift after (A)positive (p=0.002) and (C) negative (p=0.0005) conditioning. CF=Characteristic frequency, NP=Non-preferred frequency and P=preferred frequency.

5.4.2. Q values

The Q10 and Q30 data was taken from individual neurons across two time points (pre stimulus and the greatest shift post-stimulus). Statistical analysis was done using a two-tailed Wilcoxon test.

In the Rivastigmine treated animals, in both the positive (Fig5.5A, $p=0.025$) and the negative (Fig.5.5C, $p=0.006$) STDP conditions at Q10, IC neurons responded with a significant increase in Q values. In response to the STDP stimulus at Q10, these neurons showed tighter receptive fields. In these animals a similar effect was seen at Q30 in response to negative STDP conditioning (Fig.5.5C, $p=0.015$). There were no statistical differences in the Q10 and Q30 within the saline treated group ($p<0.05$).

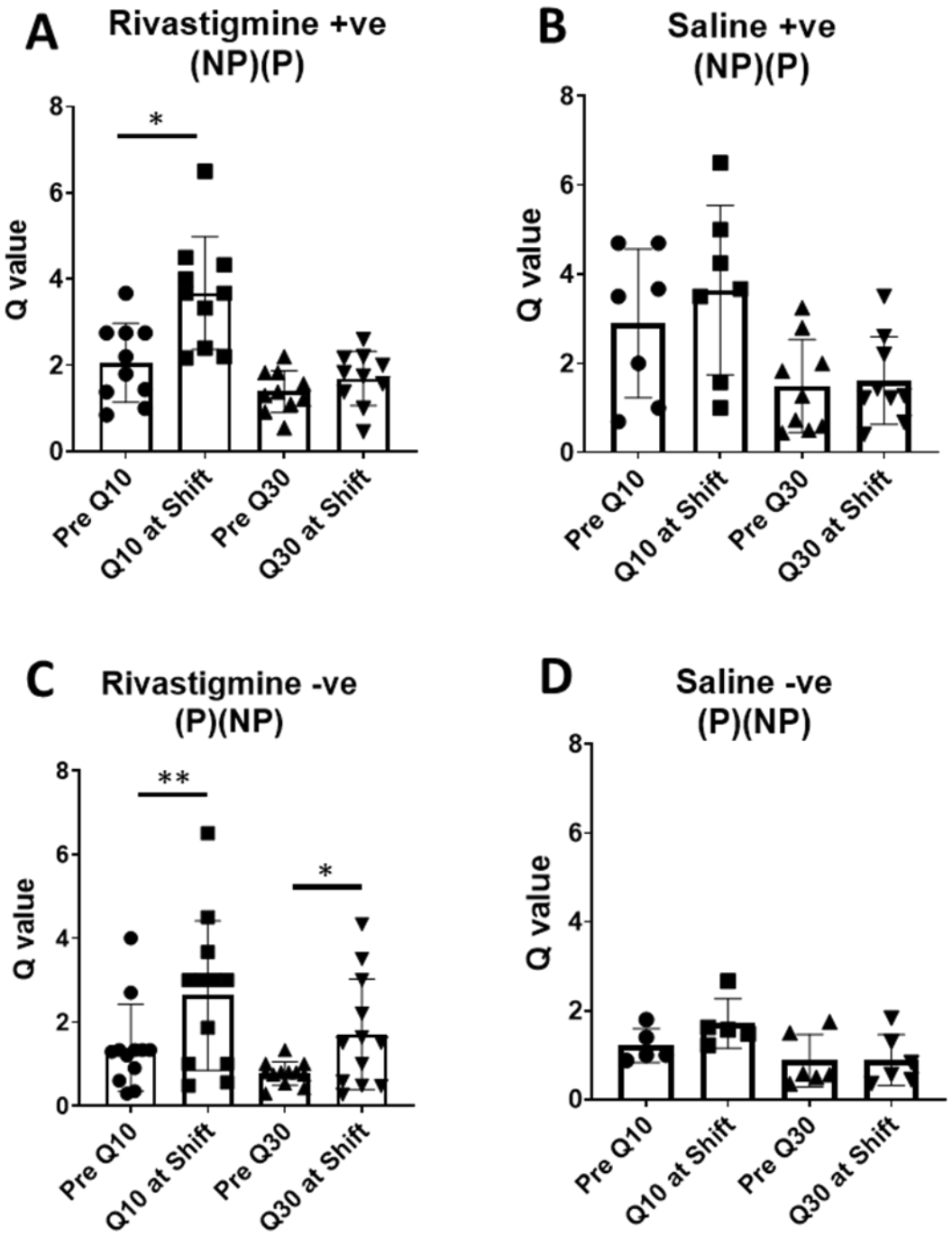


Figure 5.5. The Q10 and Q30 values of neurons pre STDP stimulus and at the greatest shift post STDP stimulus. Rivastigmine treated animals showed a significant shift after (A) positive (Q10 p=0.025 & Q30 p=0.242), and (C) negative (Q10 p=0.006 & Q30 p=0.015) conditioning.

5.4.3. Rate Level Function Test

Figures 5.6 and 5.7 show the rate level functions of IC neurons pre STDP stimulus and at the largest shift post STDP stimulus at 8kHz and 12kHz of Rivastigmine (Fig.5.6) and Saline (Fig.5.7) treated animals. The data were categorised based on STDP compliance and non-compliance. Statistical analysis was done using a two-tailed Wilcoxon test.

In positive conditioning, STDP compliant IC neurons of Rivastigmine treated animals (Fig5.6A), showed significant differences in the RLF at both 8kHz ($p=0.04$) and 12Khz ($p=0.004$) when compared to pre STDP conditioning. At the lower and higher sound pressure levels, increases of spiking activity were observed. In the non-compliant STDP IC neurons, a significant difference was seen at 8kHz in response to both positive (Fig.5.6B, $p=0.008$) and negative (Fig.5.6D, $p=0.004$) conditions when compared to pre STDP conditioning. In the STDP non-compliant IC neurons, both negative and positive conditioning at 8kHz, showed a significant decrease in spiking activity at each sound pressure level.

In saline treated animals (Fig.5.7), there were no statistical differences in the RLF of IC neurons pre and at the greatest shift after the STDP stimulus, in both positive and negative conditioning and in STDP compliant and STDP non-complaint IC neurons ($p>0.05$).

