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Using novel diffusion magnetic resonance imaging techniques to aid in the diagnosis of auditory neuropathy

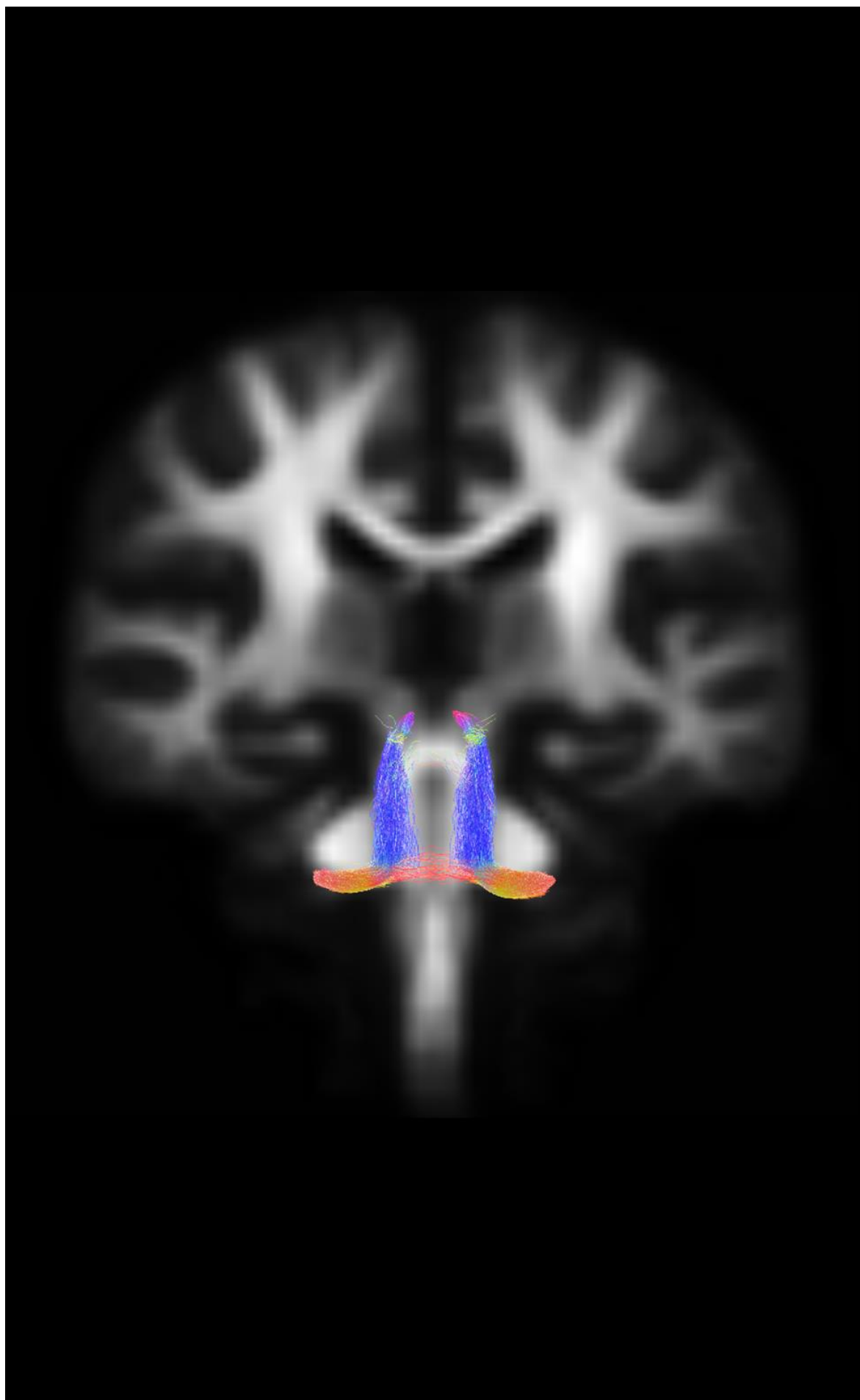
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Abstract

‘Auditory neuropathy’ is a nosological term coined by Starr et al. (1996) used to describe a group of hearing disorders, which are characterised by abnormal auditory nerve activity in the presence of normal cochlear outer hair cell function. While our understanding of this condition has advanced significantly since it was first identified almost 30 years ago, limitations in current diagnostic assessments make it difficult for clinicians to determine both the site(s) of lesion *and* the extent of dysfunction in an affected individual. As such, determining the most appropriate management strategy can be difficult. This is particularly salient for cochlear implantation, which is currently the intervention strategy of choice for individuals with auditory neuropathy. Cochlear implant outcomes, however, vary considerably and the inconsistency in post-operative speech perception ability is thought to be directly dependent on both the site(s) of lesion and extent of dysfunction.

In this thesis, advanced diffusion MRI techniques were used to investigate changes in the white matter fibres of the VIII nerve and auditory brainstem in individuals with different forms of auditory neuropathy. Three major research questions formed the basis of this thesis and subsequently the three studies contained herein: (1) Can dMRI-based tractography techniques be used to reconstruct the pathways of the auditory brainstem *in-vivo* using clinically accessible MRI field strengths?; (2) Can newly developed dMRI-based quantitative analysis techniques be used to elucidate neurodegenerative changes within the brainstem of a genetically defined group of individuals with X-linked auditory neuropathy?; (3) Can these techniques be used to further our understanding of the underlying lesion site(s) and extent of neural dysfunction in a group of individuals with auditory neuropathy due to different aetiologies? The first study investigated the use of different diffusion models and tractography algorithms. Using these techniques, the pathways between the left VIII nerve and bilateral inferior colliculi were mapped in three normal hearing control subjects. Furthermore, the accuracy of tract reconstructions was compared between two different diffusion models: (i) the classic diffusion tensor model, and (ii) a high-order diffusion model called Single-Shell 3-Tissue constrained spherical deconvolution. Finally, the benefits of using the Anatomically-Constrained Tractography algorithm were also examined. To address question two, the second study used a novel dMRI-based quantitative analysis measure called Apparent Fibre Density as obtained from Single-Shell 3-Tissue Constrained Spherical Deconvolution and analysed with the fixel-based analysis framework to investigate white matter fibre-specific degeneration in a genetically characterised group of individuals with X-linked auditory neuropathy. This case-control study showed that individuals with X-linked auditory neuropathy had axonal degeneration throughout the auditory pathways, with the most significant degeneration being localised to the VIII nerve region. The results were supported by several objective auditory measures, which showed normal cochlear sensory function but significantly disrupted auditory

nerve activity. Moreover, the results showed that the degree of temporal processing deficit was related to the severity of axonal degeneration in individuals with X-linked auditory neuropathy. Behavioural auditory assessments of sound detection, speech perception, and the processing of binaural cues were also significantly poorer in affected individuals compared to the normal hearing controls. Finally, the third study of this thesis used similar techniques to the second study, this experiment examined axonal degeneration in the VIII nerve region of individuals with auditory neuropathy from various aetiologies. Results were compared against participants with normal hearing. The Apparent Fibre Density findings showed that the auditory neuropathy group had significantly fewer auditory nerve fibres compared to controls. Overall, the Apparent Fibre Density metric was significantly correlated with auditory measures including, sound detection thresholds, speech perception and temporal processing ability, auditory brainstem response amplitudes, and binaural processing ability. Furthermore, the degree of axonal degeneration observed in each affected individual was consistent with the other available data, including clinical information, such as disease progression/duration of disease, and the known/assumed pathophysiological mechanism(s) underlying the auditory neuropathy phenotype.

The results of this thesis offer insights into the site of lesion(s) and pathophysiological process underlying the auditory neuropathy phenotype. Furthermore, the findings provide evidence for the ability of advanced diffusion MRI techniques to be used as a diagnostic tool to examine the auditory subcortical white matter pathways *in-vivo*. Hence this technique may prove to be a useful adjunct to existing auditory assessments, which may in the future guide rehabilitation options for individuals with auditory neuropathy. Importantly, these techniques may be applied to related conditions where VIII nerve axonal density may affect patient outcomes, including managing cochlear or brainstem implant candidacy.

Declaration

This is to certify that:

- (i) the thesis comprises only my original work towards the PhD except where indicated in the Preface,
- (ii) due acknowledgement has been made in the text to all other material used,
- (iii) the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Name: Julien Zanin

Signed:.....

Date:.....

List of Publications

1. **Zanin, J.**, Dhollander, T., Rance, G., Yu, L., Lan, L., Wang, H., Lou, X., Connelly, A., Nayagam, B.A., & Wang, Q. (Under review). Fibre specific changes in white matter microstructure in individuals with X-linked auditory neuropathy. *Ear and Hearing*.
2. **Zanin, J.**, Dhollander, T., Farquharson, S., Rance, G., Connelly, A., & Nayagam, B. A. (2019). Using diffusion-weighted magnetic resonance imaging techniques to explore the microstructure and connectivity of subcortical white matter tracts in the human auditory system. *Hearing Research*, 377, 1-11.
3. Dhollander, T., **Zanin, J.**, Nayagam, B. A., Rance, G., & Connelly, A. (2018). Feasibility and benefits of 3-tissue constrained spherical deconvolution for studying the brains of babies. In *Proceedings of the 26th annual meeting of the International Society of Magnetic Resonance in Medicine*, (p. 3077).

Preface

This thesis is my original work and, to the best of my knowledge, contains no material written by another person, except where due acknowledgement has been made.

Some of the material and content of this thesis has been published in Hearing Research in 2019. The following people have made significant contributions as co-authors on the publication: Dr Thijs Dhollander, Ms Shawna Farquharson, Prof. Gary Rance, Prof. Alan Connelly and A/Prof. Bryony Nayagam. The publication has been included in Appendix 1 for reference purposes. Furthermore, the entirety of Chapter 3 of this thesis is composed of the article “Fibre specific changes in white matter microstructure in individuals with X-linked auditory neuropathy” submitted for publication in Ear and Hearing and is currently under review. The following people have made significant contributions to this manuscript as co-authors: Dr Thijs Dhollander, Prof. Gary Rance, Ms Lan Yu, Ms Lan Lan, Dr Hongyang Wang, Dr Xin Lou, Prof. Alan Connelly, A/Prof. Bryony Nayagam. and Prof. Qiuju Wang.

The content of this thesis does not contain any work that was carried out prior to the commencement of my research higher degree candidature.

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List of Abbreviations

3DFT – CISS	Three-Dimensional Fourier Transformation-Constructive Interference in Steady State
4FA	Four Frequency Average
ABR	Auditory Brainstem Response
AC	Auditory Cortex
ACT	Anatomically Constrained Tractography
AFD	Apparent Fibre Density
AM	Amplitude Modulation
AN	Auditory Neuropathy
AP	Action Potential
AUNX1	X-Linked Auditory Neuropathy
AVCN	Anterior Ventral Cochlear Nucleus
CAEP	Cortical Auditory Evoked Potential
CAP	Compound Action Potential
CDTM	Chinese Definition Testing Monosyllables
CM	Cochlear Microphonic
CN	Cochlear Nucleus
CMT	Charcot-Marie-Tooth
CSD	Constrained Spherical Deconvolution
CSF	Cerebrospinal Fluid
dB	Decibel
dB HL	Decibel Hearing Level
dB nHL	Decibel Normalised Hearing Level
dB SPL	Decibel Sound Pressure level
DCN	Dorsal Cochlear Nucleus
DEC	Directional Encoded Colour
dMRI	Diffusion Magnetic Resonance Imaging
DNLL	Dorsal Nucleus of the Lateral Lemniscus
DPOAE	Distortion Product Otoacoustic Emissions
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Image
ECochG	Electrocochleography
EMF	External Magnetic Field

EPI	Echo Planar Imaging
ER	Etymotic Research
FLAIR	Fluid Attenuated Inversion Recovery
FOD	Fibre Orientation Distribution
FM	Frequency Modulated
fMRI	Functional Magnetic Resonance Imaging
FoV	Field of View
FRDA	Friedreich's Ataxia
FWE	Family Wise Error
GM	Grey Matter
HARDI	High Angular Resolution Diffusion Imaging
IC	Inferior Colliculus
IHC	Inner Hair Cell
iFOD	Integration over Fiber Orientation Distributions
ITD	Interaural Timing Difference
kHz	Kilohertz
LiSN-S	Listening in Spatialised Noise-Sentences Test
LL	Lateral Lemniscus
LSO	Lateral Superior Olive
MGB	Medial Geniculate Body
MNTB	Medial Nucleus of the Trapezoid Body
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MSO	Medial Superior Olive
MSP	Mandarin Speech Perception
NMR	Nuclear Magnetic Resonance
NMV	Net Magnetisation Vector
OAE	Otoacoustic Emissions
OHC	Outer Hair Cell
OPA1	Optic Atrophy 1
OTOF	Otoferlin
PMP22	Peripheral Myelin Protein 22
PLAGH	People's Liberation Army General Hospital
PTA	Pure Tone Audiometry

PVCN	Posterior Ventral Cochlear Nucleus
RF	Radio Frequencies
ROI	Region of Interest
RTD	Riboflavin Transporter Deficiency
SGN	Spiral Ganglion Neurons
SP	Summating Potential
SR	Spontaneous Rate
SS3T	Single-Shell 3-Tissue
T	Tesla
TEOAE	Transient Evoked Otoacoustic Emissions
TFS	Temporal Fine Structure
VCN	Ventral Cochlear Nucleus
VEMP	Vestibular Evoked Myogenic Potential
vHIT	Video Head Impulse Test
VNLL	Ventral Nucleus of the Lateral Lemniscus
WM	White Matter

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Overview

Auditory neuropathy (AN) is a condition affecting the auditory system and is characterised by the presence of normal cochlear outer hair cell function with absent or disordered auditory nerve function (Rance & Starr, 2015; Starr et al., 1996; Starr et al., 2001). Clinically, otoacoustic emissions (OAEs) and/or cochlear microphonics are used to assess outer hair cell integrity and an averaged auditory brainstem response (ABR) is used to measure neural synchrony and neural conduction time within the auditory brainstem. A clinical diagnosis of AN is therefore dependent on the presence of otoacoustic emissions and/or a cochlear microphonic (CM), in conjunction with severely disordered or absent ABRs.

Pure-tone hearing thresholds obtained from individuals with AN vary considerably. While some affected individuals have normal hearing, others have profound cochlear sensory hearing loss, affecting either one or both ears (Rance et al., 1999; Starr et al., 1996). Fluctuating hearing thresholds have also been reported in some affected individuals (Starr et al., 1998). In regard to functional deficits, the most notable are speech perception and comprehension difficulties. In particular, a significant number of individuals exhibit speech recognition deficits disproportionate to the extent of their hearing loss (Kraus et al., 2000; Rance et al., 2008). Furthermore, deficits in temporal processing and binaural sound integration are also commonly reported characteristics (Rance et al., 2012e; Zeng et al., 2005; Zeng et al., 1999).

The hallmark clinical features of AN were first described in the mid-1980's by Kraus et al. (1980) who reported findings of absent ABRs in a cohort of children with relatively normal hearing thresholds. They hypothesised that the disparity in the electrophysiological measures and behavioural audiological results were due to brainstem dysfunction. More than a decade later Starr et al. (1996) coined the term 'auditory neuropathy' to describe this type of auditory impairment. In the period since, researchers in the field have offered a number of alternate descriptors in an attempt to define the pathophysiological mechanism(s) that underlies AN. Berlin et al. (2001) have advocated to rename 'auditory neuropathy' to 'auditory dys-synchrony' as the latter term better reflects the type of neural disruption that has been posited to be taking place. Moser et al. (2006) have suggested 'auditory neuropathy/synaptopathy' based on evidence from clinical studies that show inner hair cell to auditory afferent synaptopathy in a group of individuals with the standard clinical presentation of AN. Starr et al. (2000) have also proposed 'pre-synaptic and post-synaptic nerve disorder' to reflect the varying sites of lesion that have since been categorised. In an attempt to encompass all aspects of this heterogeneous condition, the broad term 'auditory neuropathy spectrum disorder' has also been proffered (Roush, 2008). The search for an accurate descriptor is an indication of an emerging understanding of the underlying mechanism(s) and pathologies that results in individuals with similar electrophysiological results but a large variability in outcomes and functional deficits.

In Australia, AN makes up ~ 10% of all permanent childhood hearing losses (Ching et al., 2013; Rance, 2005). The prevalence worldwide has been shown to be consistent with these findings (Berlin et al., 2000; Cheng et al., 2005; Foerst et al., 2006; Vlastarakos et al., 2008) and in some regions, reportedly higher. For example, a study conducted in the Western Anatolian region of Turkey by Kirkim et al. (2008) found the rate of AN to be 15% in a group of 65 children with permanent hearing loss. Another study conducted in Singapore reviewed 52 newborns with hearing loss, 17% of this group presented with AN (Ngo et al., 2006). Although these studies are based on small sample sizes, it is possible that a higher prevalence in certain regions may be attributable to genetics, as region specific genetic abnormalities have been shown to contribute significantly to the prevalence of the AN phenotype (Manchaiah et al., 2011; Wang et al., 2003)

A multitude of risk factors and aetiologies exist, that can result in either congenital or acquired AN. The main cause of congenitally acquired AN is attributable to genetic abnormalities, occurring either as hereditary non-syndromic mutations, such as X-linked auditory neuropathy (AUNX1) (Wang et al., 2006) and Otoferlin (*OTOF*) related AN (Rodriguez-Ballesteros et al., 2003), or as part of a syndrome (Manchaiah et al., 2011). Some examples of syndromic related AN include, Friedrich's Ataxia (FRDA) (Rance et al., 2009; Satya-Murti et al., 1980), Charcot-Marie-Tooth (CMT) disease (Rance et al., 2012d; Starr et al., 2003), Leber's Hereditary Optic Neuropathy (Ćeranić & Luxon, 2004; Rance et al., 2012c), Mohr-Tranebjaerg Syndrome (Merchant et al., 2001; Santarelli, 2010), and Refsum's Disease (Bamiou et al., 2003). In regards to acquired AN, possible risk factors comprise of prematurity, oxygen deprivation, hyperbilirubinaemia, perinatal intracranial pressure, ototoxic drugs, demyelinating conditions (multiple sclerosis, HIV/AIDS), autoimmune disorders (Guillain Barre syndrome), neoplastic conditions (acoustic neuroma and other tumours), metabolic disturbances (diabetes), and infectious agents (measles, mumps) (Moser & Starr, 2016; Rance, 2005; Starr et al., 2003; Starr et al., 1996). Furthermore, recent work by Furman et al. (2013) has shown selective degeneration of low spontaneous rate (high threshold) afferent fibres due to acoustic overexposure. This fibre type is not involved in threshold detection in a quiet environment but plays a crucial role in the ability to hear in the presence of background noise (Kujawa & Liberman, 2009). Hence, the functional deficits observed in individuals with noise-induced hearing loss are markedly similar to those observed in AN and may be explained, in part, by this selective loss of these afferent fibres. Despite the comprehensive list of possible causes, in a large proportion of cases the aetiology remains unknown, which is likely to be attributable to the lack of diagnostic assessments sensitive enough to identify the site(s) of lesion (Starr et al., 2000).

Presently, audiological management for individuals with AN can include provision of hearing aids, cochlear implantation, and/or assistive listening devices (Rance & Starr, 2015). However, an evidence based systematic literature review performed by Roush et al. (2011) shows that there is currently not sufficient evidence available to guide clinicians as to the most appropriate rehabilitation strategy for

any given patient. Opinion regarding the benefit and choice of hearing aids/acoustic amplification and cochlear implants to individuals with AN has been a topic of significant debate (Moser & Starr, 2016; Rapin & Gravel, 2003). Several researchers have argued that providing acoustic amplification to increase the accessibility to sounds is ineffectual because it only serves to make an already distorted and disordered signal louder (Berlin et al., 2002; Moser & Starr, 2016; Rance, 2005; Raveh et al., 2007). Furthermore, others have taken the stance that amplification may result in acoustic trauma to functional outer hair cells in individuals with AN, which may cause a subsequent deterioration in hearing thresholds (Doyle et al., 1998; Hood, 1998). Although, there is currently no evidence to support this hypothesis, cochlear implants have been adopted as the preferred intervention approach for the majority of patients with AN. Preference for cochlear implant rehabilitation in this cohort, however, is also problematic. Evidence suggests that cochlear implant outcomes in individuals with AN is significantly more variable compared to those individuals with cochlear sensory hearing loss alone (Gibson & Sanli, 2007; Roush et al., 2011; Teagle et al., 2010). This large range and unpredictability in cochlear implant outcomes has been attributed to the variability in lesion sites that exists in AN (Rance & Starr, 2015). Individuals with AN due to inner hair cell loss/dysfunction, disruption of the synaptic terminals between the inner hair cells and the terminal dendrites or a complete loss of terminal dendrites have been shown to achieve relatively good speech perception outcomes with cochlear implantation (Gibson & Sanli, 2007; Santarelli et al., 2011). On the other hand, individuals with AN because of a site of lesion at the spiral ganglion neurons or at more central locations within the auditory brainstem have much more variable results (Rance & Starr, 2015).

Knowledge and understanding of AN has gained significant ground over the last two decades. Research in the area has led to the identification of different underlying mechanisms and sites of lesion. Consequently, a considerable framework has been established in an attempt to categorise the numerous aetiologies and link these with potential lesion sites within the auditory system (Moser & Starr, 2016; Rance & Starr, 2015; Rapin & Gravel, 2003; Santarelli, 2010; Starr et al., 2010). Gaps in our understanding, however, persist, particularly in predicting functional ability and remediation outcomes from the current models of pathophysiological processes. Further research is necessary to develop diagnostic techniques capable of determining the degree and site of dysfunction as well as categorisation of more wide-spread abnormalities that may be occurring within the peripheral and central auditory system in individuals with AN.

Recent advances in the field of diffusion weighted magnetic resonance imaging (dMRI) and the associated field of white matter tractography is providing the potential to image and map the auditory fibres within the brainstem (Sitek et al., 2019; Zanin et al., 2019). These technological advancements have produced novel ways to assess the microstructure and connectivity of white matter pathways, providing robust quantitative measures of fibre density. Hence, such techniques provide a tangible solution to the lack of diagnostic specificity available in assessing AN. It is proposed that the recent

advances in imaging and image analysis techniques offer a quantifiable way to assess neural abnormalities and the extent of fibre disruption that is occurring within the auditory brainstem of individuals with AN.

In the studies contained within this thesis, data was collected using a range of behavioural and objective auditory assessments and high angular resolution diffusion imaging (HARDI) from: (i) a group of individuals with a genetically characterised type of AN called X-linked auditory neuropathy (AUNX1) as well as a group of gender matched normal hearing controls; and, (ii) a group of individuals with the AN phenotype due to various aetiologies, and a group of age and gender matched controls. Advanced so-called ‘higher-order’ dMRI analysis techniques, such as Single-Shell 3-Tissue constrained spherical deconvolution (SS3T-CSD) and fixel-based analysis framework, were then used to investigate potential white matter changes within the subcortical auditory pathways in affected individuals.

This thesis is organised into two parts: the first part (Chapter 1) provides an overview of the concepts required to understand and contextualise the work presented within this thesis. The second part (Chapters 2 – 5) details the original contributions of this work. Specifically, Chapter 2 investigates the applicability of different diffusion modelling techniques and tractography algorithms to determine the method most suitable for mapping the fibre tracts of the auditory brainstem. The feasibility of using these techniques to study the auditory brainstem pathways in several different ages is also explored, as well as some existing limitations and future directions. Chapter 3 explores the use of the dMRI-based quantitative metric of Apparent Fibre Density (AFD), as part of the fixel-based analysis framework, to investigate white matter degeneration in the brainstem of individuals with AUNX1 (Raffelt et al., 2012b; Raffelt et al., 2017). Chapter 4 further investigates the use of the fixel-based analysis framework, specifically the AFD metric, to investigate white matter changes in the auditory brainstem tracts of individuals, including children, who present with the auditory neuropathy phenotype due to varying aetiologies. Apparent Fibre Density results are considered in the context of a wide range of behavioural and objective auditory assessments. Finally, Chapter 5 concludes the thesis by providing a discussion of the clinical relevance of this work and potential future applications.

Part I

Chapter 1: Background

1.1 The auditory system: a brief anatomical overview

In order to understand the key questions posited in this thesis, a thorough understanding of the human auditory anatomy and physiology is necessary, including potential sites of disruption that could contribute to the AN phenotype. Moreover, given the current thesis is investigating the integrity of the white matter tracts of the auditory system, a basic ability to recognise innervation patterns, and anatomical features of the central auditory nervous system is crucial in understanding this body of work.

1.1.1 The peripheral auditory nervous system: outer, middle, and inner ear

The mechanisms involved in auditory perception are some of the most intricate in the entire human body. The auditory system relies on numerous finely tuned components, which carry out transduction and translational processes to convert sound, a fundamentally mechanical occurrence, into an electrical signal for interpretation within the brain. The auditory system can be divided into three parts: the outer, middle, and inner ear (Figure 1.1). A sound wave's journey through the auditory system begins at the outer ear. Sound is collected by the pinna or auricle, after which it passes through the external auditory canal and vibrates the tympanic membrane. Vibrations from the tympanic membrane are passed onto the ossicular chain, made up of the three smallest bones in the human body – the malleus, incus and stapes. The ossicular chain ensures that sound vibrations are transmitted through the middle ear to the inner ear. Two muscles, named the musculus stapedius and the musculus tensor tympani, are also involved in this process. They serve a protective function and dampen the movement of the ossicular chain in response to high intensity sounds ($\sim >85$ dB SPL) (Katz et al., 1985; Metz, 1952). From the middle ear, sound vibrations pass to the cochlea, a fluid-filled spiral tube of the inner ear. Within the cochlea are the components necessary to transform the 'mechanical' movement of sound energy into electrical impulses, which are subsequently processed, filtered and interpreted in the brainstem nuclei and auditory cortex.

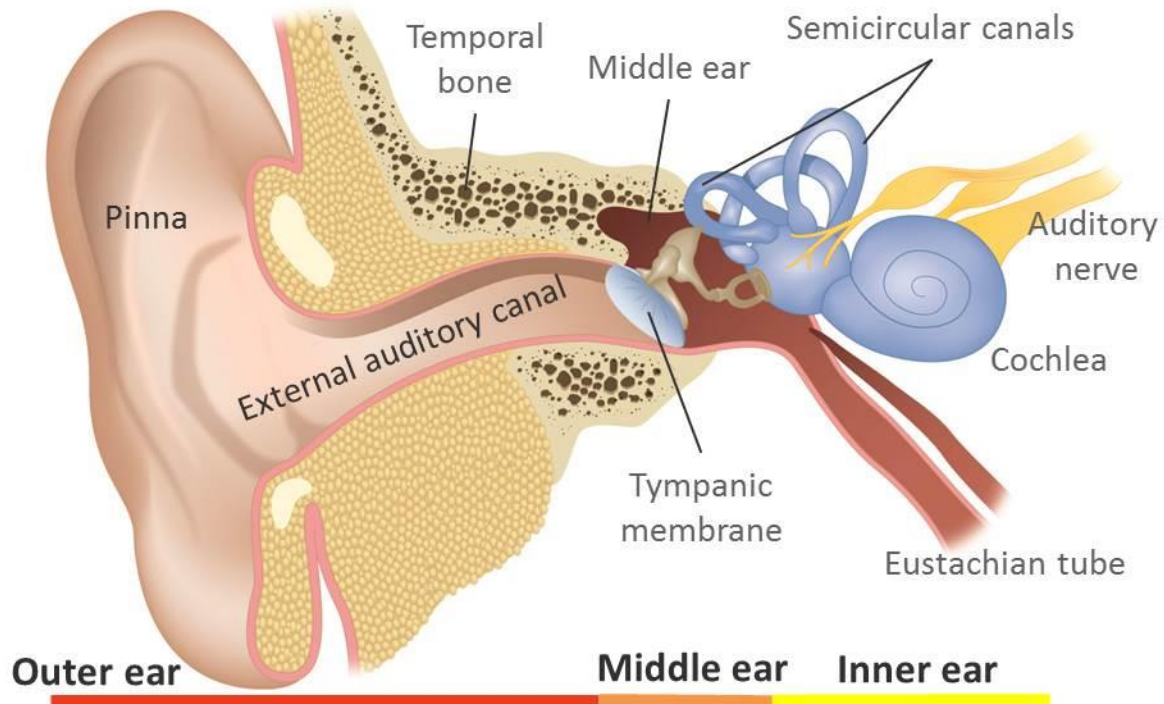


Figure 1.1 | Anatomy of the auditory system. An overview of the different components that make up the auditory system. The three major divisions, including the outer, middle and inner ear are shown.

1.1.1.1 The cochlea

The cochlea is composed of three fluid-filled compartments, the scala tympani, scala vestibuli, and scala media. The scala tympani and scala vestibuli are filled with perilymphatic fluid and are positioned either side of the endolymph filled scala media. The scala media houses the sensory epithelium of the cochlea, known as the organ of Corti (Figure 1.2), which sits on top of the basilar membrane. Within the organ of Corti lie two populations of specialised sensory cells, called the inner hair cells and the outer hair cells. In humans, the organ of Corti contains approximately 15,000 of these hair cells. They are arranged in rows with a specific formation, consisting of one row of inner hair cells and three to five rows of outer hair cells (Pickles, 2015). On the top of the sensory hair cells are tiny hair-like protrusions, called stereocilia, which facilitate the transmission of auditory information through the process of mechanotransduction. The tectorial membrane, an extracellular structure involved in hair cell depolarisation, rests on top of the stereocilia. Mechanical sound-induced movement of the ossicular chain is passed to the cochlea, whereby the vibrations produce movement of the endolymph and perilymph fluid. The resultant ‘wave-like’ motion causes deflection of the basilar membrane, forcing the stereocilia to be compressed against the tectorial membrane. The shearing force from the stereocilia pushing against the tectorial membrane results in the opening of mechanotransducer channels at their tips. A rapid influx of potassium (K^+) and calcium (Ca^{2+}) ions from the surrounding endolymph ensues, driven by a negative intracellular potential compared to the surrounding endolymph. This rapid change

in cell polarity results in depolarisation of the hair cells and the subsequent activation of the auditory nerve.

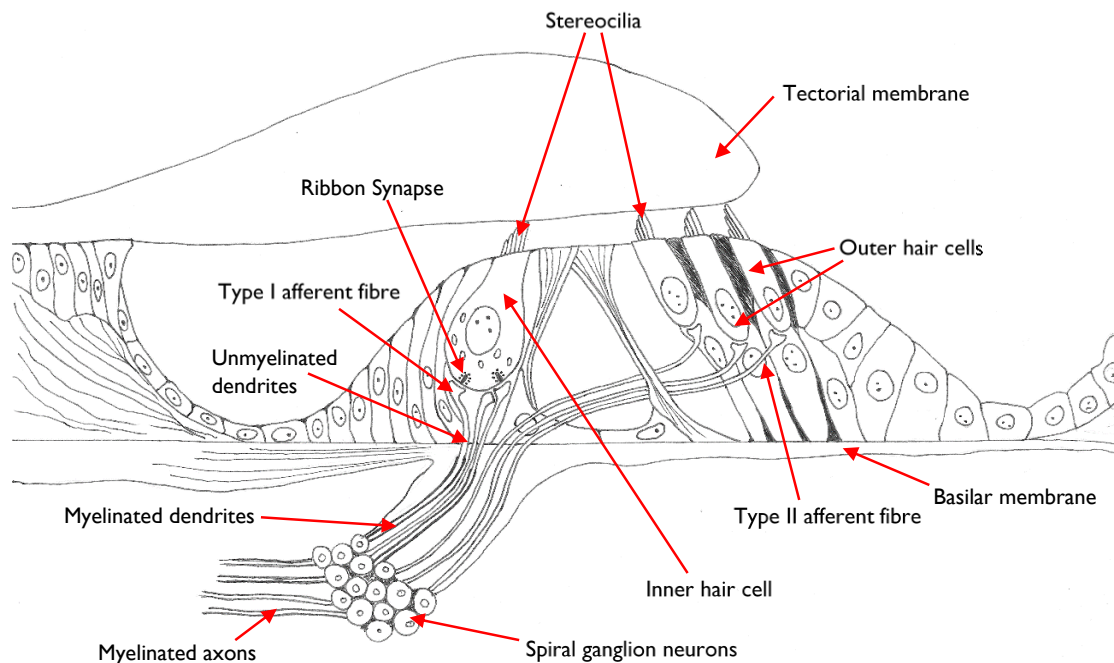


Figure 1.2 | Cross-section of the organ of Corti. Important structural features are labelled. The tectorial membrane, a gelatinous structure, sits atop the stereocilia of the cochlear amplifiers (outer hair cells) and the sensory receptors (inner hair cells) and facilitates neurotransmitter release from the ribbon synapses. This in turn, activates the afferent fibres. The type I afferents, which are the primary pathway between the organ of Corti and the cochlear nucleus within the brainstem consist of an unmyelinated dendritic portion, a myelinated dendritic portion, the spiral ganglion neurons, and myelinated axons. In comparison, the type II afferents supply the outer hair cells, are entirely unmyelinated and serve a less well-defined function in audition.

1.1.1.2 Depolarisation of the cochlear hair cells

Specialised pre-synaptic structures, called ribbon synapses (also commonly referred to as synaptic ribbons), control the depolarisation of the inner and outer hair cells. In particular, they ensure an extremely high rate of neurotransmitter release, which can be maintained in response to long auditory stimulation. This is achieved via several mechanisms: (i) by rapidly replenishing vesicles at the hair cell to auditory nerve synapse; (ii) ensuring the simultaneous release of multiple vesicles at the synaptic terminal, and; (iii) by ensuring released vesicles rapidly fuse with the hair cell membrane to facilitate rapid exocytosis and subsequent afferent stimulation (Glowatzki et al., 2008; Safieddine et al., 2012). Even though both the inner and outer hair cells are reported to have ribbon synapses (Hashimoto et al., 1990), their function and capability differs significantly, which can be explained by considerable differences in ultrastructure and functionality.

The ribbon synapses of the inner hair cells are highly ordered, with an easily identifiable ‘halo’ of vesicles surrounding the structure itself (Weisz et al., 2012). Upon depolarisation, each active zone, comprised of 1-2 ribbon synapses, release ~50 vesicles onto the afferent fibres that innervate them (Glowatzki & Fuchs, 2002). The exact functional significance of this multi-vesicular release is not known; it is likely, however, that such a response serves several purposes, such as (i) securing the activation of the auditory afferent fibres, (ii) providing a way to differentiate synaptic activation from spontaneous activity/discharge, (iii) modulating sound intensity encoding, and (iii) ensuring temporal accuracy in the generation of action potentials (Fuchs, 2005; Glowatzki & Fuchs, 2002; Khimich et al., 2005). In comparison, the ribbon synapses of the outer hair cells are thinner, poorly organised, with considerably fewer docked vesicles (Liberman et al., 1990), which ultimately results in the release of less vesicles and the production of significantly lower excitatory postsynaptic potentials (Weisz et al., 2012). In fact, depolarisation of outer hair cells rarely results in the activation of the afferents that innervate them, and instead, depolarisation activates a protein known as prestin (Kennedy et al., 2006; Zheng et al., 2000). Prestin is a motor protein that allows the outer hair cells to shorten on depolarisation and lengthen on hyperpolarisation (Ashmore, 1987; Zheng et al., 2000). This electromechanical action increases the movement of the basilar membrane in response to low intensity sounds, which in turn enhances the stimulation of the inner hair cell stereocilia. Hence, the outer hair cells play a direct role in the amplification of sounds within the cochlea. Comparatively, the inner hair cells are regarded as the sensory receptors of the cochlea, faithfully transmitting information about the movement of the basilar membrane to the auditory brainstem nuclei for processing and integration.

1.1.2 Ascending pathways: the auditory afferent fibres

Differences between the inner and outer hair cells extends beyond the ribbon synapses to the afferent fibres that innervate them. There are two types of auditory afferents, the Type I and Type II afferent fibres (Figure 1.2). The Type I afferents are the dominant fibre type, making up approximately 90 – 95% of the total population. They exclusively innervate the inner hair cells with 20 – 30 fibres making direct contact with a single cell (Liberman, 1982). Comparatively, a single Type II afferent branches to supply 10 – 30 outer hair cells and ~ 6 Type II afferents innervate a single outer hair cell (Simmons & Liberman, 1988). In regard to their structural attributes, the Type I afferent fibres are thick, myelinated and have bipolar cell bodies within the spiral ganglion (Nayagam et al., 2011). In comparison, the Type II afferent fibres are morphologically thinner, unmyelinated and have pseudo-unipolar cell bodies (Spoendlin, 1966; Spoendlin, 1973). Herein lies an important distinction between the fibre types, the contrast in myelination and fibre calibre suggests a fundamental difference in their role within the auditory system. Given the Type II afferents are unmyelinated and at a larger distance from the cochlear nucleus (CN), it is likely that Type II afferents do not directly encode auditory information (Nayagam et al., 2011). Rather, research indicates they serve as a local feedback circuit, modulating outer hair cell electromotility through communication between interconnected outer hair cells (Jagger & Housley,

2003; Nayagam et al., 2011; Thiers et al., 2008). In comparison, the presence of myelin and the larger fibre calibre of the Type I afferents suggests they are responsible for the rapid and precise encoding of all aspects of an acoustic signal, including essential features such as frequency, intensity, and timing (Pajevic et al., 2014).