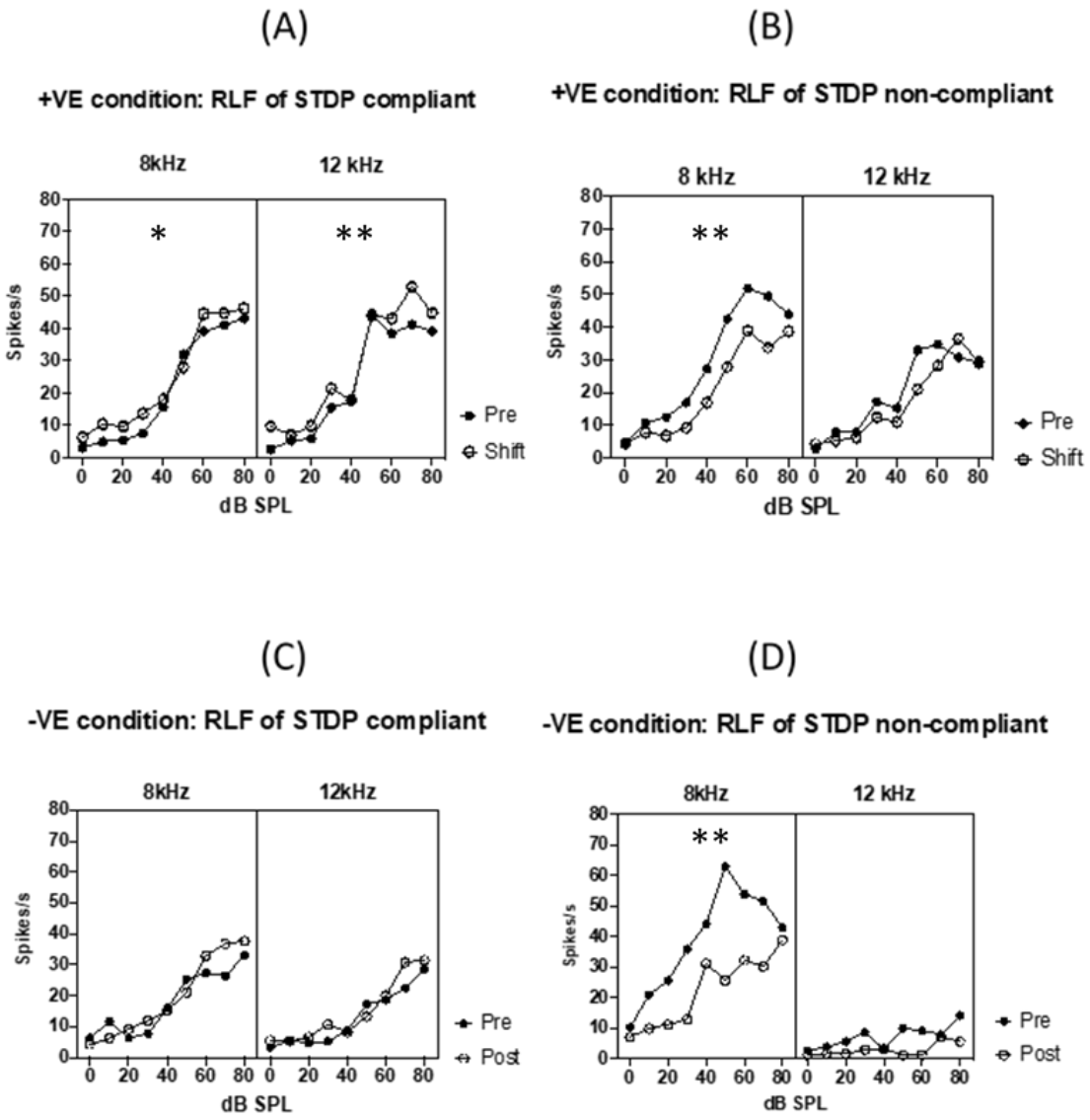


Figure 5.6. The Rate Level Function (RLF) of (A&C) STDP compliant and (B&D) STDP non-compliant IC neurons in animals treated with Rivastigmine. The Rate Level Function of neurons was obtained pre STDP stimulus and at the greatest shift post the STDP stimulus at 8 and 12Khz. In non-compliant STDP IC neurons, at 8Khz, there was a decrease in spike/s in respond to STDP stimulus (B, p=0.008) (D, p=0.004).

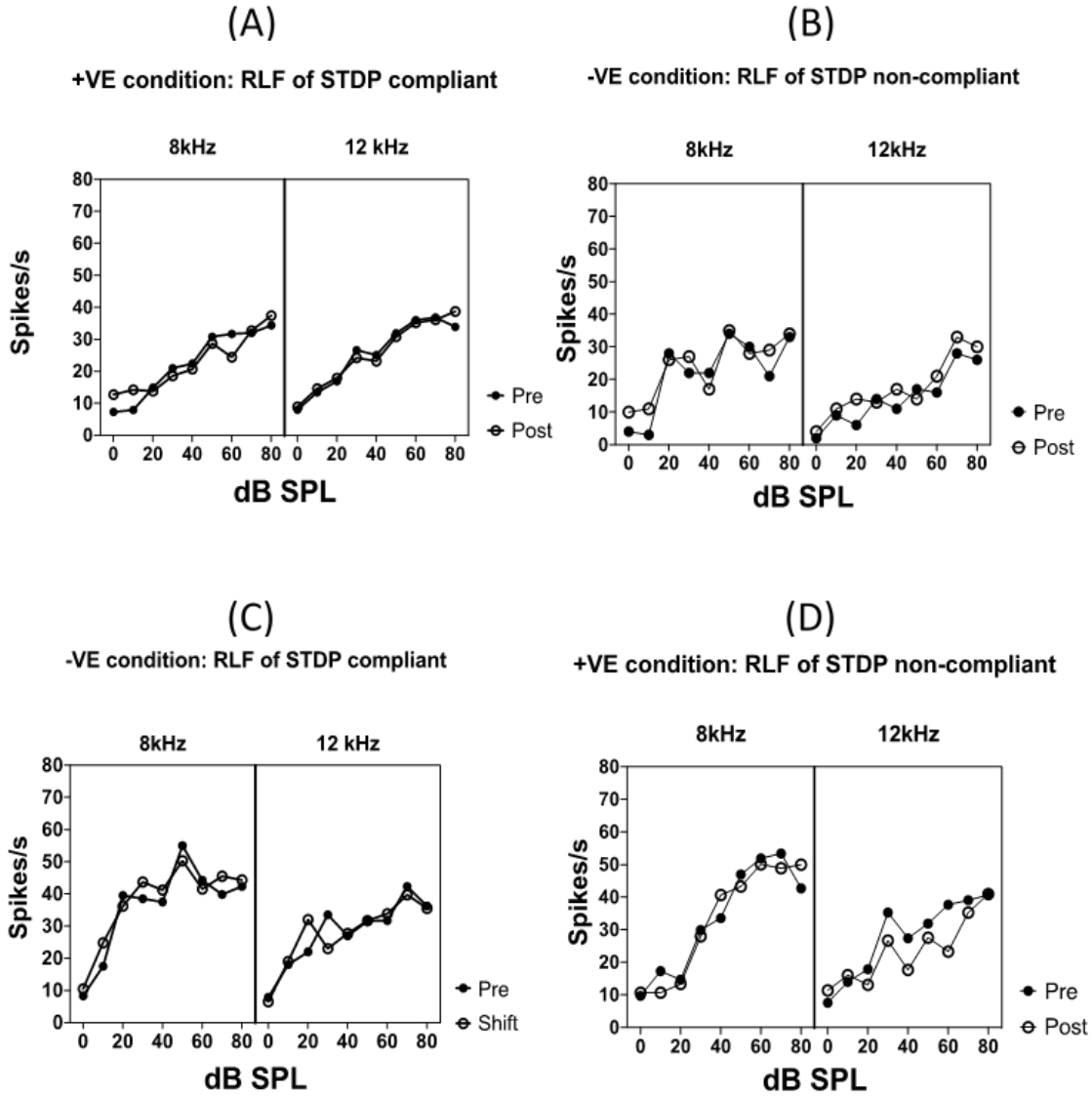


Figure 5.7. The Rate Level Function (RLF) of STDP compliant and STDP non-compliant IC neurons in animals treated with Saline. The Rate Level Function of neurons was capture pre STDP stimulus and at the largest shift post the STDP stimulus at 8 and 12Khz. There were no statistical differences in the RLF of saline treated animals.

5.5. Discussion

Studies that have investigated STDP in auditory neurons in vivo, have only been studied in the auditory cortex and the dorsal cochlear nucleus (Tzounopoulos et al., 2004b; Dahmen et al., 2008; Basura et al., 2015). For the first time, the results of STDP conditioning in the IC within an animal model of UNIHL is presented. Futhermore, we explored the sensitivity of this adaption in response to cholinergic enhancement. The results from this study indicated two major consequences, 1) IC neurons showed a capacity to respond in an STDP-like manner in vivo and 2) in a UNIHL animal model, four weeks of Rivastigmine treatment, differentially affects the response properties of IC neurons when subjected to STDP conditioning. This present study adds to the growing body of evidence that suggests cholinergic modulation generally acts to optimise the processing of signals in attention demanding contexts (Hasselmo & McGaughy, 2004; Sarter et al., 2005; Jääskeläinen et al., 2007; Ayala & Malmierca, 2015).

This present study demonstrates that IC neurons show a capacity to respond in an STDP-like manner in vivo. The IC plays an obligatory relay role for incoming auditory information (Semple & Kitzes, 1985; Oliver, 2005; Schreiner & Winer, 2005; Malmierca & Young, 2015). The results here highlights that at the midbrain level, acute and adaptive plasticity is evident and therefore would influence the ascending signal processing of central auditory information. In addition, this further supports the contention that neuroplasticity is seen not only at the cortical level but also at the midbrain level (Szczepaniak & Moller, 1996; Oliver, 2005; Ji & Suga, 2009; Papesh & Hurley, 2012; Wang et al., 2013).

The results of this present study showed that IC neurons contralaterally stimulated from the intact hearing ear have the capacity to respond in a STDP-like manner. This was seen in animals treated with either saline or Rivastigmine for four weeks. However, it is

important to note that it was the opposite ear that had been partially lesioned. In some neurons the CF shift beyond the 12kHz trainining tone. While this not predicted we can speculate the overshoot may be due to the stimulus effecting the overall FRA direction and not specifiically to the 12kHz training tone. Indeed what is signifant is the shift back to pre-timulus conditions. In Rivastigmine treated animals, there was a higher proportion of STDP-compliant neurons (76%)(Tab.5.1) and in both negative and positive STDP conditioning (Fig.5.4A&C). In contrast, in the saline treated cohort, although there were CF shifts that have moved in the predicted direction, these CF shifts were only significant in response to negative conditioning (Fig.5.4B) and not positive conditioning (Fig.5.4D). It can be inferred from the saline treated animals, that IC neurons are indeed sensitive to STDP conditioning, however its affect is less influential. This study has shown that chronic acetylcholine promotion for four weeks influenced a) a higher proportion of STDP compliant IC neurons and b) the conditioning was influential and significant in either directions.