1.1.3 Peripheral auditory nervous system: role in audition

This section provides a brief overview of the role of the peripheral auditory nervous system in frequency, intensity and temporal coding. The discussion is focused on structures which are known to be affected in individuals with AN. Hence, knowledge about how the different components contribute to sound processing will aid in the understanding of how different AN lesion sites may disrupt the coding of auditory information.

1.1.3.1 Frequency coding

The two main theories around frequency encoding within the auditory system are: (i) the place code theory; and (ii) the temporal code theory. While this section will focus predominantly on the place code theory, the contribution of temporal processing to frequency encoding will be discussed in the following section about temporal coding.

Place code theory is based on the principle that most structures of the auditory nervous system are tuned to respond to specific frequencies i.e. structures have morphological adaptations which make them resonate or respond optimally to a characteristic frequency. This ensures an incoming sound is decomposed into its fundamental frequencies for interpretation within different parts of the central auditory nervous system. Virtually all the peripheral components have morphological adaptations which are tuned to specific frequencies, giving each frequency a ‘place’ of optimal stimulation. The basilar membrane is the first structure within the auditory system that facilitates place coding. The basilar membrane is graded in mass and stiffness along its length. This gradual change in membrane compliance creates a tension gradient from the base to the apex of the cochlea, which ensures different segments of the membrane vibrate optimally to different frequencies – in particular, low frequencies are mapped at the apical end and higher frequencies are mapped towards base. Similarly, other peripheral structures also show structural adaptations. For instance, the outer hair cells progressively increase in length, and the axons of the spiral ganglion neurons (SGNs) increase in calibre from the base to the apex, reflecting the difference in their resonant frequencies along the axis of the cochlea (Anniko & Arnesen, 1988; Engel et al., 2006; Liberman & Oliver, 1984; Meyer et al., 2009; Ulfendahl & Flock, 1998). This ‘mapping’ of specific frequencies to place is called tonotopic mapping and this structure provides the basis for tonotopicity within the entire auditory pathway.

Although not directly related to the place code, the electromotility of the outer hair cells also contributes to frequency encoding. As discussed previously, the active process of the outer hair cells acts to overcome viscous damping forces and magnifies the movement of the travelling wave creating a

narrow, sharply tuned peak at characteristic frequencies of the basilar membrane (Fisher et al., 2012). Therefore, the outer hair cells ensure the accuracy and specificity of basilar membrane activation, which is passed onto the stereocilia of the IHCs and subsequently transmitted to the auditory nerve afferent fibres. Without the electromotility of the outer hair cells, basilar membrane tuning is broad, and the cochlea exhibits poor frequency resolution (Lagarde et al., 2008).

Finally, the theory of ‘place coding’ forms only one part of the way frequency is encoded in the auditory system. As mentioned previously, temporal aspects of sound are also involved in frequency encoding. In particular, a phenomenon known as phase-locking plays an important role for frequencies up to 5 kHz (Moore, 2008). Temporal aspects, however, will be discussed in further detail in the section on temporal coding.

1.1.3.2 Intensity coding

Intensity coding is vital to audition, particularly in binaural computations relating to sound localisation (Moore, 1991). It also plays an important role in the ability to discriminate speech in the presence of noise. Variations in sound intensity at the two ears, termed the interaural intensity difference, is therefore under constant scrutiny within the auditory nuclei (Bronkhorst & Plomp, 1988; Moore, 1991). Differences in interaural intensity are dependent on the length of the wavelength of a sound: low frequencies have long wavelengths allowing them to ‘bend’ around the head and arrive at each ear with approximately the same intensity. On the other hand, high frequencies have short wavelengths resulting in them being reflected off the head, causing the ear closest to the source to receive the signal at a higher intensity. Hence, the detection and neural encoding of intensity differences of high frequency sounds is a significant factor in sound localisation ability. It is mediated by several physiological factors, including the unique features of the inner hair cell synapse and properties of the SGN and afferent fibres.

The typical synapse of most primary sensory organs activates directly in response to action potentials, with a strong all-or-nothing release of neurotransmitter. In contrast, the inner hair cell afferent synapse has a graded response to changes in membrane potential (Nouvian et al., 2006). In this way the inner hair cell synapse encodes different intensity sounds by controlling the amount of neurotransmitter released, which in turn mediates the firing rate of the afferent fibres. Furthermore, each inner hair cell can be innervated by several Type I afferent fibres. These fibres have different thresholds of activation and are most typically characterised by their spontaneous firing rates (SR; the firing rates in the absence of sound). High-SR fibres are activated in response to low intensity sounds and hence have the lowest thresholds of firing, whereas the low-SR fibres are activated in response to high intensity sounds and therefore have the highest thresholds of firing. Medium-SR fibres, an intermediate between low-SR and high-SR fibres, also exist but for simplicity will be considered together with the low-SR fibres. The fibre types differ anatomically; low-SR fibres have fewer mitochondria and their axons are thinner compared to high-SR fibres (Liberman, 1982). Moreover, their patterns of inner hair cell innervation

differ, i.e. high SR-fibres have large bouton located on the pillar side of the inner hair cell, whilst low SR-fibres have small bouton located on the modiolar side. These physiological and structural differences play an important role in intensity encoding, ensuring a wide-dynamic range of hearing. For example, high-SR fibres, which are activated close to behavioural thresholds are likely to govern sound detection in quiet environments. The response of these fibres, however, saturates at levels 20-30 dB above their threshold. Their sensitivity to supra-threshold sound means that these fibres are susceptible to synaptic fatigue due to vesicle depletion, which results in a reduction in discharge rate (Meyer & Moser, 2010). Hence, low-SR fibres are necessary to extend the dynamic range and determine supra-threshold hearing. Furthermore, due to their higher activation thresholds the low-SR fibres are less susceptible to background noise and therefore play an important role in determining hearing ability in noisy environments (Bharadwaj et al., 2014).

Finally, the active process of the outer hair cells also contributes to intensity coding. This is particularly relevant for low intensity sounds. The outer hair cells increase the sensitivity to soft sounds by amplifying the vibrations of the basilar membrane via the mechanoelectrical transduction process (Pickles, 2012). The movement of the outer hair cells, however, saturates as stimulus intensity increases and therefore their contribution is negligible for high intensity sounds.

1.1.3.3 Temporal coding

An essential aspect of the auditory system is its ability to faithfully and rapidly encode temporal fluctuations in an auditory stimulus. The temporal information of most naturally occurring sounds such as speech can be separated into two main parts: the temporal fine structure (TFS) and the envelope. The TFS constitutes the fast variations in sound pressure of an auditory stimulus that determines the spectral content, whereas the envelope refers to slower amplitude fluctuations superimposed on the TFS (Figure 1.3; Moore (2008)). The envelope itself does not exist as a separate entity but is rather generated as a result of interactions between the different TFS components (i.e. constructive and destructive interference of the component frequencies making up the TFS; this is the reason that the TFS is often referred to as the carrier, because it is said to ‘carry’ the envelope (Joris et al., 2004). Both the envelope and TFS play an important role in speech perception. For example, several studies have linked envelope cues with speech perception ability in quiet and TFS cues to pitch/melody recognition and speech recognition ability in background noise (Hopkins & Moore, 2009; Lorenzi et al., 2006; Shannon et al., 1995).

As discussed, through cochlear tonotopic filtering at the auditory periphery, complex sounds are broken down into constituent frequency bands and hence each auditory nerve fibre encodes a different component of the initial signal. Encoding of envelope and TFS cues by nerve fibres occurs through modulation of the timing of their discharge rates. Auditory nerve fibres tuned to low-frequencies can encode both envelope and TFS cues; both are encoded through the synchronisation of neural spikes,

called phase locking; whereby physical excitatory deflection of inner hair cells is preserved in the neural discharge pattern of the auditory nerve fibres. For frequencies above 4-5kHz phase locking weakens and therefore auditory nerve fibres tuned to high-frequencies are not able to phase lock to the TFS, instead temporal information is encoded through phase locking to envelope fluctuations (Bharadwaj et al., 2014; Joris & Yin, 1992).

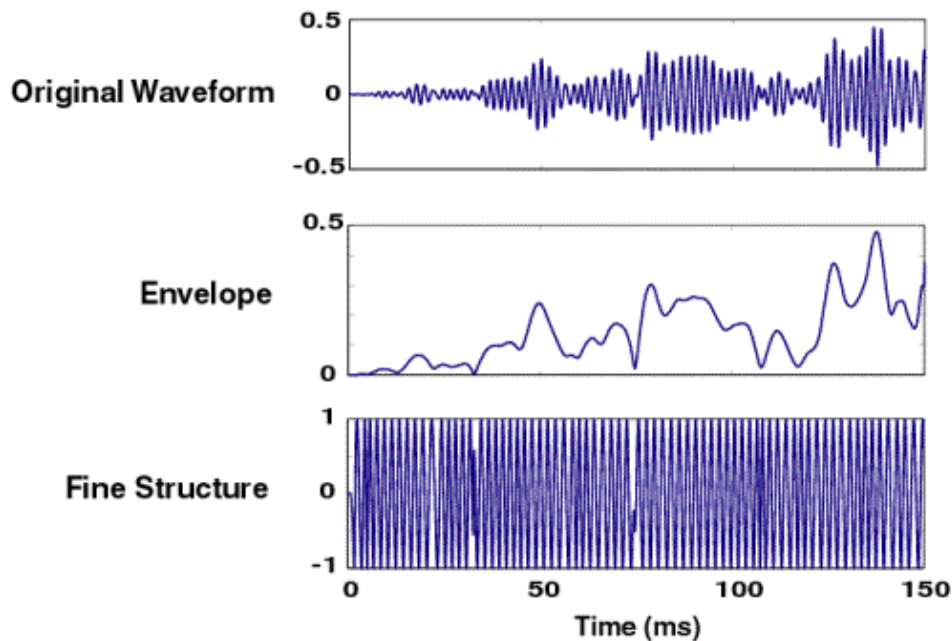


Figure 1.3 | Components of a complex acoustic signal. Temporal fine structure (*bottom*) and envelope (*middle*) extracted from the original acoustic signal (*top*). Image modified from Shen et al. (2001).

Temporal coding also plays an integral part in spatial localisation of sounds, helping to build the auditory scene. For example, interaural time differences (ITDs; the difference in time taken for a sound to arrive at the two ears) are vital for spatial localisation of sound in the horizontal plane and they play a role in the ability to detect and discriminate speech in the presence of noise. Computation of ITDs requires an extremely high level of temporal precision; in the order of tens of microseconds (Blauert, 1997). Perceptually, encoding of low frequency TFS cues is the dominant cue for source azimuth (Wightman & Kistler, 1992). For high frequency or broadband sounds, however, ITDs can be encoded by the temporal envelope alone and this can be just as precise for source localisation in the horizontal plane (Bernstein & Trahiotis, 2002). Additionally, encoding of envelope cues may be important for spatial perception when the TFS is distorted by the presence of reverberation (Dietz et al., 2013).

To determine the role of the TFS and envelope in speech perception, several studies have filtered speech to contain solely envelope or TFS cues (Dudley, 1939; Gilbert & Lorenzi, 2006; Lorenzi et al., 2006). Overall, research has shown that envelope cues are sufficient to understand speech in quiet, however with the introduction of fluctuating background noise, envelope cues on their own no longer provide enough information to ensure speech intelligibility (Hopkins et al., 2008). When listeners have access

to TFS cues, however, there is an improvement in their ability to perceive a speech signal in the presence of competing and fluctuating background noise (Hopkins et al., 2008).

In general, the remarkable acuity with which temporal coding occurs within the auditory system is dependent on the unique physiology and structure of many of the peripheral auditory structures as well as the integrity of the signal transmission within the central auditory system. For phase-locking to occur each Type I afferent is required to fire with an extremely high level of precision and synchronicity. As previously discussed, the inner hair cell ribbon synapse has the capacity for the rapid release of multiple neurotransmitter vesicles which produces a large-amplitude evoked excitatory post-synaptic current. Large amplitude currents have been shown to enhance phase-locking and increase the synchronicity of afferents (Li et al., 2014). Other mechanisms are also employed by the inner hair cell to accomplish temporal fidelity, some of which include: short membrane time constants, Ca^{2+} nanodomain control of vesicle exocytosis, and accumulation of a large readily releasable pool of vesicles (Moser et al., 2006). Furthermore, there is emerging evidence demonstrating the importance of afferent neuronal density in temporal coding and detection of speech in noisy environments (Lopez-Poveda & Barrios, 2013; Plack et al., 2014).

1.1.4 Descending pathways: the efferent fibres

Efferent tracts run in close proximity to the afferent fibres and extend throughout the entire auditory system. They are descending projections, which carry modulatory signals from the auditory cortex and all major auditory nuclei, including the medial geniculate body (MGB), inferior colliculus (IC), superior olivary complex (SOC), and CN. Projections reach both the outer and inner hair cells of the cochlea via the fibres of the olivocochlear bundle (Terreros & Delano, 2015). The efferent pathways enable complex responses from higher divisions of the auditory system to modulate activity all the way down to the organ of Corti (Leon et al., 2012; Perrot et al., 2005).

Out of all the efferent pathways, the olivocochlear bundle has been studied the most extensively (Pickles, 2012). The bundle begins in the SOC and projects to the sensory hair cells of the cochlea. It is generally organised into two divisions: the medial olivocochlear bundle and the lateral olivocochlear bundle. The fibres of the medial olivocochlear bundle originate from the medial portion of the SOC and innervate the outer hair cells. The cells of these fibres are relatively large and give rise to myelinated axons (Guinan et al., 1983). Comparatively, the fibres of the lateral olivocochlear bundle originate from the lateral SOC and innervate the dendrites of the type I afferent fibres, those connecting to the inner hair cells. The cells of the lateral olivocochlear bundle have small bodies and their axons are unmyelinated. The exact function of the olivocochlear bundle remains somewhat unclear. Neurotomy of the olivocochlear bundle in both humans (Scharf et al., 1997; Zeng et al., 2000) and animals (Igarashi et al., 1972) has had no significant effect on hearing acuity in quiet. There have, however, been reports of efferent lesions causing some perceptual difficulties in noise (Heinz et al., 2001b; May & McQuone,

1994; Zeng et al., 2000). In general, current research indicates the olivocochlear bundle plays a protective role in audition, minimising damage from acoustic trauma (Maison & Liberman, 2000; Rajan & Johnstone, 1988), a feature which has likely arisen incidentally, as a by-product of the olivocochlear bundle's ability to suppress the electromechanical movement of the outer hair cells (Kirk & Smith, 2003; Smith & Keil, 2015). Overall, further studies are still required to ascertain its exact function and the function of the other efferent connections of the auditory system.

1.2 The central auditory nervous system

This section will examine the different components of the central auditory nervous system, their role in audition, their structure, as well as the innervation patterns of the major synaptic relays of the auditory brainstem and auditory cortex. An understanding of the function of the central auditory nervous system and its prominent anatomical features are important in understanding the basis of this work. In particular, a thorough understanding of prominent anatomical landmarks within the auditory brainstem and cortex is essential for interpreting many of the MRI analysis techniques, which will be further discussed in Chapter 1.8.

1.2.1 Cochlear nucleus (CN)

The cochlear nucleus is the first 'processing and relay station' within the central auditory nervous system. It is the point at which all the afferent auditory nerve axons from the cochlea terminate. It is located at the pontomedullary junction at the level of the cerebellopontine angle, directly inferior to the flocculus, a small lobe of the cerebellum that plays an important role in motor control and the vestibulo-ocular reflex. The size and shape of the CN varies considerably between individuals but it has an approximate size of 8.01 x 1.53 x 3.76 mm (length x width x height) (Figure 1.4) (Rosahl & Rosahl, 2012). The location and orientation of the CN cannot be directly visualised on MR images obtained at clinical MRI field strengths (1.5 – 3 Tesla), due to limitations in imaging resolution and contrast. Its location, however, can be approximated based on surrounding structures such as the inferior cerebellar peduncle, the foramen of Luschka, the fourth ventricle, and the entry point of the VIII nerve into the brainstem. These structures can typically be identified on T2-weighted MR images acquired using a high spatial resolution. Since the CN lies on the superficial edge of the inferior cerebellar peduncle, at the anterior edge of the foramen of Luschka this information can be used to approximate its location (Abe & Rhoton, 2006). Figure 1.5 shows the estimated location of the CN, in relation to more easily identified brainstem structures.

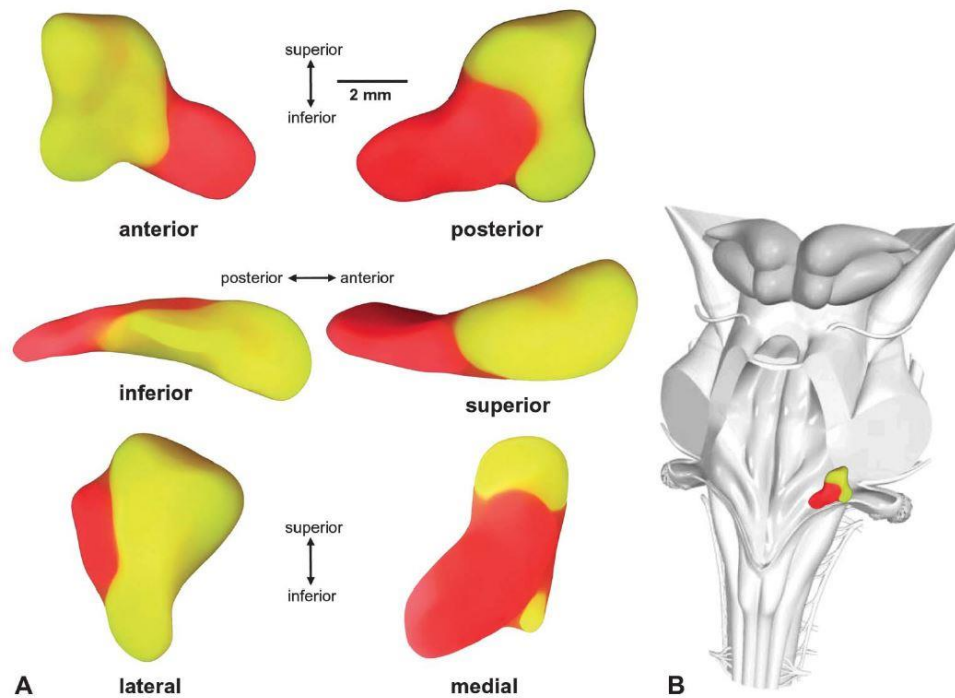


Figure 1.4 | Cochlear nucleus structure and location. (A) the cochlear nucleus has a distorted ‘jelly-bean’ or boot-like structure when viewed sagittally. Images shown are 3-dimensional reconstructions of the cochlear nucleus in several different orientations. The ventral cochlear nucleus (combination of the anterior and posterior cochlear nucleus) is shown in yellow and the dorsal cochlear nucleus in red. (B) Dorsal view of the brainstem showing the position of the cochlear nucleus complex at the ponto-medullary junction. Reproduced with permission from Rosahl and Rosahl (2012).

The auditory afferent fibres from the vestibulocochlear VIII (cranial) nerve enter the CN ventrally, via the internal auditory meatus. Upon entering the CN, the fibres bifurcate, with an ascending branch supplying the anterior ventral cochlear nucleus (AVCN) and a descending branch supplying the posterior ventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN) (Pickles, 2012). Each of these three divisions differs neuroanatomically and serves a unique function in auditory processing. Furthermore, the afferent input retains the tonotopic arrangement observed within the cochlea, generating frequency maps within each of these divisions of the CN (Nayagam et al., 2011).

Projections from the CN can be divided into two main streams, the ventral and dorsal stream, based on their location and the type of information transmitted. The ventral stream encompasses the projections running from the AVCN to the nuclei of the SOC bilaterally and is responsible for carrying information about binaural sound localisation. Comparatively, the dorsal stream consists of the projections from the PVCN and DCN which connect to the contralateral nuclei of the lateral lemniscus and inferior colliculus. The dorsal stream is responsible for carrying information about sound identification, temporal fluctuations and spectral information (Pickles, 2015). Overall, the cochlear nuclei and the subsequent nuclei of the auditory brainstem have a symmetrical arrangement and follow a logical progression throughout.

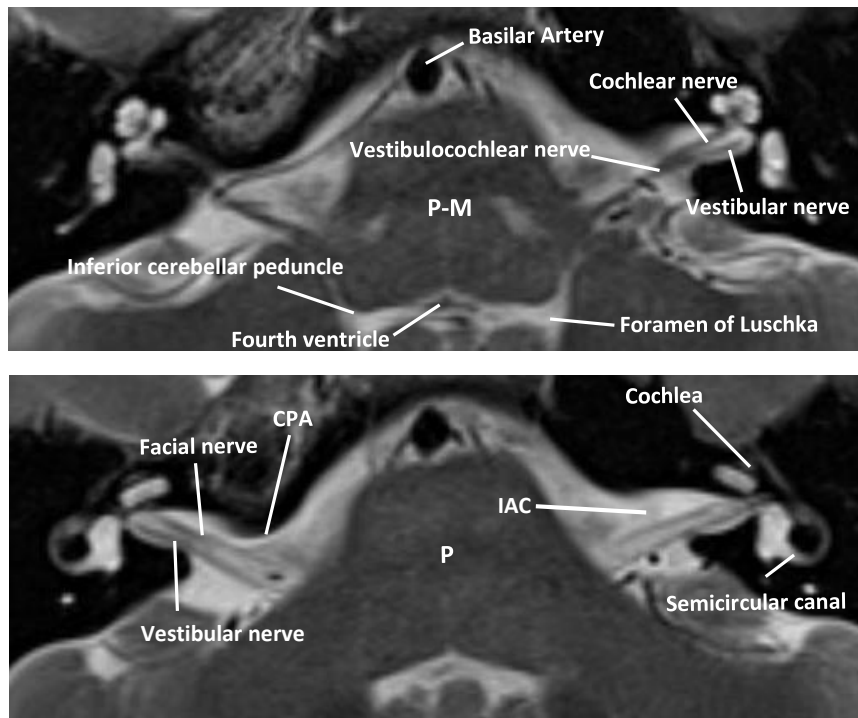


Figure 1.5 | High-resolution (voxel size of 0.5 x 0.5 x 2 mm) T2-weighted axial MR images at the level of the ponto-medullary junction (P-M) and pons (P). (A) The structural features that can be used to approximate the location of the cochlear nuclei are shown: (inferior cerebellar peduncle, fourth ventricle and foramen of Luschka. The two components of the VIII nerve, the cochlear nerve and vestibular nerve are also shown. (B) Other notable components of the auditory system are labelled. IAC: internal auditory canal; CPA: cerebellar pontine angle.

1.2.1.1 Anterior ventral cochlear nucleus (AVCN)

In terms of its cellular architecture the AVCN consists predominantly of globular and spherical bushy cells. Approximately 1-4 auditory Type I afferent fibres innervate a single spherical bushy cell through giant specialised synaptic terminals called endbulbs of Held (Ryugo & Fekete, 1982). This axon terminal is noteworthy for its size and large amount of synaptic contacts – up to 2000 in cats (Ryugo et al., 1996), which is indicative of the important role it plays in binaural sound localisation within the auditory system. The design of the endbulb of Held ensures that an action potential from an auditory fibre has a high probability of depolarising the spherical bushy cell that it innervates. As such, it facilitates the transmission of neural activity directly from the auditory nerve fibres with very low synaptic delay and a high degree of fidelity (Ryugo et al., 1996). This is a necessary physiological function for the comparison of interaural timing cues arriving at each ear, an essential component of sound localisation (Smith et al., 1993). Spherical bushy cells send ‘excitatory’ myelinated projections to the medial superior olive nuclei bilaterally. The ipsilateral lateral superior olive also receives thick myelinated projections from spherical bushy cells. This nucleus is involved in comparing the intensity of sounds from both ears. In comparison, the auditory nerve contacts the globular bushy cells through much smaller end bulbs of Held, with ~ 4 to 40 auditory nerve fibres contributing to a single synapse (Pickles, 2015). The globular bushy cells primarily send their projections to the medial nucleus of the trapezoid body, which is considered an intermediary stage in the pathway to the contralateral lateral

superior olive (Smith et al., 1991). Furthermore, the dendrites of both cell types also receive inhibitory inputs from interneurons arising within the CN and other auditory nuclei. These inhibitory inputs can modulate the sensitivity and response of both the spherical and bushy cells found within the AVCN (Pickles, 2015).

1.2.1.2 Posterior ventral cochlear nucleus (PVCN)

The PVCN is composed of a more diverse population of cells than the AVCN. The major cell types include globular bushy cells, octopus cells, and two types of stellate cells; T- and D-stellate cells (Cao & Oertel, 2010). The cells of the PVCN are involved in both the ventral binaural localisation stream as well as the dorsal sound identification stream.

The role of the PVCN has been determined through a detailed analysis of its cells and the connections these form with other nuclei within the auditory brainstem. The octopus cells have a complex structure and are dedicated to resolving temporal fluctuations in broadband stimuli such as speech (Cao & Oertel, 2010). They are particularly sensitive to the temporal structure of clicks, the onset of tones, and to periodic stimuli (Ferragamo & Oertel, 2002). The octopus cells predominantly project to the contralateral ventral nucleus of the lateral lemniscus. The T-stellate cells are the principal class of stellate cells. In terms of their physiology, T-stellates are particularly well suited to encoding the spectral shape of complex sounds and conveying envelope fluctuations of ongoing stimuli (Cao & Oertel, 2010). T-stellates receive direct afferent input from the auditory nerve and innervate the contralateral ventral nucleus of the lateral lemniscus, and the IC bilaterally. They also send collaterals throughout the ipsilateral CN and to the superior olivary complex (Cant & Benson, 2003). In humans, the distance an auditory fibre would have to travel from the PVCN to the ipsilateral IC is approximately 35mm and 46mm to the contralateral inferior colliculus (Moore, 1987). D-stellate cells make up a much smaller proportion of the stellate cells. These receive afferent innervation from the auditory nerve and subsequently send inhibitory projections throughout the CN on both sides.

1.2.1.3 Dorsal cochlear nucleus (DCN)

The DCN, like the PVCN, is composed of several different cell types, however, the DCN has fewer myelinated axons and contains only a small number of neurons (Mai & Paxinos, 2011). Overall, the structure of the DCN is like the cerebellum, particularly in its organisation and constituent cell types. Afferent axons of the DCN stem from two cell types, the fusiform and giant cells (Oertel & Young, 2004). Both these cells send projections primarily to the central nucleus of the IC; they also, however, send collaterals to other cells within the nucleus (Cant, 1992; Young et al., 2012). Both giant and fusiform cells have complex responses to sounds, modulated by numerous excitatory and inhibitory inputs from other cells within the DCN (Young et al., 2012). The DCN plays an important role in the ability to localise sounds in the environment, particularly in analysing and processing changes in the sound spectrum caused by the pinnae and head (spectral cues) (Oertel & Young, 2004; Young & Davis,

2002). Furthermore, Oertel and Young (2004) propose that the DCN corrects for distortions in auditory inputs resulting from head and body movement through the integration of information from somatosensory inputs.

1.2.2 Superior olivary complex (SOC)

The SOC is situated within the pons in the inferior portion of the pontine tegmentum and superomedial to the CN. If viewed in the coronal plane, the SOC can be found between the facial nerve root laterally, and the abducens nerve root medially (Schwartz, 1992). Three major nuclei make up the entire complex, including the medial superior olivary nucleus, lateral superior olivary nucleus, and the medial nucleus of the trapezoid body. These three structures are surrounded by periolivary nuclei, which vary in size, cellular composition, and shape between different species (Schwartz, 1992). The entire complex is dedicated to binaural hearing and is therefore responsible for the processing of intensity and timing differences at the two ears. The SOC receives innervation from the CN from both sides of the brainstem and the information carried by these afferents facilitates the binaural processing that occurs within the cells of the SOC.

The most prominent nucleus of the entire SOC is the medial superior olivary nucleus. It receives direct excitatory axonal projections from the spherical bushy cells located in the AVCN from both sides of the brainstem. The medial superior olivary nucleus is dedicated to resolving interaural timing cues and therefore it assists in the localisation of a sound based predominantly on its low frequency content (Grothe & Park, 2000). The medial superior olivary nucleus sends excitatory projections to the ipsilateral inferior colliculus and the ipsilateral dorsal nucleus of the lateral lemniscus (DNLL) (Helfert et al., 1989).

The lateral superior olivary nucleus is spherical in shape and is typically small in animals that have poor high frequency hearing and relatively large distances between the two ears (interaural distance), which includes humans (Heffner & Masterton, 1990). It receives two main inputs, one excitatory input from the spherical cells of the ipsilateral AVCN and an inhibitory input originating in the contralateral AVCN and arriving via the adjacent medial nucleus of the trapezoid body. The lateral superior olivary nucleus is tuned to extract frequency specific differences in interaural intensity and is involved in sound source localisation (Tollin, 2003). The major projections from the lateral superior olivary nucleus are to the central nucleus of the inferior colliculus bilaterally.

The medial nucleus of the trapezoid body is the final component of the SOC that will be considered. This nucleus acts as a relay point for incoming inputs from the globular bushy cells of the contralateral AVCN to both the lateral and medial superior olive (Pickles, 2012). The projections from the AVCN terminate via calyces of Held on the principal cells of the medial nucleus of the trapezoid body (Nakamura & Cramer, 2011). Furthermore, the medial nucleus of the trapezoid body has also been

shown to enhance certain features of incoming signals, including the degree of phase locking and sharpness of the onset responses.

1.2.3 Lateral lemniscus (LL)

The ascending fibres between the SOC and inferior colliculus run in a tract called the lateral lemniscus, which acts primarily as a relay station. It is located within the body of the pons and consists of two major nuclei, known as the dorsal and ventral nuclei of the lateral lemniscus (DNLL and VNLL, respectively) (Kelly et al., 2009). Both the dorsal and ventral nucleus of the lateral lemniscus contain a variety of different cell types including stellate, multipolar, globular, spherical and elongated cells (Covey & Casseday, 1986).

The VNLL receives projections from each cell type within the contralateral VCN. It also receives inputs from the ipsilateral medial nucleus of the trapezoid body. The VNLL, however, does not receive any projections from the lateral and medial superior olivary nucleus of the SOC and therefore it is not considered to be involved in the binaural sound localisation stream. Instead, the VNLL is thought to form part of the monaural sound identification stream. The cells within the VNLL are specialised to extract and encode temporal information from complex auditory stimuli, including periodic components of complex sounds (Nayagam et al., 2005). Furthermore, its helical shape and cellular organisation suggest it also plays a role in the extraction of harmonic relations between stimuli. Fibres from the VNLL run almost exclusively to the ipsilateral IC where that incoming information is thought to be ‘compared’ to the same auditory information arriving directly from lower nuclei that bypass the VNLL entirely (Pickles, 2012).

In comparison, the DNLL is part of the binaural sound localisation stream, receiving significant inputs from the ipsilateral medial superior olivary nucleus, and the contralateral lateral superior olivary nucleus. Furthermore, the DNLL also receives inputs from the contralateral CN and many inhibitory inputs from the contralateral DNLL via the commissure of Probst. In general, however, the majority of neurones within the DNLL exhibit excitatory responses from contralateral stimuli and are inhibited by ipsilateral ones. As such, the DNLL plays a role in increasing the contrast, accuracy and dynamic range of localisation information arriving from the nuclei of the SOC (Pecka et al., 2010). From the DNLL afferent fibre projections run to the inferior colliculus on both sides of the brainstem.

1.2.4 Inferior colliculus (IC)

Most ascending fibres from lower brainstem nuclei converge at the IC. As such, it is considered the primary receiving station of the midbrain and serves to re-integrate auditory information regarding sound localisation and sound identification. Compared to the nuclei at lower levels of the auditory brainstem, the IC has a much higher degree of complexity in the way it responds to auditory activity. Consequently, it also plays a pivotal role in the integration of information from lower nuclei to define

an auditory object/scene. It is also the largest structure within the auditory brainstem pathway with much greater cellular density in comparison to the other auditory nuclei (Moore, 1987).

The inferior colliculi are located on the dorsal surface of the midbrain (Figure 1.6), caudal to the superior colliculi (a pair of nuclei involved in visual processing). The IC has an irregular rounded shape, with a diameter of approximately 6- to 7 mm in every plane. There are three key divisions to the IC: the central nucleus, the intercollicular nucleus/dorsal cortex, and the external nucleus/lateral cortex. Each of the three divisions has a unique neuronal architecture, a distinct pattern of innervation, and each performs a different function.

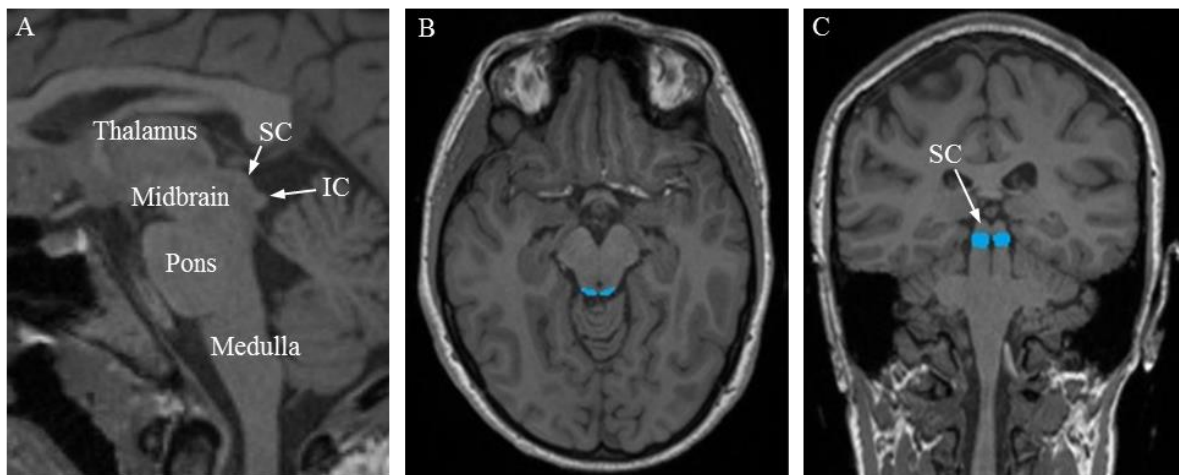


Figure 1.6 | T1-weighted magnetic resonance images showing the location of the inferior colliculi within the human brainstem at the level of the midbrain. (A) A mid-sagittal cross-section through the brainstem. The main features are labelled, including the inferior colliculus (IC), superior colliculus (SC), medulla, pons, midbrain, and thalamus. (B) A transverse cross-section through the brain and brainstem, the approximate location of the inferior colliculi are shown in blue. (C) A coronal cross-section of the human brain, with the inferior colliculi shown in blue and an arrow showing the location of the right SC.

The central nucleus, which is also referred to as the ‘lemniscal’ nucleus due to the large amount of input from fibres running from the lateral lemniscus, consists predominantly of disc-shaped neurons. Stellate cells make up the remainder of the cells present throughout the central nucleus. Both cell types send axons to the nuclei of the medial geniculate body, however bifurcations from the main axons results in innervation patterns across the nucleus itself. These collaterals act as interneurons, which are thought to have a modulatory function. The central nucleus also receives excitatory (glutamatergic) afferent projections from the contralateral CN, contralateral lateral superior olive, ipsilateral medial superior olive, and the ipsilateral ventral nucleus of the lateral lemniscus (Cant & Benson, 2003). Additionally, the central nucleus receives several inhibitory afferents from the DNLL, ipsilateral lateral superior olive, and the ipsilateral VNLL (Merchán et al., 2005). Cells within the central nucleus exhibit directional selectivity and are typically only activated in response to sounds arriving from the contralateral side. Comparatively, ipsilateral inputs play an important role at refining and enhancing the incoming signals (Kuwada et al., 2011).

The external and intercollicular nucleus surround the central nucleus and are considered extra-lemniscal nuclei with non-specific, multi-sensory interactions. Inputs to the external nucleus come from the contralateral DCN and from the central nucleus. Projections also run between the dorsal columns of the trigeminal nuclei to the external nucleus, which carry somatosensory information (Irvine, 2012). Comparatively, the intercollicular nucleus receives no direct ascending connections but receives inputs from the contralateral IC and efferent (descending) inputs from the auditory cortex. Overall, there is limited evidence detailing the function of these two nuclei, however it is likely that the external nucleus acts to integrate somatosensory and auditory inputs impacting spatial reflex responses to different auditory stimuli (Lumani & Zhang, 2010). Connections between the external nucleus and the superior colliculi have also been identified. This makes it likely that the processed information in the external nucleus contributes to a joint visual and auditory map of surrounding space, used for behavioural orientation (Burnett et al., 2004).