Neurons stimulated from the intact hearing ear of the saline treated animals showed small affects to STDP conditioning. It may be expected that a similar result would be seen in the hearing control animals. It was observed that cholinergic enhancement promotes STDP compliance as well as the direction and degree of the STDP conditioning. It is important to make the distinction that the Rivastigmine treatment was done orally and over four weeks and therefore direct exogenous acetylcholine was not directly applied to these neurons. The implication of this method, is that its quite possible, STDP is occurring in A1, and the observed effects in the IC are downstream of this change or a separate pathway. A limitation of this study is that the direct measure of acetylcholine was not done in response to the STDP conditioning. Molecular studies have shown that there were no cholinergic neurons within the IC (Motts et al., 2008; Motts & Schofield, 2009; Schofield & Motts, 2009; Schofield et al., 2011), which suggests the changing electrophysiological properties of IC neurons were influenced by acetylcholine that is exogenous in origin. (Farley et al., 1983;

Raza et al., 1994; Habbicht & Vater, 1996; Ayala & Malmierca, 2015; Ayala et al., 2016). Paired tracer studies with immunohistochemistry investigated cholinergic input into the IC, and showed that the IC receives its cholinergic input from descending projections (Schofield & Motts, 2009; Schofield, 2010; Schofield et al., 2011). The authors suggested that the IC receives cholinergic input from pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT) via descending projections from the auditory cortex converge into the PPT and LDT (Schofield, 2010). The results of this present study is consistent with other electrophysiology studies in the IC that suggest the role of acetylcholine is to regulate IC response properties (Farley et al., 1983; Habbicht & Vater, 1996). The hypothesis that the cholinergic influences that are seen electrophysiologically in IC, is being modulated by the auditory cortex via cholinergic projections of the PPT and PPD is indeed attractive. However, this hypothesis requires further in vivo electrophysiological studies that involve multiple electrodes combine with molecular studies are needed.

The results of this present study may have implications for the development of therapies associated with hearing dysfunction. The results of this study showed that treatment with a human equivalent dose of Rivastigmine for four weeks (Ballmaier et al., 2002; Abdel-Aal et al., 2011; Voss et al., 2016), in a physiological animal model of UNIHL can influence the central auditory system, in that acetylcholine promotion resulted in IC neurons with an increased capacity to respond in an STDP-like manner. This short-term acute plasticity has the potential to be further investigated and ultimately exploited for therapeutic means. Further work in regards to the underlying molecular mechanisms with correlating electrophysiological data, is required in order to strengthen the novel results of this study. The use of more complex and sensitive investigations may reveal a potential mechanism which could be exploited for therapeutic means. Future investigations should be aimed at understanding the physiological capacity of manipulating auditory neurons of different nuclei within the context of maladaptive central auditory conditions.

Chapter 6
The Effect of Rivastigmine paired with Auditory Training on Acetylcholine levels in Brain Homogenates using UHPLC-MS/MS

6.1 Introduction

Acetylcholinesterase and cholinesterase (AChE/ChE) inhibitors are the only class of drugs that are approved to manage and treat the symptoms associated with Alzheimer's disease (Waldemar et al., 2007; Colovic et al., 2013; Birks & Evans, 2015). The rationale behind the use of these drugs is that by impeding the catabolism of acetylcholine, there is an increase of synaptic availability of acetylcholine and thus an enhancement of cholinergic neurotransmission. The symptomatic alleviation through the use of AChE/ChE inhibition is considered modest and temporary, but may stabilise or slow the progressive decline of cognitive function and functional ability characterised by Alzheimer's disease (Felician & Sandson, 1999; Colovic et al., 2013).

Cholinergic dysfunction has also been implicated in disorders of the central auditory system (Farley et al., 1983; Metherate & Weinberger, 1990; Morley & Happe, 2000; Kaltenbach & Zhang, 2007; Gómez-Nieto et al., 2014; He et al., 2014; Forrest et al., 2019). Unilateral noise induced hearing loss (UNIHL) is a physiological animal model used to study tinnitus. These studies have shown that the associated maladaptive plasticity is implicated by cholinergic receptor upregulation (Kaltenbach & Zhang, 2007; Forrest et al., 2019). Forrest et al. (2019) reported that, as a consequence of UNIHL there are changes in the cholinergic receptor expression in the auditory cortex (A1) and rostral belt (RB). Indeed, contralateral to the lesion, the authors reported a downregulation of the muscarinic receptor (mAChR) expression in A1 and RB. Furthermore, there was an upregulation of the nicotinic receptor (nAChR) expression in the RB. Although there was an upward trend in A1 of nAChR expression, this upward trend was not statistically significant (Forrest et al., 2019). In the dorsal cochlear nucleus (DCN), as a result of intense noise exposure, all superficial neurons in the DCN, except for the fusiform cell layer, showed an increase in hyperactivity (Kaltenbach & Zhang, 2007). The application of a cholinergic agonist, carbachol, resulted in suppression of hyperactivity in the fusiform cell layer, with this suppression observed more profoundly in the sound exposed rats. The authors inferred that sound exposure resulted in an increased sensitivity to the inhibitory influence of cholinergic inputs (Kaltenbach & Zhang, 2007). Together, the value of these two studies showed that following sound exposure, there were significant changes in the auditory system that are implicated in cholinergic interactions.

In the auditory system, acetylcholine has a neuromodulatory role (Metherate & Weinberger, 1990; Irvine, 2007; Voss et al., 2016). Previous authors have shown that the pairing of acetylcholine promotion and the manipulation of sensory inputs have central consequences specific to the manipulation. They reported that the delivery of a cholinergic agonist to the cortex or electrical stimulation of Nucleus Basalis (NB) causes cortical receptive fields to expand around the stimuli that are associated with the release

of acetylcholine (Metherate & Weinberger, 1990; Rasmusson, 2000). Furthermore, Voss et al. (2016) reported two-tone frequency discrimination training combined with cholinergic promotion resulted in rats learning tasks more quickly, compared to their age-matched training-saline cohort. In addition to their behavioural results, their electrophysiological results indicated there was a reduction in the overlap of the two tones in the auditory cortex (A1), which suggests an enhancement of cortical segregation. This in turn inferred that there was enhanced discriminability of the two tones (Voss et al., 2016). Overall, these findings demonstrated that combining sensory inputs with the neuromodulation of the cholinergic system, significantly influences the auditory system. Mechanisms of synaptic plasticity, which are highly sensitive to the timing of sensory input, are likely responsible for the differential plasticity seen in these studies.

In the previous two chapters, the results showed that Rivastigmine treatment for four weeks influences the response properties of neurons in the inferior colliculus (IC). The changes observed were associated with a more selective and narrower tuning of the receptive field, suggestive of tighter control at the characteristc frequency (CF). Although this result is novel, Motts and Schofield (2009) reported that there are no cholinergic neurons in the IC . The authors reported that cholinergic influences are likely sourced from an exogenous origin namely from cholinergic neurons projecting from two large tegmental nuclei – the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT) (Schofield & Motts, 2009). Furthermore, Schofield (2010) paired anterograde and retrograde tracer injections in A1 and IC with ChAT immunohistochemistry staining to determine if A1-IC acetylcholine interactions occurred via PPT and LDT. The results showed ChAT-positive PPT and LDT neurons had anterograde FluoroRuby-labelled cortical axons in close contact with retrograde Fast Blue-labelled inferior colliculi boutons. These results inferred that it is possible A1 may influence the IC via the cholinergic projections of PDT and LDT.

6.2 Hypothesis

The mechanism of action of Rivastigmine is to inhibit acetylcholinesterase and thus increase free acetylcholine for synaptic availability. However, it is unknown if this is a global response or regionally specific within the brain. This chapter test the hypothesis that an increase of acetylcholine in the IC will occur as a result of Rivastigmine treatment. The aim of this study is to measure the levels of acetylcholine in rat brain homogenates of different regions (frontal lobe, temporal lobe and IC) in response to four weeks of the human equivalent dose of (0.2mg/kg/daily) of Rivastigmine (Voss et al., 2016) compared to a saline treated cohort. Within the half-life of Rivastigmine, both cohorts were subjected to auditory training during those four weeks. The methodology for this study has been adapted from previous authors (González et al., 2011; Bergh et al., 2016) that used ultra high performance liquid chromatography in tandem mass spectrometry (UHPLC-MS/MS) to measure acetylcholine in rodent brain tissue.

6.3 Methods

6.3.1. Animals and Ethics

The experiments described in this study complied with the Australian code for the care and use of animals for scientific purposes, the New South Wales Animal Research Act and were approved by the Western Sydney University Animal Care and Ethics Committee (A12671). Six three-month old male Wistar rats, were obtained from Animal Resources Centre (ARC) Canning Vale, Western Australia. The animals were subjected to the quarantine procedures of the Animal Facility, School of Medicine, Western Sydney University and subsequently housed and cared for in the facility. The animals were housed and cared for as described in the general methods (Section 2.1.1). Rats were subjected to a pre-screen ABR to quantify normal hearing (Section 2.1.2) prior their allocation into Saline (n=3) or Rivastigmine treated (n=3) cohorts. Gel pellet habituation and training was achieved as described in Chapter Four (Section 4.3.2). Animals were housed in pairs and subjected to the daily monitoring, handling and auditory training (Section 4.3.2). Of each pair, one animal received the Rivastigmine and the other a saline vehicle. Individual rats were identified using AVID microchips placed subcutaneously on the dorsal aspect of the neck whilst under anaesthesia.

6.3.2. Macrosection of Brain and Sample Storage

Thirty minutes after consuming the gel pellet, the animals were placed within an isoflurane chamber. Once deeply anesthetised, they were rapidly sacrificed with a guillotine. The head was rapidly submerged in a shallow beaker containing ice cold 1% formic acid (FA) in saline solution of ~4°C and placed on ice. All surgical tools, equipment and vials were cold and sterile. The removal of the brain from the skull was completed whilst submerged within the 1% FA solution. The whole brain was placed on a petri dish on ice to be macrosectioned (Fig.6.1).

The sectioning procedure has been adapted from the methodology of previous authors (Fig.6.1)(Chiu et al., 2007; Lamas et al., 2017). The cerebellum was removed (Fig.6.1.1), and the brain was bisected into left and right hemispheres (Fig.6.1.2). The midbrain was sectioned away from the brain (Fig.6.1.3). The inferior colliculus was isolated by a superior incision, at the superior colliculus (Fig.6.1.4), and an inferior incision from the lower brainstem (Fig.6.1.5). To obtain the frontal lobe, with the medial surface facing up and the outer cortical surface facing down, the olfactory bulb was sectioned away (Fig.6.1.6) and a 90° incision slightly posterior to the anterior tip of the corpus callosum was made (Fig.6.1.7). With anterior section isolated and the medial surface facing up, at a 45° angle at the anterior tip of corpus callosum the sample was bisected (Fig.6.1.8), in order to isolate the superficial frontal lobe from the deeper tissue. To obtain a section of the temporal lobe, the middle section of the brain was placed medial surface down and cortical surface facing up. The rhinal fissure was located and using a sterile 4mm punch biopsy tool, sampling was done 1mm above the midpoint of the rhinal fissure (Fig.6.1.9). The punch of brain tissue was removed from the tool and horizontally bisected to remove the deep white tissue and to isolate the temporal cortical tissue (Fig.6.1.10). This procedure was completed for both left and right hemispheres.

All samples were weighed and collected into clearly labelled microcentrifuge tubes followed by snap freezing in liquid nitrogen and stored at -80°C. The brain tissue was subsequently, thawed on ice, prepared and analysed the same day. On the day of analyses, brain samples were homogenised in 20mL/g of 1%FA in MilliQ water with a pellet pestle cordless motor on ice.

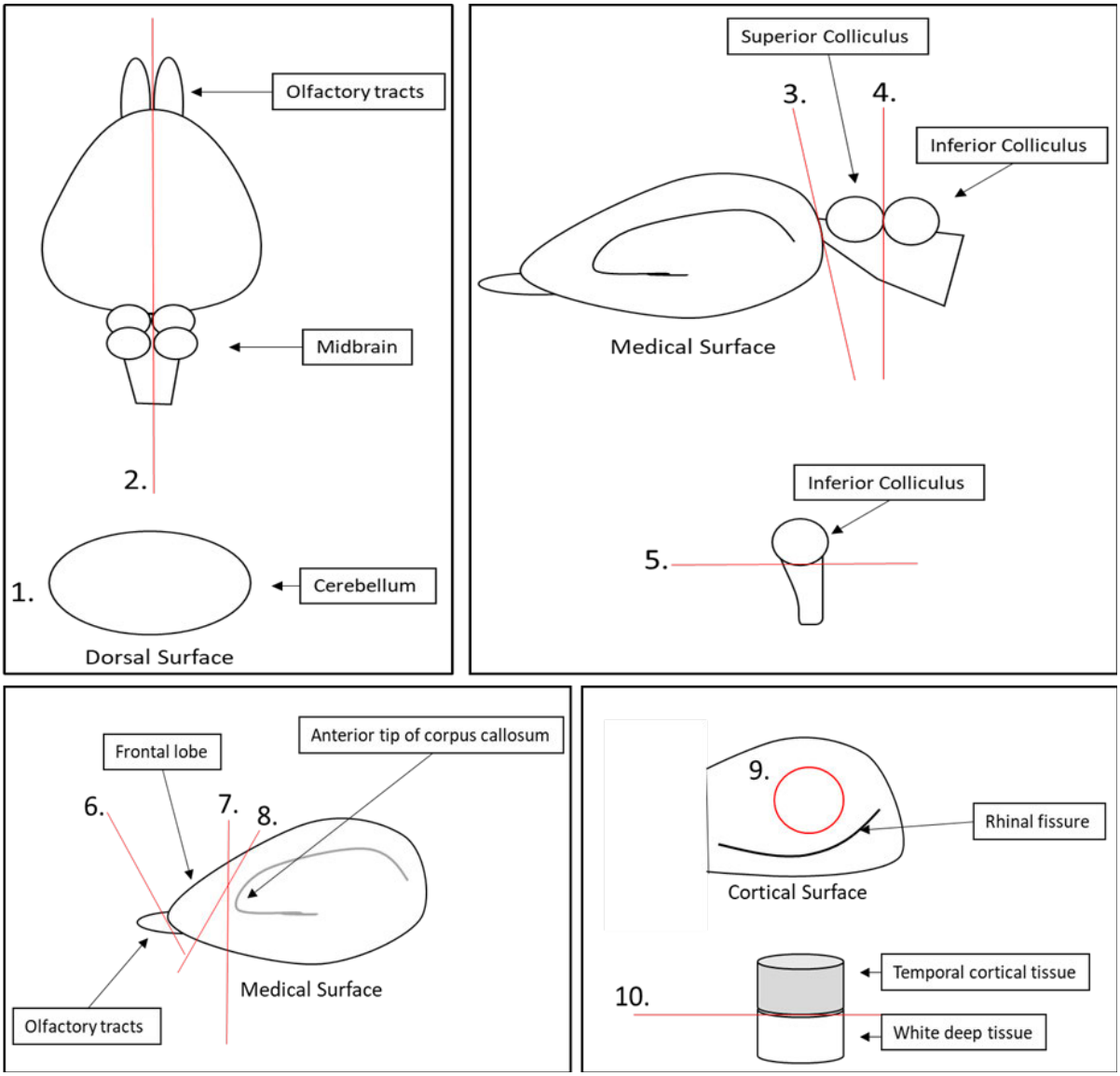


Figure 6.1. Schematic of the macrosectioning procedure.

6.3.3. Preparation of Chemicals, Internal Standards and Quality Controls

A stock solution of the protein precipitate, internal standards and method blanks (solutions minus the brain homogenates) were prepared using 250mM FA in MilliQ with appropriate dilutions. A 1:10 dilution of the stock solution made-up the diluent of 25mM FA for the internal standards, standard curve and method blanks. The internal standard was Ach-D9 chloride (Novachem Pty Ltd, Heidelberg West, VIC, AU) and acetylcholine chloride (Sigma-Aldrich Pty Ltd, North Ryde, NSW, AU) was used for the standard curve. A working stock of 80ppm Ach-D9 chloride and 80ppm of acetylcholine chloride in methanol was prepared. The Ach-D9 internal standard was diluted to 50ppb with the diluent of 25mM FA. The Ach-Chloride standard curve of 500 to 0.1 ppb was prepared using the same method as the brain homogenate, with 1% FA in MilliQ. All solutions were prepared on the day of analysis.

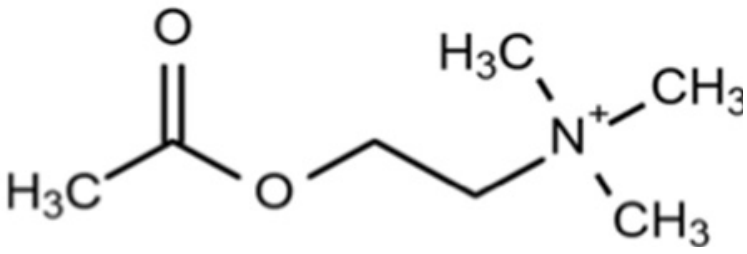


Figure 6.2. Structural Formula of Acetylcholine. Molecular mass 146.21.

6.3.4. Protein Precipitation with FA

Rat brain homogenates, acetylcholine chloride standard curve or method blank (150 µL), IS of Ach-D9 (75 µL) and ice-cold 250mM FA (150 µL) were added to pre-chilled microcentrifuge tubes kept on ice and vortexed for 20s. The samples were centrifuged for 10 min at 14 000 g at 4 °C. The supernatant was transferred to MS grade injection vials (Thermo Fisher Scientific Australia Pty Ltd, Scoresby, VIC, AU).

6.3.5. Instrumentation

Samples were analysed with an Acquity™ UPLC system (Waters, Milford, MA, USA) comprising of an Acquity Binary Solvent Manager (Waters) and an Acquity Sample Manager (Waters), coupled to a Xevo TQ MS tandem quadrupole mass spectrometer (Waters) fitted with an electrospray ionization source (ESI). The collision gas was Argon with a 0.15mL/min flow. MS-analysis was done in positive mode with multiple reaction monitoring mode parameters (MRM) optimised for Ach-d and Ach (Tab.6.1). The capillary voltage was 0.6 kV, sourced temperature was 120°C and desolvation temperature was 450 °C, cone gas cell flow was 100 L/h and desolvation gas flow was 800L/h. Waters MassLynx 4.1 SCN 917 software was used for acquisition and processing data. A Waters Acuity™ HSS T3 (2.1 mm i.d x 100mm, 1.8 µm) column was used with mobile phase A consisting of 0.1% FA in MiliQ water and mobile phase B consisting of 0.1% FA in acetonitrile (Tab.6.2). The column temperature was 35°C and the flow rate conditions was maintained at 0.5mL/min. The injection volume was 5 µL and the injection technique of partial loop needle overfill was used.