Furthermore, the left and right hemispheres of the IC are connected by a true commissure (Binns et al., 1992). The commissure is located dorsally to the aqueduct and consists of axons originating from the neurons within the dorsal nucleus. These axonal projections terminate within the contralateral intercollicular nucleus. The commissure is the last subcortical connection between both sides of the auditory midbrain, and it provides the final opportunity for any interaction or refinement of the auditory signal between the two sides (Burnett et al., 2004).

1.2.5 Medial geniculate body (MGB)

The MGB is the thalamic relay station of the auditory system and is considered the gateway to the primary auditory cortex. It is found at the level of the midbrain on the same axial slice as the red nucleus. A nucleus which can be readily distinguished on high-resolution T2-weighted MR images, as shown in Figure 1.7. As a result, the red nucleus can be used as a marker to help identify the location of the MGB on structural MRI scans.

The medial geniculate body is a small oval eminence and is approximately 5 mm wide, 4 mm deep and 4-5 mm long (Winer, 1984). It receives afferent inputs directly from the inferior colliculus bilaterally and sends projections to many parts of the auditory cortex. A study of the cytoarchitecture reveals three main divisions; the ventral or 'principal' division, which is surrounded by the medial and dorsal divisions.

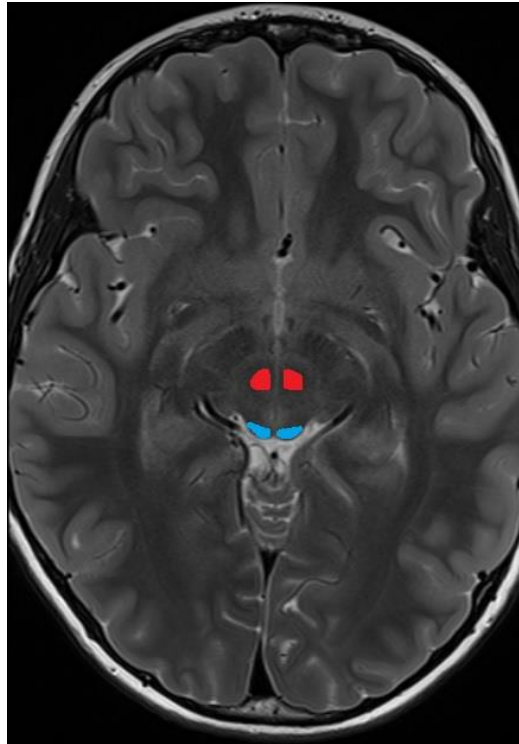


Figure 1.7 | T2-weighted MR image (axial slice) showing the approximate location of the medial human geniculate body (MGB) at the level of the midbrain. The MGB (approximate location shown in blue) is not easily identified on structural scans obtained at 1.5 – 3 T, however it is located on the same axial slice as the red nucleus (shown in red), which is typically more clearly defined on T2-weighted images.

The ventral division is considered part of the lemniscal auditory pathway as it acts to process and relay auditory inputs from the central nucleus of the ipsilateral inferior colliculus. These fibre projections run within the brachium of the IC, a protrusion found between the IC and geniculate bodies on the lateral surface of the brainstem (Pickles, 2012). From within the ventral division of the MGB fibres project directly to the ipsilateral core areas of the auditory cortex. Comparatively, the dorsal and medial divisions are categorised as extra-lemniscal as they play a role in the integration of both auditory and non-auditory (somatosensory) information.

The medial division receives inputs from the external and intercollicular nucleus of the IC, the lateral tegmental system (comprising of fibres from the superior colliculus, nucleus sagulum and cuneiform nucleus), the vestibular system and from the spinal cord (Pickles, 2015). The medial division projects to the different auditory cortices, including the core. Furthermore, a connection also exists between the amygdala and the medial division of the MGB, indicating its involvement in emotional learning and conditioning (LeDoux, 1993).

The dorsal division of the MGB predominantly receives inputs from the dorsal cortex of the IC and sends projections to the auditory areas surrounding the primary auditory cortex. Responses from its neurons show strong stimulus-specific adaptation, which can be modified through training or experience. For example, when conscious guinea pigs were exposed to a tone which was coupled with

an aversive behavioural stimulus (electric shock) the response from the neurons within the dorsal nucleus were enhanced to the specific frequency of the tone used in the experiment (Edeline & Weinberger, 1991). These findings suggest that the dorsal division plays a role in preparing the auditory cortex to respond to sounds that are of particular significance to an organism.

1.2.6 Auditory cortex (AC)

Until recently, knowledge about the organisation of the AC was based on post-mortem studies performed on the cat. The nomenclature established during this period differs compared to current definitions in use. According to the original viewpoint, the AC consisted of three distinct subdivisions; the primary (AI), secondary (AII) and tertiary auditory cortex (AIII). Once studies were conducted on a broader range of species the nomenclature was reassessed as it no longer adequately described the organisational hierarchy that was observed within the AC. According to the modern definition, the AC consists of a primary receiving area known as the core, adjacent regions known as belt areas, and peripheral regions called parabelt areas.

In humans, the entire AC is located on the upper surface of the temporal lobe, positioned on a region called the superior temporal plane, which is found deep within the Sylvian sulcus. The core occupies a posteromedial part of Heschl's gyrus, within the Sylvian sulcus, and is surrounded by the belt and parabelt areas. Fundamentally, the AC plays a vital role in sound detection and pitch discrimination (Heffner & Heffner, 1986; Tanaka et al., 1991). It has been shown, however, that lesions within the AC degrade the ability of an individual to perform a range of complex auditory tasks, including the ability to perceive and understand speech (Goll et al., 2010) and the ability to localise sounds (Tian & Rauschecker, 2004). Finally, the AC also functions to represent auditory objects, with differing patterns of neurons activating for different objects. More significant or important objects are likely to elicit a greater response, which has been shown to vary depending on the behavioural state of the animal (Pickles, 2012). A simplified schematic of the main ascending auditory pathways is shown in Figure 1.8.

1.2.6.1 The core

The core is made up of several regions including what was previously considered AI. These regions are grouped together according to several criteria: (i) Each core region shares similarities in cytoarchitectonic appearance, characterised by a 'koniocortical' structure, defined as having a predominance of evenly distributed small cells with dense myelination (Pickles, 2012); (ii) The core areas receive direct input from the lemniscal stream of the ventral division of the MGB and each core area is densely interconnected with neighbouring regions, indicating a strong reciprocal influence (Kaas & Hackett, 2000). Furthermore, projections also exist between core areas and some adjacent belt areas, indicating that the belt is a necessary secondary stage of auditory cortical processing (Kaas & Hackett, 2000); (iii) In each of the regions that constitute the core, neurons are tonotopically organised with one

area tuned to a single progression of characteristic frequencies, from low frequencies to high or vice versa (Pickles, 2012); (iv) Finally, the core regions share common histochemical characteristics, with a dense expression of metabolic enzymes called cytochrome oxidase, and acetylcholinesterase, as well as a dense reaction for the calcium-binding protein, parvalbumin.

1.2.6.2 Belt and parabelt areas

The belt areas lie adjacent to the core and rely on inputs from the core for activation (Rauschecker et al., 1997). Belt regions also form connections to adjacent and distant belt and parabelt regions, as well as to the frontal lobe (Kaas & Hackett, 2000). In general, less is known about the parabelt, however, these regions are cytoarchitecturally distinct from belt regions. The parabelt receives dense connections from the belt areas and minimal connections from the core (Hackett et al., 1998). Moreover, the parabelt also forms connections with areas within the adjacent parts of the temporal and parietal lobe and with several regions within the frontal lobes (Hackett et al., 1998; Kaas & Hackett, 2000). In comparison to the core, belt and parabelt regions are defined according to another set of criteria: (i) these regions receive inputs from either the dorsal or medial divisions of the MGB; (ii) they receive either minimal or no connections from the ventral division of the MGB; and (iii) they have recordable auditory responses, with neurons in these regions being particularly responsive to complex stimuli (Pickles, 2012).

1.3 Auditory assessments and anatomical correlates

Several diagnostic assessments allow audiologists, neurologists and otologists to investigate the integrity and the health of the auditory system. These tests can be used to assess the function of different components, including the sensory hair cells of the organ of Corti, auditory afferent fibres, auditory brainstem nuclei, and the auditory cortex. In general, the available assessments are minimally invasive, relatively time-efficient and easily administered. They have been frequently used, with varying degrees of success, to help identify lesion sites in AN. Figure 1.8 presents a simplified schematic of the auditory system beginning at the organ of Corti and ending at the auditory cortex. Available diagnostic assessments and the sites/structures they evaluate are indicated along the pathway.

1.3.1 Pure tone audiometry (PTA)

Pure tone audiometry is a behavioural auditory assessment, which is used routinely in clinical practice to determine the sound detection threshold of hearing at several test frequencies ranging from 250 Hz to 8 kHz. Pure tone audiometry uses both air and bone conduction methods to differentiate between conductive hearing loss (a shift in hearing thresholds due to issues in the outer or middle ear) and cochlear sensory hearing loss (threshold shifts due to problems in the inner ear or more centrally). In practice, PTA is also used to determine the impact of an individual's hearing loss on their ability to access speech sounds, all of which fall into the frequency range commonly assessed (250 Hz – 8 kHz). As such, speech intelligibility is directly related to the degree that hearing thresholds are elevated

(Yellin et al., 1989). In individuals with auditory neuropathy, PTA thresholds can vary considerably, and speech perception ability is often disproportionately worse compared to their cochlear sensory hearing loss peers (Rance et al., 2012e; Zeng et al., 2005).

1.3.2 Otoacoustic emissions (OAEs)

Otoacoustic emissions are sounds produced exclusively by the movement of the outer hair cells in response to low intensity sounds (Kemp, 2002). They are typically inaudible to the subject; however, they can be recorded by placing a microphone in the external auditory canal. Since recording OAEs relies on the transmission of sound from the organ of Corti back through to the external auditory canal, recordings are sensitive to middle ear pathologies. Hence, they cannot be recorded in the presence of middle ear effusion or wax occlusion. Robust repeatable OAEs indicate healthy outer hair cell function and are reportedly present in ~ 95% of individuals with normal audiometric thresholds (<20 dB HL) (Kemp, 1978; Lonsbury-Martin et al., 1990). Frequency-specific regions of outer hair cells can also be stimulated and assessed using a dual-tone evoked distortion product assessment paradigm, commonly referred to as distortion product otoacoustic emissions (DPOAEs). This technique allows the condition of the outer hair cells to be assessed at different points along the basilar membrane, providing frequency specific information about damaged outer hair cells. In practice, measuring OAEs is a useful tool to assess the integrity of the outer hair cells and typically, as the severity of cochlear sensory hearing loss increases, there is an associated reduction in the probability of recording these emissions. For example, DPOAEs are absent for hearing losses greater than 50 to 60 dB HL but may still be present for losses between 20 – 50 dB HL (Gorga et al., 1997; Lonsbury-Martin et al., 1990). While in ~50% of AN cases OAEs are preserved irrespective of hearing loss, they have been shown to diminish over time and/or through ongoing amplification use (Sininger & Oba, 2001). Starr et al. (2010) have suggested that this gradual loss of emissions may also form part of the disease progression in AN, whereby outer hair cell function deteriorates over time due to a disruption or loss of function.

1.3.3 Electrocochleography (ECochG)

Electrocochleography is considered a near-field electrophysiological assessment as it provides a way to record auditory-event related neural potentials generated from within the cochlea and the early portion of the auditory nerve. These potentials include, (i) the cochlear microphonic (CM), (ii) the summing potential and, (iii) the compound action potential (CAP) (Eggermont, 1976). As with all evoked potentials, ECochG waveforms are described according to measures of amplitude and latency. The amplitude and latency of these potentials reflect the number of neural elements stimulated, the synchronous firing of these elements and the overall timing of neural activation (Hall, 1992). Hence, this information can be used to assess the integrity of the neural populations responsible for a given potential.

Electrocochleography is performed by placing a needle electrode through the tympanic membrane and onto the cochlear promontory or near the round window. This is the most commonly used technique and it is called the transtympanic approach. This acquisition method ensures the recording electrode is as close as possible to the source of the neural potentials, enabling a clear and robust response to be recorded. It is also possible to obtain recordings from the surface of the eardrum itself, which is referred to as the extratympanic approach. Naturally, a reduction in amplitude of the neural potentials, however, occurs at recording sites more distant from the cochlea. Once the electrode is in place, a transient broadband click stimulus is most often used to activate the sensory cells and the auditory nerve afferents (Eggermont, 1976). In comparison to the auditory brainstem response, discussed later, ECochG can provide detailed information regarding the health of the sensory hair cells within the organ of Corti and the distal portion of the auditory nerve.

Overall, using ECochG to elucidate AN lesion sites has shown some promise; however, results have not been consistent across studies (McMahon et al., 2008; Santarelli et al., 2013; Stuermer et al., 2015). The variability in findings may be explained by differences in electrode placement, the type of recording electrodes used within each study and the stimulus used to elicit a response (Roland & Roth, 1997; Stuermer et al., 2015). Important information regarding the integrity of the sensory cells and auditory nerve afferents, however, can still be obtained using ECochG and therefore it remains a valuable tool in the diagnosis of AN (Santarelli & Arslan, 2002; Santarelli et al., 2015c).

1.3.3.1 Cochlear microphonic (CM)

The CM, a robust alternating current potential, is the earliest recordable pre-neural potential of the auditory system. The origin of the CM is a contentious issue in the literature; experimental evidence suggests that the outer hair cells are likely to be the predominant contributors because there is a higher proportion of outer hair cells than inner hair cells within the organ of Corti (Dallos, 1983; Dallos & Cheatham, 1976; Dallos & Wang, 1974). Attempts to resolve this issue, however, have not been successful and as a result it is generally assumed that the CM reflects the summed pre-neural response of *both* the outer and inner hair cells (Cheatham et al., 2011; Dallos & Cheatham, 1976; Santarelli et al., 2006; Schoonhoven et al., 1999).

Recording the CM is possible from several sites, at locations both near and far from the cochlea. The amplitude of the CM, however, is directly dependent on the proximity of the recording electrode to the sensory hair cells, and therefore a clearer and larger amplitude response can be obtained with recordings obtained as close to the organ of Corti as possible. A distinguishing feature of the CM is its direct phasic relationship with the waveform of the auditory stimulus (Starr et al., 1996). This means that the polarity of the CM is directly dependent on the polarity of the acoustic stimulus. Therefore, acoustic stimuli of opposite polarity, such as rarefaction and condensation clicks, can help distinguish the CM from the other neural potentials in evoked potential recordings, particularly in those containing large amounts of

extraneous noise (Starr et al., 1991; Starr et al., 1996). In comparison, the other neural potentials obtained with the ABR remain unchanged regardless of stimulus polarity.

In general, ECoChG and ABR recordings from individuals with AN show clearly identifiable CMs even though neural potentials are absent or severely disordered (Rance & Starr, 2015; Starr et al., 2010). Comparatively, CMs are absent in individuals with severe to profound cochlear sensory hearing loss reflecting the extensive reduction of both sensory hair cell populations that occurs to produce this chronic condition (Rance & Starr, 2015; Santarelli et al., 2006; Starr et al., 1996). Hence, the CM has an important diagnostic role in differentiating between AN and cochlear sensory hearing loss. For instance, a diagnosis of profound cochlear sensory loss will typically be made if the ABR *and* CM are completely absent at maximum presentation levels. A differential diagnosis of AN is therefore reliant on the presence of the CM with an absent or severely disordered ABR, indicating at least some sensory hair cell function in the absence of ordered neural activity of the auditory afferents (Starr et al., 1991; Starr et al., 1996).

1.3.3.2 Summating potential (SP)

Electrocochleography can also be used to record potentials generated from depolarisation of the inner hair cells. These are direct current potentials known as the cochlear summating potentials (SPs), which originate from the inner hair cells situated in the basal portion (i.e. the high-frequency region) of the cochlea (Durrant et al., 1998). The SP is generally not identifiable in ABR recordings because the recording electrodes are located too far away from the inner hair cells. Hence, this makes ECoChG the only assessment available that provides information about the integrity or activation of the inner hair cells.

In auditory neuropathy, a recordable SP is dependent on the lesion site. For instance, if the function of the inner hair cells was disrupted the SP may be absent or reduced in amplitude (Rapin & Gravel, 2003; Santarelli et al., 2013). Comparatively, a lesion site occurring beyond the inner hair cell synapse would leave the amplitude and latency of the SP unaffected but may disrupt the compound action potential (CAP). Therefore, the quality of the SP can be helpful in determining the site of lesion in individuals with AN. This is discussed in further detail in the following section on the pathophysiological mechanisms of AN.

1.3.3.3 Compound action potential (CAP)

The last neural response recordable with ECoChG is the CAP. It reflects the summed neural activity of the auditory nerve fibres and is analogous to Wave I of the ABR. Although both assessments can be used to evaluate the CAP, its amplitude is up to 30 times greater on ECoChG recordings, making it much easier to identify (Santarelli et al., 2008). This difference in amplitude occurs because the recording electrode is placed much closer to the auditory nerve afferents in ECoChG.

Like the other measures discussed, the presence, amplitude and latency of the CAP in individuals with AN is dependent on the site of lesion. For example, any lesion(s) occurring at sites between (and including) the inner hair cells and the auditory nerve afferents will affect the generation of the CAP. A lesion site within the brainstem, however would not be expected to have any effect on the presence of the CAP.