Table 6.1. MRM acquisition settings for Ach and Ach-D9.

Compound	MRM transitions (m/z)	Cone Volt (V)	Collision Energy (eV)	Dwell time (s)	Retention time (min)
ACh	146.20 > 43.00	25.0	21.0	0.1	0.81
ACh	146.20 > 60.10	25.0	11.0	0.1	0.81
ACh	146.20 > 87.00	25.0	13.0	0.1	0.81
ACh-d9	154.20 > 87.00	25.0	13.0	0.1	0.81

Table 6.2. Gradient parameters for mobile phase A and B.

Time (min)	%A 0.1% FA in Mili Q water	%B 0.1% FA in acetonitrile	Curve
Initial	100	0	Initial
1.00	100	0	6
1.10	5	95	6
3.50	100	0	11

6.4. Results

6.4.1 Method Development and Optimisation

Prior to analyses of samples, an optimisation step was conducted to: 1) to determine if the brain homogenate matrix had an effect on the peak of Ach, 2) if the recovery of Ach-D9 IS or Ach spiked within the brain samples was consistent and, 3) if the parameters used on the instrument were producing consistent results.

To determine if the Ach-D9 had a suppression or enhancement effect of the peak of Ach, two standard curves were tested. A standard curve without Ach-D9 IS was compared to the another standard curve with Ach-D9 IS (Fig.6.4). To determine if the brain homogenate matrix had an effect of the peak of Ach and Ach-D9, a brain sample with no IS was tested (Fig.6.3.A1) and compared with a sample with IS Ach-D9 (Fig.6.3.A2), as well as a sample spiked with a known amount of Ach (Fig.6.3.B2). To determine if the sample preparation and the instrument had reliable recovery of known amounts of Ach-D9 and Ach in the brain homogenate matrix, a recovery test was done. Different points in the experiment where Ach-D9 could be added were tested: 1) at the homogenising step (Fig.6.3.blue) and, 2) at the protein precipitation step (Fig.6.3.green). Recovery was also tested with a sample where a known amount of Ach in the brain homogenate was added at the protein precipitation step (Fig.6.3.B2). For quality control, all brain homogenate samples were replicated with method blanks and compared. These optimising steps were completed using brain tissue from the frontal lobe, temporal lobe and IC. It was determined from the testing of the recovery samples that the sample preparation and parameters used on the instrument were reliable. In addition, the matrix effect could be compensated by using a consistent known amount of Ach-D9 that would be added to all the samples at the protein precipitation step (Fig.6.3A2).

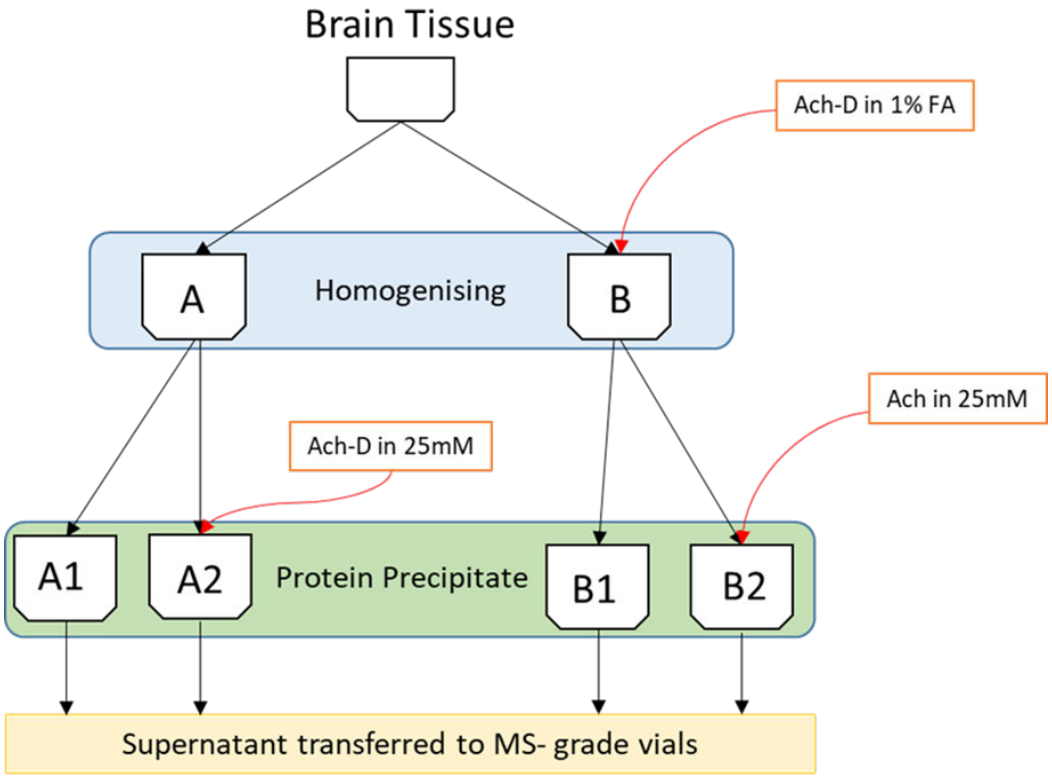


Figure 6.3. Schematic of the matrix and recovery experiments. These step were tested using brain tissue from the frontal lobe, temporal lobe and inferior colliculus. The effect of the brain homogenate matrix on the peaks Ach-D9 and Ach was determined by comparing A1, A2 and B2. The recovery of known amounts of Ach and Ach-D9 in the samples was done by comparing A2, B1 and B2 to determine that the sample preparation and the parameters used on the instrument were reliable.

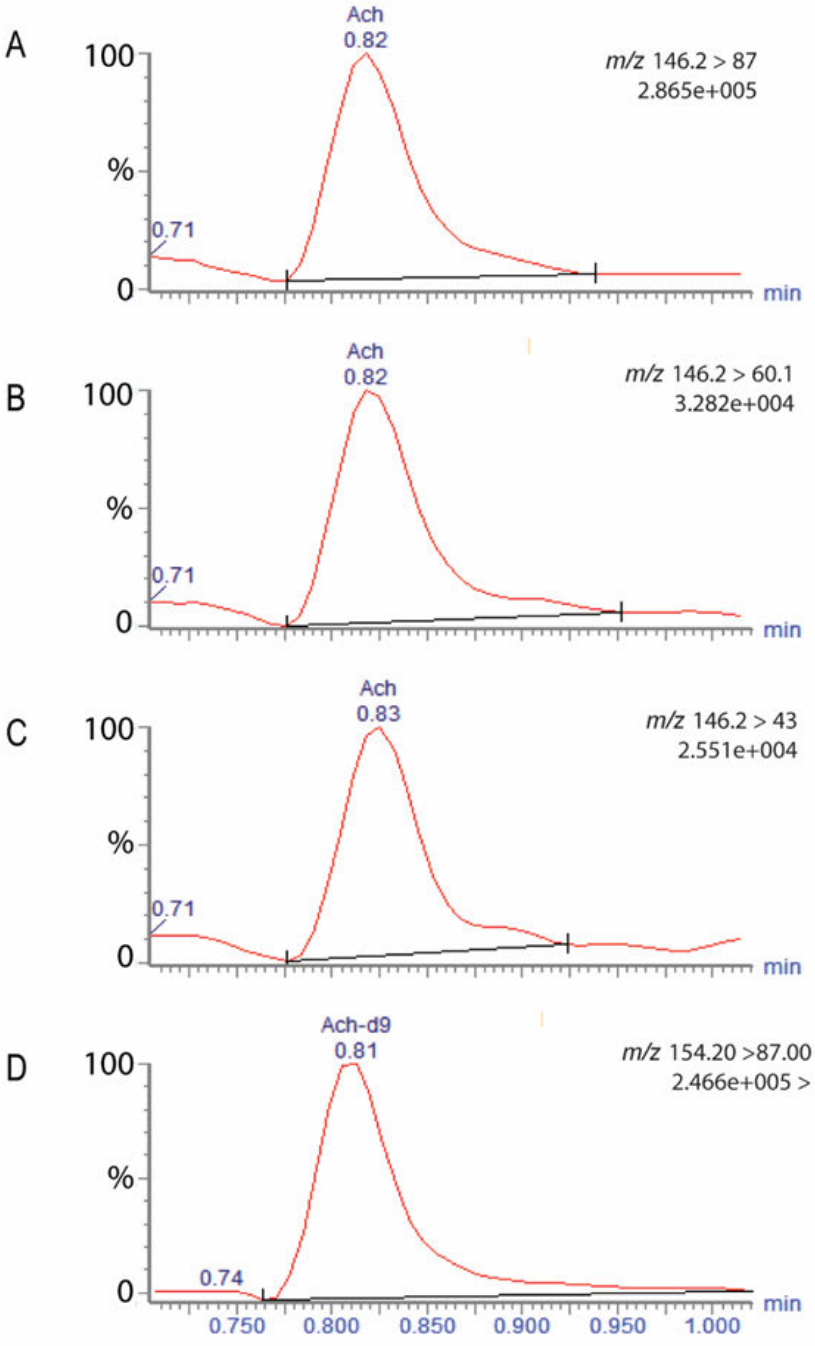


Figure 6.4. Chromatograms of 0.2ppb of Ach in a method blank solution, representative sample used for the Ach standard curve. (A), (B) & (C) MRM transitions of Ach and (D) Ach-D9.

6.4.2 Acetylcholine in Brain Homogenates

Table 6.1 shows the mean concentration and ratio of Ach in the different brain regions of rats in response to four-weeks of Rivastigmine and saline treatment paired with auditory training using HPLC-MS/MS. For statistical analysis, a t test was used to compare the mean concentration and percentages between the two cohorts.

Two major consequences are seen as a result of the auditory training paired with Rivastigmine treatment. There was a significantly higher proportion of acetylcholine in the temporal cortex ($p=0.03$). There was also a significant decrease in the amount of acetylcholine in the inferior colliculus in Rivastigmine treated animals compared to the saline control animals ($p=0.03$).

Table 6.3. Mean con. (ug/g) and percentage (%) of acetylcholine of each region over the total amount measured for all three regions. Brain regions analysed were the frontal Lobe, temporal Lobe and inferior colliculus. aSEM is given in brackets

Brain Region	Saline[a] (n=6)		Rivastigmine[a] (n=6)	
Frontal Lobe	0.56 (0.108)	41% (0.05)	0.31 (0.04)	36% (0.10)
Temporal Lobe	0.24 (0.02)	18% (0.02)	0.33 (0.06)	36% (0.02)
Inferior Colliculus	0.55 (0.09)	41% (0.05)	0.25 (0.04)	28% (0.01)

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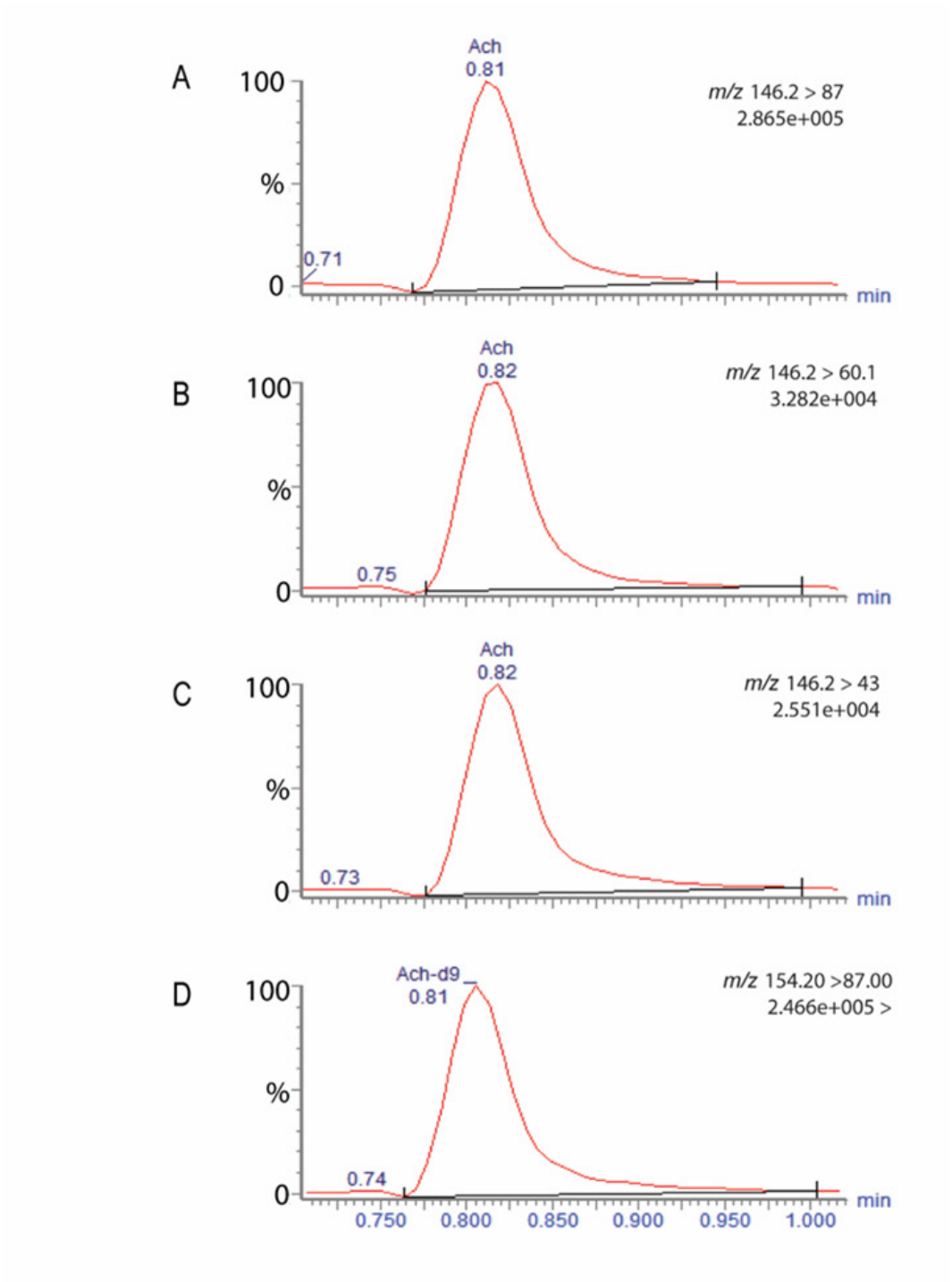

Figure 6.5. Chromatograms of real samples of the left inferior colliculus. (A), (B) & (C) MRM transitions of Ach and (D) Ach-D9.

6.5. Discussion

In the previous two chapters, Rivastigmine treatment paired with auditory training, resulted in significant changes to the response properties of IC neurons. These changes included a higher proportion of narrowed and tighter frequency tunning curves in ipsilaterally driven IC neurons. In addition, in response to stimulus-timing dependent plasticity (STDP) conditioning, IC neurons showed an increase in the capacity to acutely adapt, in a manner that was specific to the conditioning input. Although these results were novel, it remains to be determined if the electrophysiological changes were a direct result of an increase of acetylcholine. To investigate if it was indeed an increase of acetylcholine, rats were subjected to the treatment with Rivastigmine, accompanied by auditory training for 4 weeks, as previously described in Section 4.3.2. The levels of acetylcholine in specific brain regions were analysed using UHPLC-MS/MS.

The process of synthesis, storage and release of acetylcholine requires the expression of specialised structures, including vesicular acetylcholine transporter (VAChT) and the acetylcholine biosynthetic enzyme, choline acetyl transferase (ChAT). In a study in male Wistar rats, Tayebati et al. (2004) showed that, a seven-day treatment of Rivastigmine (2.5mg/kg/day) resulted in an increased expression of VAChT and ChAT in the frontal lobe, striatum and hippocampus. In addition, Voss et al. (2016) showed in rats, treatment with Rivastigmine (0.2mg/kg/daily) paired with perceptual and auditory learning for an average of 11 training days effects the structure and function of cortical A1 neurons. The authors showed that the paired interventions, resulted in a greater number of somatostatin positive cells in the A1. Somatostatin positive cells are a GABAergic class of interneurons, which are the primary target of cortical cholinergic projections. Together, these results showed that prolonged treatment with Rivastigmine alters the expression of cholinergic associated proteins in the brain. Although the relationship between Rivastigmine treatment and the changes in the expression of cholinergic associated proteins is valuable, the

question remained as to effect of four weeks' treatment with Rivastigmine paired with AT, on the levels of acetylcholine in the different areas of the central auditory system.

The results in this chapter showed that there was a significant decrease in the relative amount of acetylcholine in the IC of Rivastigmine treated rats compared to the saline control ($p=0.03$). This finding did not support the hypothesis that there would be an increased amount of acetylcholine in the IC. Ayala and colleagues (2015) investigated stimulus specific adaption in the IC using paired in vivo electrophysiology and iontophoresis injections of acetylcholine antagonists in order to increase acetylcholine levels in IC. Their results showed that, increased levels of acetylcholine contributed to the maintained encoding of repetitive sounds in a more selective manner. This resulted in the decreased capacity of the IC neurons to adapt to the novel tone (Ayala & Malmierca, 2015; Ayala et al., 2016). In line with the findings of Ayala and Malmierica studies, the resulted presented in Chapter Five showed that saline treated animals showed a decreased capacity to adapt to the STDP conditioning and in this present study a relative higher amount of acetylcholine detected in their ICs (Tab.6.1). On the other hand, in Rivastigmine treated animals, the IC neurons displayed an increased capacity to adapt to STDP conditioning and lower acetylcholine levels where relatively detected in their IC when compared to the control saline cohort. Taken together, these results supports the findings of Ayala and Malmierca (2015) to suggested that an increase of acetylcholine in the IC would result in a decreased capacity of IC neurons to acutely adapt.