1.3.4 Auditory brainstem response (ABR)

The ABR provides a measure of the synchronised neural activity of the auditory nerve and brainstem. A transient click stimulus is typically used to generate the action potentials, however, tone bursts can also be used to obtain frequency specific information. The neural potentials that are generated are recorded using electrodes placed onto the surface of the skin. Tracings obtained from an ABR recording normally consist of five ‘waves’ or ‘peaks’, which correspond to different sites of neural potentials along the auditory pathway. Wave I of the ABR is generated by the peripheral portion of the VIII nerve, in the region of the SGN nearest to the cochlea (Moller et al., 1988), whereas Wave II reflects the neural activity of the central to late portion of the VIII nerve (Ananthanarayan & Durrant, 1991). Wave III is generated by the CN complex (Hashimoto et al., 1981), Wave IV is likely to correspond to the activity of the superior olivary complex and/or lateral lemniscus (Moller & Jannetta, 1983) and Wave V is thought to reflect the neural activity of the IC (Durrant et al., 1994; Parkkonen et al., 2009). Aside from providing a measure of neural synchrony, the ABR also provides information regarding the conduction velocity along the auditory nerve and within the brainstem, which is reflected in the absolute latencies between Wave I and V. Furthermore, Rance et al. (2012d) reported that relative amplitudes of Wave V and I (ratio of V/I) can be used as a measure of brainstem dysfunction. For the appropriate auditory evoked potentials to be recorded the ABR relies on an averaging process. The potentials produced within the auditory system remain time-locked to the stimulus used and are therefore preserved. In comparison, random ‘extra-encephalic’ noise present within the recording (i.e. non-time-locked potentials produced by other neural elements within the body) are averaged out. In the presence of any disruption in the neural activity or loss of neural potentials, however, the recorded auditory evoked potentials can become obscured because of this averaging process. Temporal variations in the synchrony of neural firing of as little as 0.5 ms have been shown to disrupt the averaged ABR (Starr et al., 1991). Since AN is thought to be caused by dys-synchrony and/or deafferentation within the auditory system (Rance & Starr, 2015), the averaged ABR is typically absent or disordered in affected individuals. It is this clinical finding in conjunction with other assessments that is used to confirm a diagnosis of AN.

1.3.5 Acoustic reflex testing

As discussed in section 1.1.1, loud sounds cause the stapedius muscle to contract, which increases the stiffness of the middle ear system and dampens the movement of the ossicular chain. This change in

compliance can be measured using the middle ear acoustic reflex test. It is routinely used in audiological practice to confirm behavioural results and aid in the diagnosis of middle ear, cochlear or retrocochlear sites of lesion. The test can be used to probe the integrity of the acoustic reflex arc, which involves the VIII vestibulocochlear nerve, brainstem (cochlear and medial superior olivary nucleus), and the VII facial nerve (Borg, 1973). Hence, in an ear with normal middle ear function, absent acoustic reflexes can be attributed to a pathology affecting the inner hair cells, SGNs/auditory nerve, auditory brainstem nuclei (CN and MSO), facial nucleus or VII facial nerve. Acoustic reflexes have been shown to be absent or significantly impaired in individuals with AN, irrespective of their audiometric thresholds (Starr et al., 2010).

1.3.6 Cortical auditory evoked potentials (CAEPs)

Cortical auditory evoked potentials are an indication of the conscious perception of sound by an individual and can be evoked using complex stimuli such as speech. Through measures of amplitude and latency, CAEPs can be used to assess the salience and timing of sound processing, respectively. Cortical auditory evoked potentials are recorded using surface electrodes placed on the scalp of the subject. The potentials are generated from stimulus-locked post synaptic dendrites of pyramidal neurons from several cortical regions, including the primary auditory cortex and associated cortical regions, auditory thalamo-cortical as well as cortico-cortical pathways (Ceponiene et al., 1998; Ponton et al., 2002). The event related potentials that are recorded from these regions are separated into those that are ‘obligatory’ or ‘exogenous’ in nature (brain activity modulated by acoustical features) and those that are ‘discriminative’ or endogenous (measure of brain activity dependent on cognitive processing such as attention and learning) (Katz et al., 1985). In comparison to the ABR, cortical auditory evoked responses are much less susceptible to temporal fluctuations within the auditory system, with normal CAEPs being recordable despite changes of temporal synchrony of up to 20 ms (Michalewski et al., 1986).

An example of an obligatory event-related evoked potential is the P1-N1-P2 complex, which is used clinically in the estimation of functional hearing thresholds in patient populations that are unable to undergo standard audiometry or are otherwise non-compliant (Katz et al., 1985). It is believed that the P1-N1-P2 complex reflects the neural encoding of an acoustic signal, but it provides no information regarding speech discrimination. Comparatively, P300 is an example of an endogenous CAEP, which is elicited using an “oddball” paradigm. An oddball paradigm typically utilises two stimuli, whereby the target stimuli is presented infrequently among frequent standard stimuli. Active attention is required from the subject and therefore P300 provides a measure of the physiological processing involved in the discrimination of sound.

The literature is inconclusive regarding the prognostic value of CAEPs in individuals with AN. As discussed, it is generally accepted that CAEPs do not require the precise neural synchrony that is

necessary for the acquisition of an ABR and therefore they are more likely to be extant in affected individuals (Rapin & Gravel, 2003). For instance, Rance et al. (2002) found a strong positive correlation between the presence of obligatory CAEPs and aided open-set speech perception scores. In the study, all children with AN who showed reasonable speech perception performance had obligatory CAEPs of normal latency, amplitude and morphology. Comparatively, absent CAEPs were correlated with poor speech perception ability in affected individuals. Hence, the authors concluded that CAEPs might provide a suitable method for predicting aided speech perception ability in newly diagnosed children. Furthermore, Sharma and Cardon (2015) also advocate for CAEPs to be performed on all infants and children diagnosed with AN to assess auditory cortical maturation, as this information could be used as a prognostic marker for possible intervention strategies. Lee et al. (2001), however, reported on two cases of children with AN who showed recordable CAEPs but had poor aided speech perception scores. Therefore, even though CAEPs may be of some prognostic value for individuals with AN, further research needs to be undertaken before this assessment is adopted in routine clinical practice.

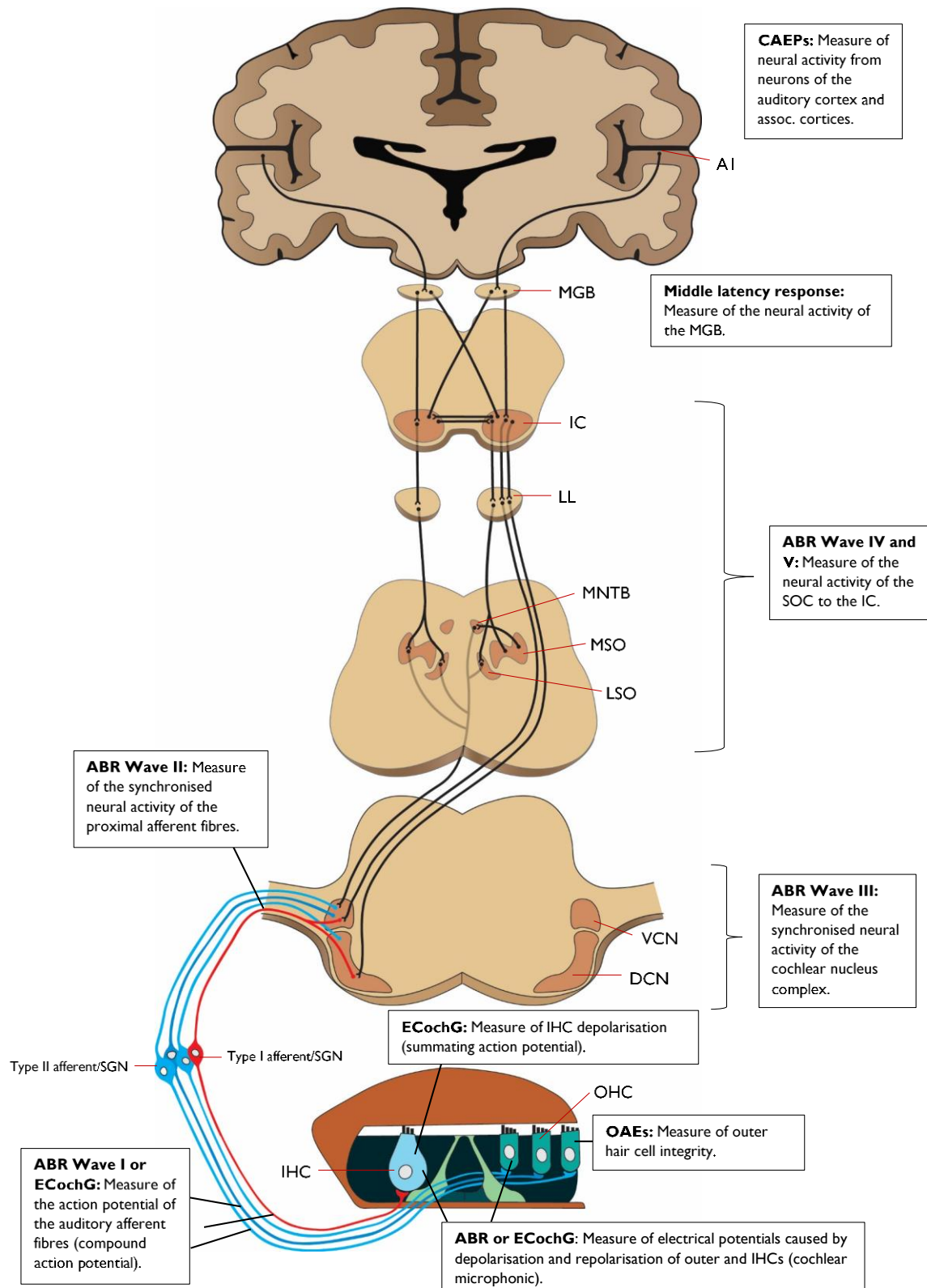


Figure 1.8 | Simplified schematic of the human afferent auditory system beginning at the level of the organ of Corti and ending at the auditory cortex. Diagnostic assessments and their target sites are labelled. ABR: auditory brainstem response; ECochG: electrocochleography; OAE: otoacoustic emissions; IHC: inner hair cell; OHC: outer hair cell; CAEP: cortical auditory evoked potential; A1: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; LL: lateral lemniscus; SOC: superior olivary complex; MNTB: medial nucleus of the trapezoid body; MSO: medial superior olive; LSO: lateral superior olive; VCN: ventral cochlear nucleus; DCN: dorsal cochlear nucleus.

1.4 Pathophysiological mechanisms underlying auditory neuropathy

The pathophysiological basis of AN stems from the disruption or distortion of the neural activity of the auditory afferent fibres (auditory nerve). This is reflected by the absence of an averaged ABR and typically extant but typically delayed cortical responses (Berlin et al., 2003; Starr et al., 1996). The underlying cause of this phenomenon is attributable to: (i) dyssynchrony: a loss of synchronous neural activity; and/or (ii) deafferentation: a reduction in neural discharge (Rance & Starr, 2015; Starr et al., 2010; Zeng et al., 2005).

Dyssynchrony of the auditory afferents is likely to disrupt the function of the brainstem nuclei, compromising their ability to appropriately filter, relay, and analyse incoming auditory information (Carr, 2004). Comparatively, deafferentation reduces the amplitude of the neural signals received at the level of the brainstem nuclei, making it harder for the nuclei to process the incoming information. The effects of dyssynchrony and deafferentation on the summed neural response of the auditory afferents (measured as the CAP or Wave I of the ABR) are shown in Figure 1.9. As illustrated, deafferentation decreases the amplitude of the measured response, whereas dyssynchrony occurs when the speed of neural conduction varies across the population of neuronal elements, therefore producing a ‘smearing’ of the averaged neural response. In either case, even if a neural response is present, it is much more susceptible to being obscured by extraneous electro-encephalic noise.

Our current understanding of lesion sites that produce the AN phenotype has come from several sources, including studies of animal models of AN, genetic conditions/mutations, and neuropathic disorders. In general, these lesion sites can be divided into two groups based on their locations: (i) a pre-synaptic site of lesion affects the processes and structures prior to the inner hair cell synaptic terminal; and (ii) post-synaptic lesion sites affect structures of the auditory system after the inner hair cell synapse. Different AN lesion sites affecting pre- and post-synaptic processes and structures are illustrated in Figure 1.10.

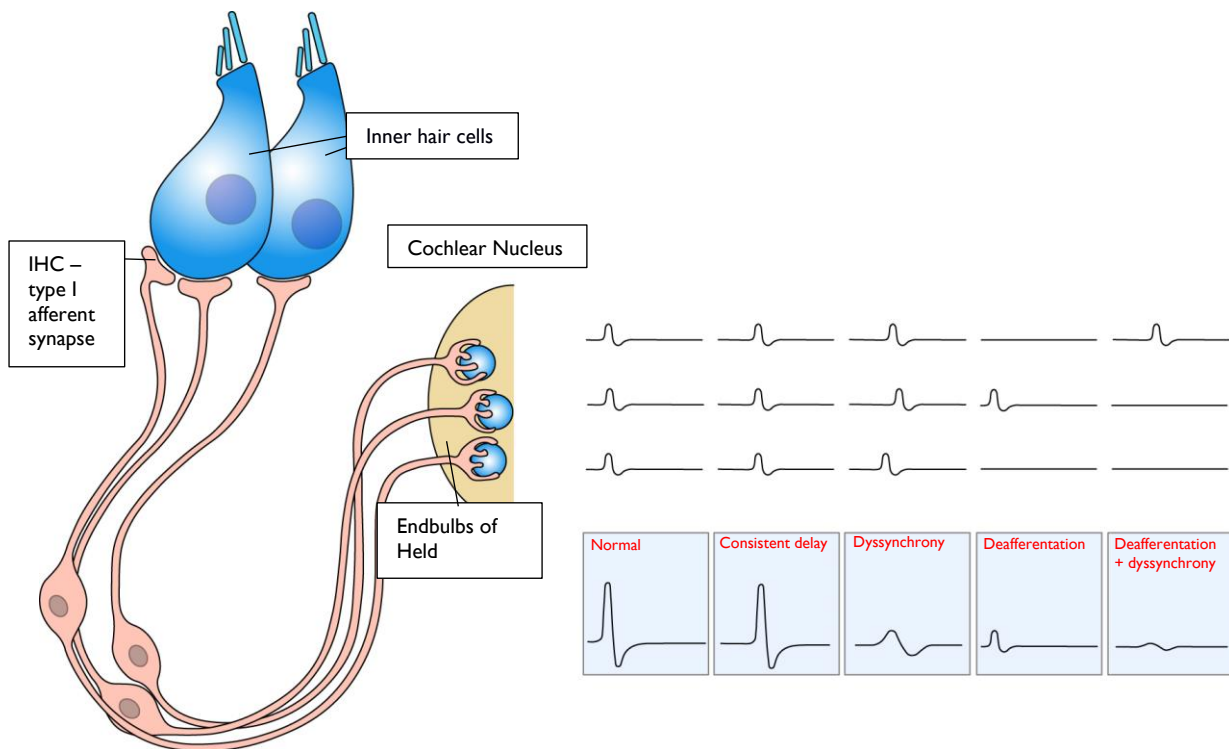


Figure 1.9 | The auditory brainstem response (ABR) and compound action potential (CAP) is a measure of the averaged summed response of neural firing. If the auditory nerve is firing synchronously the resultant averaged response will be clearly observed (normal). If there is a consistent delay in the production of the action potentials in each fibre the overall latency of the averaged response increases, however the quality of the response remains unaffected (i.e. conductive hearing loss). If the fibres are no longer firing synchronously (dyssynchrony), due to variable delays, the quality of the averaged response will deteriorate – amplitude of response decreases, and latency increases – a ‘smearing’ of the averaged response is observed. If there is a loss of neurons (deafferentation) the amplitude of the averaged response will decrease but the latency will likely be unaffected. The combination of the loss of neural synchrony and loss of neurons will result in an increase in latency and a decrease in amplitude – typically the recorded ABR or CAP will be obscured due to extraneous electro-encephalic noise. Figure adapted from (Moser & Starr, 2016).

1.4.1 Pre-synaptic mechanisms

As discussed, the inner hair cell is the structure responsible for the transmission of auditory information from the organ of Corti to the central auditory nervous system. Several mechanisms within the inner hair cells dictate the successful and consistent release of neurotransmitter at the synapse site. Disruption to these processes can lead to disordered or reduced activation of the auditory nerve afferents, resulting in the AN phenotype. The most common pre-synaptic lesion sites will be discussed in this section and are depicted in Figure 1.10.

1.4.1.1 Inner hair cells

A selective loss of inner hair cells has been implicated as a potential cause of AN. As discussed, depolarisation of the inner hair cells provides the impetus for auditory nerve activation, hence pre-synaptic lesion sites affecting the integrity of the inner hair cells are likely to result in a reduction in the number of auditory nerve afferents activated. Additionally, if the lesion site(s) cause inconsistencies in the depolarisation of the inner hair cells, a loss of synchrony in the neural discharge of the afferent fibres

may also occur. Overall, the clinical presentation of such lesion sites is expected to be dependent on the extent of inner hair cell loss/disruption, however it is likely that the ABR, compound action potential and SP would be absent or markedly reduced in amplitude in an affected individual. Comparatively, OAEs and the CM would be unaffected because the outer hair cells remain intact. This pattern of audiological results has been reported by El-Badry and McFadden (2009) who recorded ECoChGs, ABRs and OAEs from chinchillas treated with the chemotherapeutic agent, carboplatin, which causes isolated destruction of inner hair cells. The amplitudes of the ABRs and CAPs were significantly reduced in affected animals, whereas OAEs and CMs were normal.

In general, selective loss of inner hair cells is likely to be a rare phenomenon; research suggests that the outer hair cells are much more vulnerable and are often damaged first (Liberman & Kiang, 1978; Sha et al., 2001). For example, in most occurrences of cochlear sensory hearing loss (due to noise trauma, ototoxicity, presbycusis, or genetic abnormalities) inner hair cell degeneration occurs secondary to marked outer hair cell loss (Liberman & Kiang, 1978; Taylor et al., 2008). In humans and animal models, however, selective inner hair cell loss has been shown to occur in conditions such as hypoxia (Amatuzzi et al., 2011; Sawada et al., 2001; Shirane & Harrison, 1987), thiamine deficiency (Attias & Raveh, 2007; Liberman et al., 2006) and genetic mutations in diaphanous homolog 3 (DIAPH3)(Schoen et al., 2013; Starr et al., 2004).

The integrity of the organ of Corti obtained from premature infants who suffered a host of perinatal and postnatal complications, such as hyperbilirubinaemia, meningitis, septicaemia, hyaline membrane disease, and hypoxia has been studied in detail using histological analysis techniques (Amatuzzi et al., 2011; Amatuzzi et al., 2001). Selective inner hair cell loss in these infants occurred in conjunction with elevated or absent ABRs. Furthermore, histopathology results showed no associated degeneration of outer hair cells or auditory nerve afferent fibres. The researchers noted, however, that the longest duration of survival post-natally was only one month, which may not have been enough time for the degeneration of the afferent fibres to occur. Nevertheless, emerging evidence suggests that long-term survival of the SGNs and auditory afferents may be possible as long as supporting cells within the organ of Corti remain intact (Zilberstein et al., 2012). In temporal bone studies of adults with AN, however, inner hair cell loss is consistently accompanied by both outer hair cell and SGN degeneration (Bahmad et al., 2007; Starr et al., 2003).

1.4.1.2 Neurotransmitter release/inner hair cell synapse

Alongside the selective loss of the inner hair cells, disruption or dysfunction in the events modulating neurotransmitter release at the inner hair cell synapse can result in the AN phenotype. This type of lesion is typically referred to as a synaptopathy and is likely to cause inconsistencies in the activation of the auditory nerve afferent fibres (Moser & Starr, 2016; Santarelli, 2010; Zeng et al., 2005). An example of a synaptopathy linked to AN is caused by mutations in the OTOF gene (Varga et al., 2003).

The OTOF gene codes for otoferlin, a protein responsible for the replenishment of vesicles at the inner hair cell to auditory nerve synapse (Pangrsic et al., 2010). Mutations in OTOF result in a reduction in otoferlin expression, which in turn decreases the quantity and rate of synaptic vesicle replenishment. The subsequent decrement in neurotransmitter release is likely to compromise the consistency of synaptic transmission, thus disrupting the synchronised firing of afferent fibres and reducing the amplitude of neural potentials (Pangrsic et al., 2010). In support of the pathophysiological process described, Wynne et al. (2013) used psychophysical and electrophysiological measures of auditory adaptation to show that individuals with OTOF missense mutations exhibit auditory neural ‘fatigue’. In particular, their results revealed that the auditory nerve afferent fibres in affected individuals exhibited a progressive failure in their ability to fire synchronously as the auditory load was increased.

Transtympanic ECochG results in children with OTOF gene mutations typically show normal CMs and SPs, indicative of normal outer and inner hair cell activation. Compound action potentials, however, are often abnormal/absent, which in this case is likely indicative of a loss of neural synchrony in the firing of the auditory nerve afferents (Santarelli et al., 2015b; Santarelli et al., 2009; Santarelli et al., 2013). Auditory brainstem responses have also been reported to be abnormal/absent, however OAEs are commonly preserved (Pangrsic et al., 2010; Rodriguez-Ballesteros et al., 2003; Santarelli et al., 2009). This demonstrates the importance of a suitable battery of audiometric measures in discriminating the clinical presentation or manifestation of a particular genetic mutation.

1.4.2 Post-synaptic mechanisms

As much as the pre-synaptic components of the auditory system ensure consistent auditory nerve activation, many post-synaptic structures are also vital to the accurate and precise transmission of auditory information. Hence, dysfunction/disruption in a range of post-synaptic structures and physiological processes can also lead to dyssynchrony or reduced auditory nerve activity. Figure 1.10 provides an illustrative overview of these lesion sites and provides examples of some related disorders.

1.4.2.1 Dendritic nerve terminals

The most distal portion of Type I auditory nerve afferent fibres (dendritic terminals) are unmyelinated and populated by glutamatergic synapses. When these synapses bind with glutamate, the excitatory neurotransmitter released from the inner hair cell synaptic vesicles, the afferent fibres depolarise, producing an action potential (measured by CAP or ABR Wave I). Hence, the dendritic terminals are the first post-synaptic region in the peripheral auditory pathway susceptible to disruption.

Individuals with mutations in the gene Optic Atrophy 1 (*OPA1*), which is involved in regulating mitochondrial function, have been shown to exhibit hearing difficulties consistent with the AN phenotype (Santarelli et al., 2015c; Santarelli et al., 2011). The exact cellular mechanism(s) involved have not yet been elucidated, however evidence from electrophysiological studies show result patterns consistent with a lesion site at the dendritic portion of type I afferent fibres (Huang et al., 2009). For

instance, electrocochleography results for individuals with *OPA1* mutations typically show normal SPs and absent CAPs, indicative of normal inner hair cell function but reduced or disordered neural activity. This pattern of results alone is not sufficient to identify the terminal dendrites as the primary site of lesion. Importantly, Huang et al. (2009), however, also identified an abnormally prolonged low amplitude negative potential, which the authors attributed to the incomplete depolarisation of the unmyelinated terminal dendrites. Auditory brainstem responses are also typically absent or abnormal in affected individuals (Huang et al., 2009; Santarelli et al., 2015c). Furthermore, axonal loss and demyelination have been implicated in advanced stages of the *OPA1* disease progression and therefore it is likely that loss of terminal dendrites is the initial point of dysfunction, with greater degrees of disruption occurring over time (Kjer et al., 1983). This is consistent with findings reported by Santarelli et al. (2015c), whereby individuals with advanced disease progression performed poorly with a cochlear implant compared to those in earlier stages.

Another source of damage to the terminal dendrites is exposure to high levels of noise (Kujawa & Liberman, 2009). In particular, noise trauma has been shown to have harmful effects on the synapses at the terminal dendrites of the auditory nerve afferents. When the auditory system is subjected to harmful levels of noise, there is a significant expulsion of neurotransmitter from the inner hair cell synapse. The release of excessive neurotransmitter can have a toxic effect on the synapses at the dendritic terminals of the afferent fibres (Spoendlin, 1971). Kujawa and Liberman (2009) studied the neural components of the auditory system in mice who had been temporarily exposed to damaging levels of noise. They found a marked loss of post-synaptic terminals with eventual degeneration of affected Type I auditory afferent fibres. This occurred even in the absence of a shift in the hearing thresholds or sensory hair cell loss. Furthermore, Kujawa and Liberman (2009) also reported a decrease in ABR amplitudes, reflecting a reduction in neural elements contributing to the recorded response.

Furman et al. (2013) and Kujawa and Liberman (2015) have since shown that the neuronal loss selectively affects low-SR/high threshold fibres, while high-SR/low threshold fibres are unaffected. This selective susceptibility has been attributed to the comparatively fewer mitochondria that low-SR fibres possess, which makes them less able to withstand glutamate excitotoxicity (Liberman, 1980). As discussed in section 1.1.3.2, low-SR fibres play an important role in the ability to hear in the presence of background noise (Costalupes et al., 1984), whereas high-SR fibres determine audibility thresholds. Hence, selective deafferentation of low-SR fibres likely explains the difficulties hearing in the presence of background noise frequently reported by individuals with a history of noise exposure, despite often retaining normal hearing thresholds, which are assessed in quiet conditions (Furman et al., 2013; Plack et al., 2014). This type of peripheral hearing impairment has been termed ‘hidden hearing loss’ because current diagnostic assessments are not able to identify these subtle changes within the human auditory system. Hidden hearing loss has also been implicated in the functional deficits reported

among the elderly, suggesting age-related degeneration of low-SR afferent fibres (Kujawa & Liberman, 2015).

1.4.2.2 Demyelination of auditory nerve afferent fibres

Myelin plays a vital role within the central auditory nervous system. It acts to significantly increase the conduction velocity along axons and reduce their refractory period, ultimately ensuring the timely transfer of action potentials from the site of propagation to the site of termination. Hence, the integrity of the myelin sheath is pivotal for relaying and processing temporal information along auditory circuits (Long et al., 2017). Even a slight loss of myelin can result in a reduction in temporal precision, which in turn can distort the perceived auditory signal. The role of myelin in audition has been investigated using experimental studies of myelin-deficient animals. For instance, rodents exposed to diphtheria toxin (Zhou et al., 1995a; Zhou et al., 1995b) and those with peripheral myelin protein 22 (*PMP22*) gene mutations (Naito et al., 1999, 2003) show extensive myelin and neuronal loss, whereas the sensory hair cells of the cochlea remain unaffected. The auditory phenotype in affected animals is consistent with AN; auditory brainstem responses show elevated thresholds, reduced amplitudes, and prolonged latencies (Naito et al., 2003; Zhou et al., 1995b). Prolongation of ABR latencies reflects the loss of myelin and provides evidence as to the important role of myelination in nerve conduction velocity (Long et al., 2017), whereas changes in ABR amplitude could be attributable to both demyelination or associated neuronal loss (Figure 1.9). While these animal models typically exhibit gross demyelination affecting all auditory nerve afferent fibres, varying degrees of demyelination are also possible. In such cases, the speed of neural conduction within each auditory nerve afferent fibre is likely to differ, which would impact the synchrony of neural discharge (Rance, 2005). Similarly, disruption in the initial action potential generators, the heminodes (myelin-sheath gaps), close to the inner hair cells (see Figure 1.10) can significantly impair the synchronised conduction of action potentials (Kim & Rutherford, 2016; Wan & Corfas, 2017). Demyelination can also cause prolonged nerve refractory periods resulting in an intermittent or complete inability of the nerve to propagate action potentials. This phenomenon is known as a conduction block and is likely to occur in instances of repetitive neural activation (Rasminsky & Sears, 1972; Wynne et al., 2013).

Rance et al. (2012d) studied the auditory function in individuals with a demyelinating condition known as Charcot-Marie-Tooth disease type 1 (CMT1). Charcot-Marie-Tooth is one of the most common inherited neuropathies with over 50 subtypes characterised and 35 causative genes identified. Individuals with CMT1 who have mutations in their *MPZ* and *PMP22* genes typically present with perceptual issues consistent with the AN phenotype. Rance et al. (2012d) reported that ABR amplitudes were normal in affected individuals but conduction time was delayed between Waves I and III (auditory nerve to CN – refer to Figure 1.8 for a diagrammatic representation) compared to healthy controls. Conversely, normal conduction times were reported between Waves III and V (CN to IC). These

findings suggest affected individuals have normal neuronal populations and their myelin loss may be limited to the auditory nerve afferent fibres.

Although neuronal populations appeared unaffected, as evidenced by electrophysiological assessments, in the individuals studied by Rance et al. (2012d), there are reported cases where axonal loss occurs in conjunction with demyelinating disorders, such as CMT1 (Dyck et al., 1989), multiple sclerosis (Trapp et al., 1998), and chronic inflammatory demyelinating polyneuropathy (Nagamatsu et al., 1999). This is unsurprising given myelin plays an important role in axonal maturation and long-term axonal survival (Yin et al., 1998). As a result, it can often prove difficult to determine whether functional deficits are attributable to demyelination alone, or due to a combination of both demyelination and deafferentation (Rapin & Gravel, 2003).

1.4.2.3 Axonal neuropathies

Axonal neuropathies or axonopathies, can cause degeneration of the axonal portion of the auditory nerve afferent fibres, resulting in a reduction in neural activity and a possible concomitant loss of neural synchrony. Genetic factors are the most common cause of axonopathies, and a host of mutations have been identified that involve the axons of the auditory system. Some of these genetic mutations are presented in Figure 1.10. In the majority of these conditions, axonal loss is widespread, with other neural systems involved. Hence, affected individuals can exhibit a broad range of sensory and/or motor dysfunctions in conjunction with the AN phenotype.

Although the mechanism leading to axonal degeneration can vary between disorders, most neurodegenerative conditions exhibit retrograde degeneration or ‘Wallerian-like degeneration’ (Wang et al., 2012). This term reflects the ‘dying back’ of the axons from their synaptic terminals towards the cell body. Typically, axonal degeneration progresses from axonal swelling and microtubule disassembly to complete disintegration of the axonal cytoskeleton (Cavanagh, 1964). The degenerative process is accompanied by macrophage infiltration, which are recruited to phagocytose the myelin and axonal debris (Martini et al., 2008).

In axonopathies, nerve conduction velocities and refractory times are expected to be unaffected (Rance et al., 2009; Rance et al., 2012d). Hence, measures of adaptation and assessments of saltatory conduction can theoretically be used to differentiate between purely axonal and myelin related neuropathies (Rapin & Gravel, 2003). In practice, however, the matter is complicated by the intricate relationship between axons and their myelin sheaths, making it unlikely that severe axonal loss occurs without any associated myelin sheath degeneration (Kalaydjieva et al., 1998; Rapin & Gravel, 2003). Therefore, the relevance of the aforementioned diagnostic assessments can be limited. Audiological tests, however, generally show a specific pattern of results in individuals with axonopathies involving the auditory nerve afferent fibres. Since such neuropathies rarely involve the sensory cells of the cochlea, measures of inner and outer hair cell activity, such as OAEs, the cochlear microphonic, and

summating potential amplitudes and latencies are expected to remain normal in affected individuals (Rance & Starr, 2015). There is, however, at least one report indicating reduced SP amplitudes in individuals with Friedreich's Ataxia (FRDA; an autosomal recessive hereditary neurodegenerative disorder caused by a loss of function mutation in the frataxin gene (*FXN*) that affects normal mitochondrial function), which could reflect the disease sequelae in FRDA, whereby inner hair cell loss occurs secondary to auditory nerve terminal degeneration (Santarelli et al., 2015a). Comparatively, indirect measures of axonal integrity/synchronised firing within the auditory system, such as the CAP and ABR, are expected to be reduced in amplitude or altogether absent depending on the severity of deafferentation (Rance & Starr, 2015; Santarelli et al., 2015a).

In addition to FRDA, other neurodegenerative conditions are thought to cause axonal degeneration of the cochlear nerve. Some of these conditions also involve genetic mutations which impact normal mitochondrial function including, X-linked auditory neuropathy or X-linked sensorineural deafness (AUNX1/DFNX5; Wang et al. (2006)), Charcot-Marie Tooth disease type 2 (CMT2; Rance et al. (2012d)), Leber's hereditary optic neuropathy (Ćeranić & Luxon, 2004; Rance et al., 2012c), and Mohr-Tranebjaerg syndrome (Merchant et al., 2001), while other conditions, such as riboflavin transported deficiency (RTD) or Brown-Vialetto-Van Laere syndrome (Menezes et al., 2016), involve other physiological and molecular processes.

1.4.2.4 Spiral ganglion disorders

The cell bodies of auditory nerve fibres are situated in the modiolus of the cochlea and are known as the SGNs. Like the sensory hair cells of the cochlea, once damaged these neurons do not regenerate in mammals. Bilirubin toxicity has been shown to specifically impair SGNs and their axons (Shaia et al., 2005). The current pathogenic theory of bilirubin neurotoxicity posits that increased levels of bilirubin disrupts intracellular calcium homeostasis by damaging calcium-binding proteins (Shapiro & Nakamura, 2001). Subsequently, this results in an accumulation of extracellular or intracellular calcium which is incompatible with neuronal viability (Hegarty et al., 1997). Bilirubin neurotoxicity has also been shown to damage the auditory brainstem nuclei with documented disruption in calcium-binding proteins of the VCN and LSO (Shapiro & Nakamura, 2001). Hyperbilirubinaemia and kernicterus have been linked to the AN phenotype. Chisin et al. (1979) and Yilmaz et al. (2001) reported absent ABRs and extant pre-neural responses (cochlear microphonics and OAEs) in a group of neonates born with hyperbilirubinaemia. Similar results were reported by Santarelli and Arslan (2002) who performed transtympanic ECoChG on two infants born with hyperbilirubinaemia. Both of these infants had normal CMs and summating potentials but absent CAPs. As discussed, the findings support the hypothesis that hyperbilirubinaemia affects the ability of the auditory nerve afferents to generate action potentials.

1.4.2.5 Auditory nerve hypoplasia

Congenital auditory nerve hypoplasia/aplasia can occur unilaterally or bilaterally and in the absence of any cochlea or brainstem involvement (Buchman et al., 2006; Roche et al., 2010). It is also commonly associated with stenosis of the internal auditory canal (Ai et al., 2016; Miyasaka et al., 2010). Magnetic resonance imaging (MRI) is the preferred method of diagnosis (Casselmann et al., 2001). Typically, T2-weighted para-sagittal MR images are obtained perpendicular to the direction of the internal canal and axial three-dimensional Fourier transformation-constructive interference in steady state (3DFT – CISS) images are used to determine the size of the cochlear nerve (Casselmann et al., 1997). The integrity of the cochlear nerve is subjectively assessed based on its comparative size to the other nerves within the internal auditory canal. In ~ 90% of normal cases the cochlear nerve is larger than the superior or inferior vestibular nerve (Glastonbury et al., 2002). Furthermore, in ~ 64% of cases it is considered similar in size or larger than the facial nerve (Kim et al., 1998). In individuals with cochlear nerve hypoplasia/aplasia cochlear function may be normal, i.e. present OAEs, cochlear microphonic and summing potential (depending on the state of the cochlea), however the ABR is typically absent (Ai et al., 2016; Liu et al., 2012).

1.4.2.6 Brainstem dysfunction

Conditions that affect the propagation of neural signals through the brainstem can also cause the phenotypic features of AN. Tumours of the cerebellopontine angle (e.g. acoustic neuromas and vestibular schwannomas), for example, can compress the auditory nerve fibres, disrupting neural propagation and synchrony within the auditory system. The ABR can be normal, completely absent or Wave I-V conduction times can be prolonged. Results are dependent on tumour size and degree of auditory nerve compression (Matthies et al., 1997). Cochlear function is also disrupted in a large portion of individuals with cerebellopontine tumours, caused by compression or obstruction of the labyrinthine artery which supplies blood to the cochlea (Prasher et al., 1995). Hence, OAEs and EcochG results are variable and no characteristic pattern of diagnostic results exists.

1.4.3 Considerations regarding diagnostic assessments

Given the significant variation in the pathophysiological processes that cause AN, clinical evaluation is dependent on a battery of diagnostic assessments, some of which have been discussed in Chapter 1.3. It is important to note, however, that while there is currently no standardised assessment protocol in the diagnosis of AN several guidelines have been published (Feirn et al., 2013; Moser & Starr, 2016; Rajput et al., 2019) (Table 1.1). Non-routine assessments are recommended if concomitant neurological conditions are suspected. Furthermore, as genetic testing becomes more readily available and affordable it is likely that it will play a greater role in guiding rehabilitation options and may supplement routine audiological assessments in all individuals diagnosed with AN (Shearer et al., 2010; Shearer et al., 2017).

Table 1.1 | A brief overview of the recommended clinical investigations for auditory neuropathy. Adapted from Moser and Starr (2016).

Routine audiological assessments
Audiometry (pure tone/play/visual reinforcement)
Speech audiometry (Open set speech perception)
Tympanometry (1000Hz/226 Hz; dependent on age)
Otoacoustic emissions (DPOAE/TEOAE)
Auditory brainstem response (Separate waveforms to rarefaction and condensation clicks for identification of the cochlear microphonic)
Acoustic reflex testing
Non-routine assessments
Electrocochleography (trans-tympanic/extra-tympanic)
Electric auditory brainstem response
Speech and language assessments (including speech in noise assessments)
Psychoacoustic assessments (gap detection/amplitude modulation, frequency discrimination)
Measures of adaptation as described in Wynne et al. (2013) – i.e. ABR adaptation or subjective adaptation assessments
Cortical auditory evoked potentials
MRI scan (structural imaging – typically a 3DFT-CISS sequence of the IAC)
Neurological assessment
Genetic testing
Ophthalmologic assessment
Vestibular assessment (vHIT, VEMP, calorics, videooculography)
Evaluation of hearing aid/assistive listening device/cochlear implant outcomes

DPOAE: distortion product otoacoustic emissions; TEOAE: transiently evoked otoacoustic emissions; MRI: magnetic resonance imaging; 3DFT-CISS: 3-dimensional Fourier transform constructive interference in the steady state; IAC: Internal auditory canal; vHIT: video head impulse test; VEMP: vestibular evoked myogenic potential.