Furthermore, Motts and Schofield (2009) reported that there are no cholinergic neurons located within the IC. The authors suggest that the cholinergic interactions within the IC are originating from cholinergic projections of other nuclei. Indeed, in a separate study, Schofield (2010), showed that cholinergic interactions from A1 via PPT/ LDT into the IC are likely to be a source of cholinergic activity within the IC. In this present study there

was a significant increase in the proportion of acetylcholine in the temporal lobe, the area where A1 is located. Previous in vivo studies that combined cholinergic promotion and electrophysiological techniques in A1, have shown that cortical neurons expand their receptive field at CF and this expansion was accompanied by the release of acetylcholine (Kilgard & Merzenich, 1998; Kilgard et al., 2001; Shepard et al., 2013). Therefore, A1 excitatory activity is associated with the release of acetylcholine in the context of cholinergic promotion. Taken together, cholinergic interactions from A1 to IC via PPT/LDT may be driving the responses properties seen in the IC. Further in vivo molecular techniques that can directly quantify levels of cholinergic activity paired with simultaneous in vivo multi-electrode array recordings of A1 and IC within this animal model will be needed to confirm if this is indeed the interaction occurring.

In conclusion, although the original hypothesis of this present study was not supported, the reasoning of the results supplements the results of Chapter Five. In line with Ayala and Malmierca (2015) findings, an increase of acetylcholine in the IC resulted in a decreased capacity of IC neurons to acutely adapt. In a potentially similar manner, saline treated animals had higher levels of acetylcholine in their IC, and showed a decreased capacity to adapt to the STDP conditioning (Chapter Five). Although this study reports an increase of acetylcholine in the temporal lobe, further investigations are needed to confirm that A1 to IC cholinergic interactions via PPT and LDT are indeed driving the electrophysiological results of Chapter Four. In line with previous authors who have used the same dose and oral administration of Rivastigmine (Voss et al., 2016), this study provides further evidence that when paired with training, the chronic low doses of Rivastigmine indeed influences cholinergic activity in the central auditory system. The significance of this study is that chronic systemic oral administration of cholinergic enhancement paired with auditory training interacts with the central auditory system in a manner that may have value for therapeutic means.

Chapter 7
General Discussion

7.1 General Discussion

Tinnitus is a common and potentially debilitating condition that is often associated with hearing loss. Noise induced hearing loss is a frequently utilised animal model, in studies investigating the underlying mechanisms of tinnitus (Dobie, 2003; Heller, 2003). In the central auditory system, the inferior colliculus (IC) is a near obligatory nucleus for the ascending processing of auditory signals (Schreiner & Winer, 2005). It has been comprehensively investigated in animal models of unilateral noise induced hearing loss (UNIHL) (Robertson & Irvine, 1989; Illing et al., 2005; Dong et al., 2010; Mulders & Robertson, 2011; Papesh & Hurley, 2012; Mulders & Robertson, 2013; Coomber et al., 2014; Ropp et al., 2014). Previous studies have mostly investigated the dominant, contralateral pathway with little attention being paid to the ipsilateral path. In normal hearing animals, contralaterally driven IC neurons are primarily excitatory while ipsilaterally driven IC neurons are primarily inhibitory (Semple & Kitzes, 1985; Hutson, 1997; Oliver, 2005). However, there are also ipsilaterally driven IC neurons that are excitatory. Literature that have investigated the response properties of these neurons, and their consequential response properties within an animal model of UNHIL is very limited. To date, this is the first study that has investigated the response properties of both contralaterally and ipsilaterally excitatory IC neurons, and the consequences that occur in both the left and

right ICs in an animal model of UNIHL.

Several studies have shown that following UNIHL, significant changes in the response properties of contralaterally driven IC neurons occurs. These changes include altered hyperactivity (Ma et al., 2006; Mulders & Robertson, 2013), spontaneous firing rates (Moore & Kowalchuk, 1988; Wang et al., 2013), changes in the receptive field (Szczepaniak & Moller, 1996; Wang et al., 2013; Coomber et al., 2014) and the presence of a lesion projection zone (LPZ)(Snyder et al., 2000; Schreiner & Winer, 2005). In line with these finding from previous authors (Snyder et al., 2000; Eggermont & Roberts, 2004), in this study, a LPZ was observed in IC neurons excited contralaterally from the lesioned ear. Adding to these findings, the present study provides evidence that, in addition to changes observed in the contralaterally driven IC neurons, significant changes were also found in the ipsilaterally driven IC neurons. Characteristic frequency analysis of the tonotopic organisation of the IC neurons ipsilaterally excited from the lesioned ear indicated that these neurons were largely unaffected by UNIHL. Indeed, there were no discontinuities observed in the CF distribution. However, as a variety of response properties were investigated in normal hearing animals, these ipsilaterally excited IC neurons showed characteristics of central gains in excitatory activity, evidenced by the dominant monotonic response at CF and the dominant binaural EE interactions that were observed in this population of neurons. Conversly, in animals that had been subjected to an acoustic trauma, ipsilaterally driven IC neurons showed that this function of a central gains in excitatory activity, was less so. In fact, the changes indicated an increase of inhibitory drive within the response properties observed. These changes were also observed in IC neurons that were excited ipsilaterally from the intact ear.

Furthermore, previous authors have shown that the removal of input from the dominant, excitatory, contralateral ear produces a marked increase in the level of excitation in response to ipsilateral stimulation of the intact ear (McAlpine et al., 1997; Mossop et

al., 2000). The results of the present study are in agreement with these authors, in that, the number of IC neurons ipsilaterally excited from the intact ear increased when compared to normal hearing controls. In fact, as a percentage these neurons comprised 47% of excitatory neurons found in the right ICs of treated animals, whereas in normal hearing animals, typically only 30% of excitatory neurons were found to be ipsilaterally driven. The significance of these results suggests, even though there is hearing loss on the left side of the animals, there are profound consequences that occur in both the IC neurons that are excited from the lesioned and intact ear.

Several studies have suggested that acetylcholine plays a modulatory role in the central auditory system, potentially by affecting the auditory system in ways that primes the system to consider certain sounds to be behaviourally or physiologically relevant (Hasselmo & McGaughy, 2004; Sarter et al., 2005; Jääskeläinen et al., 2007; Ayala & Malmierca, 2015; Voss et al., 2016). These studies also highlighted the great potential for exploiting cholinergic modulation in central maladaptive conditions that manifests as a result of peripheral sensory deficits. The results reported in Chapter 3, suggested that any attempt to remediate the neural consequences of UNIHL will need to also consider the interactions between the contralateral and ipsilateral driven IC neurons. In Chapter 4, within an animal model of UNIHL we investigated the effect of paired environmental interventions, in the form of auditory training (AT), which consisted of plugging the intact ear and exposing the affected ear with training tones, and long-term cholinergic promotion with the aim of altering and guiding the malleability that occurs after an acoustic trauma.

In the UNIHL animals that were subjected to AT and cholinergic enhancement, there was a significant change in the response properties of the ipsilaterally driven IC neurons. In IC neurons ipsilaterally excited from the lesioned ear, cholinergic enhancement with AT resulted in a higher proportion of this population to display tighter and narrower receptive fields when compared to UNIHL control. Furthermore, in IC neurons that were

contralaterally excited from the lesioned and intact ear, an almost equal proportion of the EI binaural interaction was found, 84% and 82%, respectively. In contrast, in the saline control cohort that were subjected with the same AT, in IC neurons contralaterally excited from the lesioned and intact ears, the percentage of EI binaural interactions were 94% and 74%, respectively. In these animals, there was 20% difference in EI binaural interactions between the left and right ICs. This demonstrates that the cholinergic system combined with AT can influence auditory processing in the IC. More specifically, it is shown here that the cholinergic system influences the proportion of EI binaural interactions between the two ICs, even though there is profound hearing loss to the left side of these animals. The significance of these results show that ipsilaterally driven IC neurons are profoundly influenced by AT paired with Rivastigmine which is observed in their receptive fields and in their contribution in EI binaural interactions.

Interestingly, even though ipsilaterally driven IC neurons were profoundly affected by AT and Rivastigmine treatment, contralaterally driven IC neurons were affected by AT and this effect was independent of cholinergic enhancement. This finding highlights the importance of looking at both the contralaterally and ipsilaterally driven IC neurons. The interpretation of the effect of long term cholinergic enhancement would have otherwise yielded a false negative conclusion, had only the contralaterally driven IC neurons been analysed. Schofield (2010) (Motts & Schofield, 2009) reported that cholinergic contacts from the auditory cortex (A1) via the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT) to the IC are seen contralaterally and ipsilaterally. In addition, the authors reported there was a higher density of cholinergic modulation found ipsilaterally. This suggested that physiologically, there may be more 'scaffolding' in the descending ipsilateral pathway that can be exploited pharmacologically, a possible explanation for the differences seen in the response properties of contralaterally and ipsilaterally driven IC neurons in Chapter 4.

Another pathway that may be implicated is the corticocolluicular (CC) pathway. While the exact function of the CC pathway remains largely unknown, activation of this pathway can rapidly and profoundly change the tuning properties of neurons in the IC (Yan & Suga, 1998; Sun et al., 2007; Bajo et al., 2010; Anderson & Malmierca, 2013). Furthermore, in the ferret, CC neurons are observed to be critical with regards to learning-induced auditory plasticity (Bajo et al., 2010). Taken together, because Rivastigmine was administered orally in this study, the systemic effects may indeed influence the interactions within the CC pathway. In the rat, A1 or anterior auditory field injections produce CC terminations on mainly two strips of labelling; one located on the external cortex (EC) and the dorsal cortex (DN) of the IC and although some projections are seen in the central nucleus (CNIC) it is generally considerably smaller in size (Saldaña et al., 1996; Winer et al., 2002). Although smaller in size, it is possible A1/CC/CNIC interactions are influencing the results seen Chapters 3 and 4, however, histological staining of AChE of this pathway reveals significant concentrations of AChE modules within the EC and DC only and not the CNIC (Chernock et al., 2004), which indicates there maybe microcircuits within the IC that the CC pathway itself is influencing.

Jen et al. (2001) tested the hypothesis that A1 stimulation will result in inhibitory activity from the lateral cortex of the IC (LC) to the CNIC. Their results revealed three significant findings; 1) AC stimulation resulted in an increased of acoustic responsiveness within LC neurons, in terms of spatial response area, rate level functions and frequency response areas, 2) AC stimulation results in a decrease of acoustic responsiveness in CNIC neurons, 3) stimulation of LC results a similar decrease of acoustic responsiveness of CNIC neurons. Specifically, azimuth responsiveness was reduced in all directions, a decrease in rate level function and FRAs showed significant narrowing (Jen et al., 2001). Taken together, histologically as there is the presence of AchE within CC terminations in LC and electrophysiologically, LC to CNIC projections result in inhibitory interactions, it is possible that the Rivastigmine intervention systemically affected the CC pathway which

lead to an increase of LC/CNIC inhibitory activity. Indeed, the result of this study observed, an increase of narrowed FRAs as well as an increase of the EI binaural interactions in ipsilaterally driven IC neurons. Furthermore, as the CC pathway is a primarily an ipsilateral descending projection, this may explain why the increase of inhibitory response characteristics was not observed in contralaterally driven IC neurons.

The effect of AT in contralaterally driven IC neurons observed in Chapter 4 are in line with other authors who also observed changes in the auditory system when the auditory environment immediately after the acoustic trauma was controlled (Noreña & Eggermont, 2005; 2006). Noreña and Eggermont (2005) demonstrated that immediately after an acoustic trauma, the central auditory system is in a malleable state, particularly primed to experience-dependent plasticity. After acoustic trauma, cats were placed in an enriched acoustic environment (EAE) and passively exposed to content representative of a wide range of frequencies and level combinations. Their results showed that in A1, the re-organised tonotopic map and the accompanied response characteristics that are typically seen as a consequence of acoustic trauma, was prevented. In this present study, in the contralaterally driven IC neurons, the percentage of monotonic and non-monotonic responses, as well as the onset and tonic responses at CF, were not statistically different to the percentages typically observed in normal hearing animals. This suggests that similar to A1, in contralaterally driven IC neurons that were excited from the lesioned ear, some of the accompanied response characteristics that are a consequence of acoustic trauma, were also prevented.

Schofield (2010) reported cholinergic contacts from the auditory cortex (A1) via PPT/LDT to the IC are seen contralaterally and ipsilaterally. This suggested that anatomically, there are cholinergic interactions that could occur in contralaterally driven IC neurons. Furthermore, several in vivo electrophysiological studies in the IC, have shown that more

than 80% of IC neurons showed a change in their response property in the prescence of acetylcholine (Watanabe & Simada, 1973; Farley et al., 1983; Habbicht & Vater, 1996). A possible explanation as to why, the effects of cholinergic promotion was not observed in contralaterally driven IC neurons (Chapter 4), is that the response properties measured were likely not sensitive enough. Indeed, the aforementioned authors reported their in vivo electrophysiological findings based on acute IC responses to acetylcholine, where an increase of acetylcholine was produced using microiontophoretic injections of acetylcholine antagonists or agonists. With a consideration to acute IC responses, an investigation of stimulus-timing dependent plasticity (STDP) in the IC was explored. By subjecting the system to STDP conditioning, insights to the ability of the synapses to adapt acutely was explored and further tested to determine if this adaption was sensitive to cholinergic enhancement.

In the auditory system, STDP has been demonstrated using paired frequency tones and paired somatosensory and auditory stimulation (Tzounopoulos et al., 2004; Dahmen et al., 2008; Basura et al., 2015). The areas of the auditory system that have been studied include A1 (Dahmen et al., 2008; Basura et al., 2015) and the DCN (Tzounopoulos et al., 2004). For the first time, STDP in the IC was investigated. The acute capacity of STDP was opportunistically explored using paired frequency tones that were delivered to the intact ear, in an animal model of UNIHL. The results indicated two major consequences; 1) IC neurons respond in an STDP-like manner in vivo and 2) in a UNIHL animal model, four weeks of Rivastigmine treatment, differentially affects the response properties of IC neurons when subjected to STDP conditioning. In that, acetylcholine promotion resulted in IC neurons having an increased capacity to respond in an STDP-like manner.

The results presented in Chapters 4 and 5 add to the growing body of evidence to suggest that in the central auditory system, cholinergic modulation generally acts to optimise the processing of signals in attention demanding contexts (Hasselmo & McGaughy, 2004;

Sarter et al., 2005; Jääskeläinen et al., 2007; Ayala & Malmierca, 2015). Although the results of Chapters 4 and 5 were novel, it remains to be determined if the electrophysiological changes were a direct result due to an increase of acetylcholine levels.

Previous studies in rats have shown that Rivastigmine treatment prolonged for over seven days alters the expression of cholinergic associated proteins in the brain (Tayebati et al., 2004; Voss et al., 2016). Although the relationship between Rivastigmine treatment and the changes in the expression of cholinergic associated proteins in the brain is valuable, the question remains as to the direct effect the treatment had on the levels of acetylcholine in the different areas of the central auditory system. It was hypothesised that Rivastigmine treatment paired with AT would increase levels of acetylcholine in the IC. To investigate if this was true, homogenised brain regions were analysed using UHPLC-MS/MS. As a result of four weeks of Rivastigmine treatment paired with auditory training, the results showed two consequences; 1) in the IC of Rivastigmine treated animals, a significant decrease in the amount of acetylcholine in the IC was observed when compared to saline treated animals and, 2) there was a higher proportion of acetylcholine in the temporal lobe, when compared to saline treated animals.

Ayala and Malmierca (2015) investigated stimulus specific adaption (SSA) in the IC using paired in vivo electrophysiology and iontophoresis injections of acetylcholine antagonists to increase acetylcholine levels locally in the IC. The authors showed that increased levels of acetylcholine resulted in IC neurons to maintain the encoding of repetitive sounds more selectively. Therefore, there was a decrease in the ability of the IC neurons to adapt to the novel tone (Ayala & Malmierca, 2015; Ayala et al., 2016). In this study, the saline treated animals showed a decreased capacity to adapt to the STDP conditioning (Chapter 5) with higher levels of acetylcholine in their ICs (Chapter 6). Although SSA and STDP are different response properties, both paradigms tested IC

neurons in an acute and adaptive manner. Taken together, these results support the findings of Ayala and Malmierca (2015) to suggested that an increase of acetylcholine in the IC would result in a decreased capacity of IC neurons to acutely adapt. Although the hypothesis was not supported, a possible explanation is that, Rivastigmine paired with AT results in an increased capacity for IC neurons to adapt to the STDP conditioning and therefore this is reflected in the decreased amount of acetylcholine analysed in the IC of these animals.

When compared to saline treated animals, Rivastigmine treated animals had significantly higher levels of acetylcholine within their temporal lobe, an area where A1 is located (Chapter 6). In vivo studies that combine cholinergic promotion and electrophysiological techniques in A1, have shown that the receptive field of the cortical auditory neurons are indeed malleable. In the context of cholinergic promotion, cortical neurons expand their receptive field at CF and this expansion was accompanied by the release of acetylcholine (Kilgard & Merzenich, 2001; Kilgard et al., 2007; Shepard et al., 2013). Thus, A1 excitatory activity is associated with the release of acetylcholine within the context of cholinergic promotion. It is unclear if the increase of acetylcholine in A1 is driving the altered response properties of IC neurons seen in Chapters 4 and 5. Further in vivo molecular techniques that can directly quantify levels of cholinergic activity paired with simultaneous in vivo multi-electrode array recordings of A1 and IC within this animal model will be needed to confirm if this is indeed the interaction occurring.

These findings have shown that in an animal model of UNIHL, cholinergic enhancement paired with AT profoundly affected the response properties of IC neurons. The consequences of UNIHL and the impact of the interventions were different in contralaterally and ipsilaterally driven IC neurons. This highlights the importance of investigating both dominant and non-dominant pathways of the central auditory system. From a general perspective, the results of the studies in this thesis may have implications

for the development of therapies for tinnitus. By using a human equivalent dose of an approved drug, paired with a well-known neuro-rehabilitation intervention, this body of work provides evidence that in a physiological animal model of tinnitus, these inventions can profoundly influence the consequential response properties of IC neurons following acoustic trauma. In a condition where currently there are no reliable cures, further development of these findings may provide insights that could lead to the generation of novel therapeutic approaches.

8.1 References

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