Regardless of the lesion site, however, dyssynchrony and/or deafferentation results in specific and measurable functional deficits (Moser & Starr, 2016; Zeng et al., 2005). These typically relate to the ability to integrate temporal cues to localise and discriminate complex or rapidly fluctuating sounds (i.e. speech) and distinguish auditory signals in the presence of background noise (Rance & Barker, 2009; Rance et al., 2009; Rance & Starr, 2015).

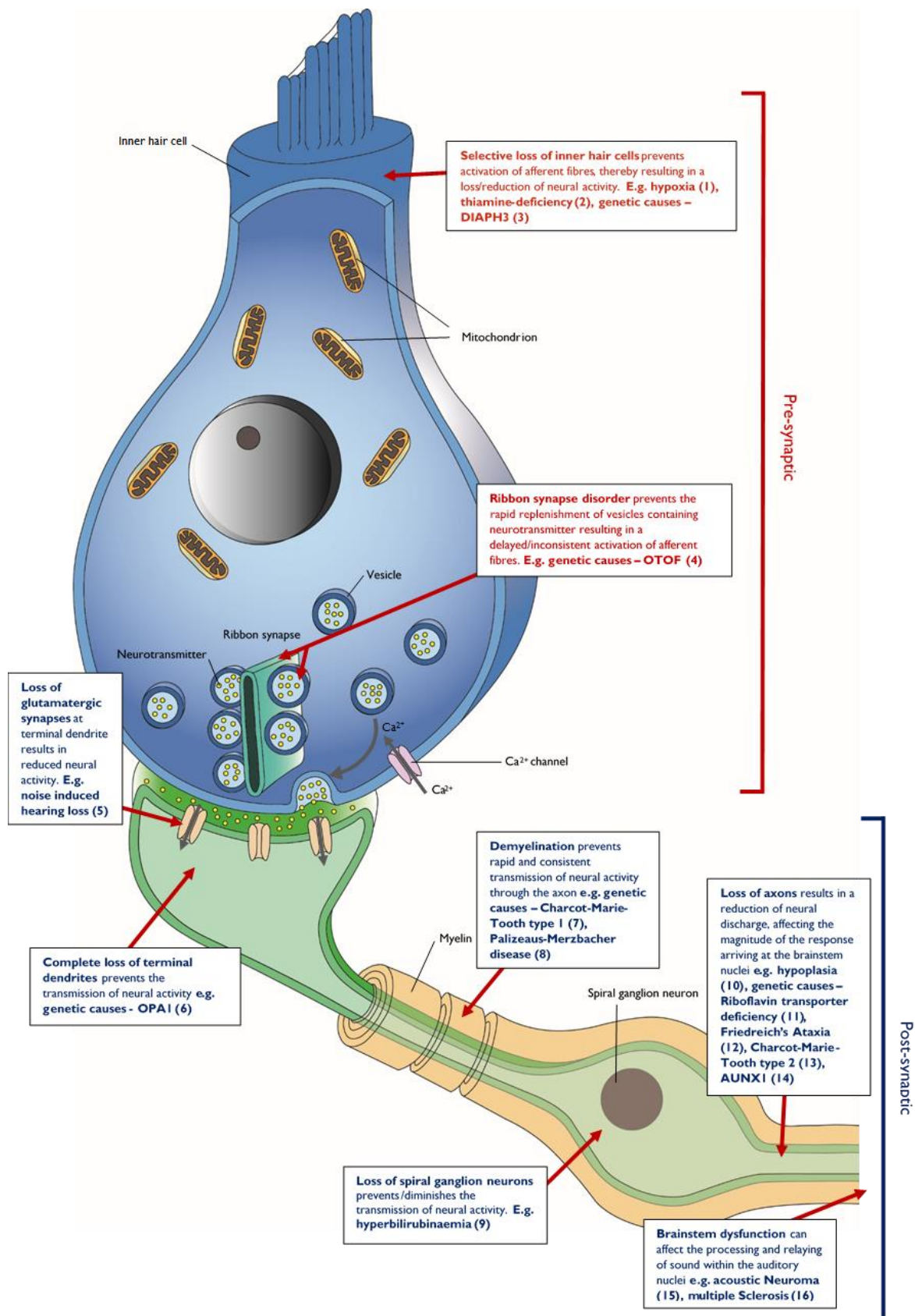


Figure 1.10 | Illustrative overview of the varying sites of lesion (pre- and post-synaptic) known to cause the auditory neuropathy phenotype. A single inner hair cell and accompanying auditory afferent fibre is shown. Examples of aetiologies are indicated for each site of lesion and key findings are provided: (1)(Amatuzzi et al., 2011; Amatuzzi et al., 2001), (2)(Attias et al., 2012), (3)(Starr et al., 2004), (4)(Santarelli, 2010; Santarelli et al., 2015b; Starr et al., 1998), (5)(Kujawa & Liberman, 2009, 2015), (6)(Huang et al., 2009; Santarelli et al., 2015c), (7)(Bertorini et al., 2004; Rance et al., 2012d), (8)(Kuan et al., 2008), (9)(Shaia et al., 2005), (10)(Buchman et al., 2006), (11)(Menezes et al., 2016), (12)(Rance et al., 2012b; Rance et al., 2008), (13)(Rance et al., 2012d), (14)(Wang et al., 2006; Zong et al., 2015), (15)(Laury et al., 2009; Selesnick & Jackler, 1993), (16)(Drulović et al., 1994).

1.5 The auditory neuropathy phenotype: functional hearing deficits

Functional deficits can vary significantly between individuals with AN. There are, however, some characteristic auditory perceptual consequences that are present in all affected individuals regardless of lesion sites. This section provides an overview of some of the impaired auditory domains and discusses how these relate to reported perceptual difficulties.

1.5.1 Hearing thresholds

Hearing thresholds vary significantly in individuals with AN. In general, there is a relatively even distribution of individuals with normal audiometric thresholds (≤ 20 dB HL) and those with profound cochlear sensory hearing loss (≥ 90 dB HL) (Rance & Starr, 2015; Sininger & Oba, 2001). There is no clear association between the severity of audiometric thresholds and aetiology, however, elevation in hearing thresholds is likely to indicate a greater severity of disruption and is more likely associated with a significant loss of inner hair cells and/or auditory nerve fibres (Starr et al., 2010).

Bourien et al. (2014) have shown that extensive loss of low-SR fibres has a negligible effect on audiometric thresholds and CAP amplitude, whereas a loss of medium and high-SR fibres results in a deterioration in both. Hence, it is possible that individuals with AN who have normal or near-normal thresholds may have a selective loss of low-SR fibres. Comparatively, individuals with AN who have greater degrees of cochlear sensory loss may have a reduction in the complete gamut of SR fibres. Sagers et al. (2017) studied the cochlear histopathology in 30 individuals with AN and found severe neuronal loss was correlated with elevated hearing thresholds, particularly in the lower frequencies (< 1 kHz). Similar findings have been reported for inner hair cell loss. For example, Lobarinas et al. (2013) studied the effect of carboplatin induced inner hair cell loss on behavioural audiometric thresholds in chinchillas. They found only very large inner hair cell lesions caused discernible audiometric threshold shifts. Even chinchillas with only 10-20% of their inner hair cells intact exhibited normal pure-tone thresholds (Lobarinas et al., 2013). Results of the study suggest that only a small population of inner hair cells is necessary for normal audiometric thresholds if the outer hair cells remain intact. It is likely, however, that any degree of inner hair cell loss would produce other functional deficits in hearing ability, which are not apparent by pure-tone audiometry testing alone.

Fluctuating hearing thresholds have also been reported in individuals with AN. For instance, individuals with mutations in the *OTOF* gene exhibit a transient cochlear sensory hearing loss brought about by a rise in core body temperature (i.e. due to fever, physical exertion etc.) (Marlin et al., 2010; Starr et al., 1998). The physiological cause of the transient threshold shift has not been elucidated, however it is likely that the rise in core temperature disrupts neurotransmitter release at the ribbon synapse, affecting the synchrony of neural discharge and/or preventing the activation of auditory nerve afferents (Santarelli et al., 2011; Varga et al., 2003).

1.5.2 Frequency discrimination

In individuals with AN, frequency discrimination is selectively impaired for low frequency tones but not above 4 kHz (Rance et al., 2004; Zeng et al., 2005). While individuals with cochlear sensory hearing loss also exhibit poorer than normal frequency discrimination, albeit to a much lesser extent, the mechanisms underlying the impairment are likely to differ between the two conditions (Freyman & Nelson, 1991; Heinz et al., 2001a; Zeng et al., 2005).

Animal lesion studies and computational models of the auditory system offer a possible explanation for the selective impairment in the discrimination of low frequency tones reported for individuals with AN. Computational models of auditory nerve activity proposed by Heinz et al. (2001b) indicate that frequency discrimination, particularly for low frequency sounds, relies on phase-locking and the collective information from a large population of afferent fibres. In AN, the presence of deafferentation and dyssynchrony impairs the phase-locking ability at the auditory periphery, which is likely to reduce the fidelity of frequency coding. In support of these computational models, are the findings of Nienhuys and Clark (1978) who investigated the effect of inner hair cell loss on frequency discrimination in the cat. These authors reported frequency resolution to be severely disrupted when more than half of inner hair cells were damaged. Results from this study suggest that frequency discrimination ability is dependent on the presence of at least some normal functioning inner hair cells. As the number of inner hair cells reduces there is likely a subsequent reduction in the amount of auditory nerve afferents being activated.

1.5.3 Intensity discrimination

As discussed in Section 1.1.3.2, intensity discrimination is dependent on many factors, including the different virtues of the high and low SR-fibres, the sensitivity of the inner hair cells and their complex ribbon synapse, and the contribution of the outer hair cells. These components work together to ensure sound intensity is encoded faithfully to provide a large dynamic range of hearing. Given that several AN lesion sites disrupt the inner hair cells or cause degeneration of afferent fibres it could be expected that the ability to resolve intensity would be impaired in at least some affected individuals. For example, Plack et al. (2014) have proposed that the selective loss of low-SR fibres associated with ‘hidden hearing loss’ results in an impaired ability to discriminate intensity. Moreover, Wynne et al. (2013) and Santarelli et al. (2015b) have also reported abnormal loudness adaptation in individuals with AN due to disruption of their ribbon synapses (i.e. mutation in the *OTOF* gene). Rance et al. (2012e), however, found no significant deterioration in intensity discrimination ability in individuals with the AN phenotype resulting from FRDA and CMT1. Hence, to date, there is no conclusive evidence to suggest processing of intensity cues is impaired in individuals with AN (Rance & Starr, 2015).

Comparatively, in cochlear sensory hearing loss, perception of intensity is significantly altered. For example, cochlear damage results in an abnormally steep growth of loudness with increasing stimulus

intensity (loudness recruitment) (Fowler, 1936). Furthermore, Turner et al. (1989) have shown that individuals with cochlear sensory hearing loss are better able to discern changes in intensity compared to individuals with normal hearing at equal sensation levels.

1.5.4 Temporal processing

Deficits in processing temporal cues is one of the defining characteristics of AN. The degree and nature of temporal coding deficits have been studied using a range of psychophysical tasks, including measures of binaural integration (assessments of interaural time differences and binaural beats), gap detection, forward and backward masking, temporal and amplitude modulation detection, and signal detection in noise (Zeng et al., 2005). Individuals with AN have been shown to be impaired in all domains investigated. Reported deficits are unique to AN and are typically not present with cochlear sensory hearing loss as long as intensity impairment and non-linear compression is accounted for (Oxenham & Bacon, 2003).

The results of psychophysical assessments used to investigate the temporal processing ability of individuals with AN can be used to explain some of their perceptual difficulties experienced outside of the controlled test environment. For instance, assessments of binaural integration show an inability to resolve interaural timing differences, which reflects an impaired ability to localise low frequency sounds. Normal hearing listeners can typically resolve $< 3^\circ$ of change in the direction of low frequency sounds, whereas individuals with AN struggle to hear differences of up to 90° (Zeng et al., 2005). In addition to contributing to localisation ability, accurate processing of interaural timing differences also improves speech intelligibility in the presence of competing noise (Bronkhorst & Plomp, 1988). Hence, the deterioration in the ability to process interaural timing differences goes some way towards explaining why individuals with AN also commonly report significant difficulties in their ability to understand speech and converse in noisy environments (Rance et al., 2012e). Comparatively, the processing of interaural intensity differences, crucial for the localisation of high frequency sounds, remains unaffected in AN.

Other psychophysical assessments provide information regarding the ability to resolve rapid changes in complex acoustic stimuli. Poor performance on these assessments translates to difficulties in speech perception in quiet *and* in the presence of background noise. For instance, gap detection investigates the shortest period of silence that can be discerned in a constant acoustic stimulus. Individuals with normal hearing and cochlear sensory loss can resolve gaps of $\sim 3 - 5$ ms at high sensation levels (>30 dB), whereas some individuals with AN can only identify gaps > 20 ms in duration (Nelson & Thomas, 1997; Zeng et al., 2005). This has implications for speech perception ability, specifically, the ability to discriminate voice onset times. The voice onset time is the period between air release and vocal cord vibration. It is the only cue available to listeners to distinguish voiced and unvoiced stop consonants (i.e. /ta/ and /da/, and /pa/ and /ba/). For these consonants the voice onset time differs by only 30 – 70

ms, which means that individuals with AN often struggle to differentiate between these stop consonants (Rance et al., 2010; Rance et al., 2008).

Individuals with AN also have significant difficulties in identifying both slow and fast amplitude fluctuations. The peak sensitivity for amplitude modulation detection thresholds in normal hearing listeners is ~ -19.9 dB (10% modulation), whereas peak sensitivity is reduced to -8.7 dB (37% modulation) in individuals with AN (Zeng et al., 2005). Such deficits have implications for speech processing. In particular, poorer amplitude modulation detection translates to deficits in the processing and integration of the temporal fine structure and envelope of speech. As discussed in Section 1.1.3.3, the temporal fine structure carries information that contributes to speech intelligibility in noisy environments. Hence, the findings reported by Zeng et al. (2005) further explain why individuals with AN typically have significant difficulties understanding speech in the presence of background noise.

The temporal processing deficits discussed can eventuate from both pathophysiological models of AN - dyssynchrony or deafferentation. As mentioned previously and illustrated in Figure 1.9, a loss of neural synchrony results in the smearing of timing information within the auditory system. It is less clear how deafferentation can disrupt temporal processing, however, Oertel et al. (2000) and Joris et al. (2004) suggest that the convergence of multiple stochastic auditory nerve fibre inputs leads to enhanced temporal precision in the firing of the cells within the cochlear nucleus. Therefore, a loss of neuronal input could also reduce the temporal precision in the discharge of the cells within the auditory brainstem nuclei.

1.6 Restoration of functional hearing

The most suitable intervention strategy for individuals with AN is a topic of significant debate within the literature (Miyamoto et al., 1999; Moser & Starr, 2016; Rance et al., 1999; Rance et al., 2002; Rance & Starr, 2015; Rapin & Gravel, 2003; Roush, 2008). Hearing aids, assistive listening devices, and cochlear implants are all viable options. Outcomes, however, can vary significantly for the different devices available. This variability is likely to be explained by the heterogeneity in lesion sites and the wide range of functional deficits exhibited by affected individuals.

1.6.1 Hearing aids and assistive listening devices

Conventional hearing aids can increase speech audibility and reduce the detrimental effects of background noise, but they are not able to improve access to the temporal aspects of an auditory stimulus. As a result, hearing aids are often not effective in restoring functional hearing in individuals with AN (Berlin et al., 2010; Rance et al., 2002; Raveh et al., 2007; Roush, 2008). Furthermore, the fitting process itself can be problematic because hearing thresholds can often fluctuate and are often a poor representation of actual hearing deficits, making it difficult to select the appropriate amplification targets for the initial setup and ongoing management (Berlin et al., 2010; Ching et al., 2013). In infants

this problem is exacerbated as behavioural assessments cannot be administered and infant objective assessments (ABR and auditory steady-state response testing) do not yield viable results, hence actual audiometric thresholds cannot be accurately established. As a result, a significant amount of time can elapse before behavioural thresholds are obtained (at around 10 months of age) and hearing aids are fitted. While this scenario is certainly not ideal, Rance and Starr (2015) still recommend hearing aids should be trialled in the first instance and cochlear implantation should be considered only if conventional amplification is not successful. Similarly, the guidelines regarding the management of individuals with AN published by the British Society of Audiology (Georga & Lightfoot, 2019) also recommend fitting hearing aids from the outset as long as behavioural thresholds are reliably elevated. In support of this recommendation are studies conducted by Ching et al. (2013), Rance et al. (1999), and Rance et al. (2002) which showed good outcomes for a proportion of individuals with AN fitted with hearing aids. All the participants fitted with hearing aids in these studies, however, had audible hearing thresholds as measured by behavioural audiometry and/or CAEPs.

Assistive listening devices designed to reduce the deleterious effect of background noise, such as remote microphone systems, have also been shown to provide substantial benefit to some individuals with AN. These types of devices are particularly suitable for individuals with AN, who have relatively good speech perception ability in quiet but experience considerable difficulty in noisy environments (Hood et al., 2003). Depending on an individual's hearing threshold, these devices can be fitted in conjunction with hearing aids or separately, without any amplification. Rance et al. (2012a) reported remote microphone systems improved the speech perception ability of children with FRDA who presented with the AN phenotype. The use of remote microphone systems enhanced performance of this cohort to the level of their normal hearing peers in a controlled test environment designed to simulate the acoustical environment of a mainstream classroom (Rance et al., 2012a). The benefit of remote microphone systems in other AN patient populations, however, remains to be shown.

1.6.2 Cochlear implants

Given that a portion of individuals with AN receive no benefit from hearing aids, many specialists have sought an alternative solution. Cochlear implantation has been recognised a viable option and is currently the intervention option of choice to restore functional hearing ability irrespective of hearing thresholds (Moser & Starr, 2016; Rance & Starr, 2015). Cochlear implantation involves substituting the function of the inner hair cells and terminal dendrites with an electrode array that is surgically implanted inside the cochlea. An ear level device receives, processes and filters auditory signals from the environment and transmits the information to the implant. Within the implant, this information is converted into an electrical current and delivered to different channels of the electrode array, where it stimulates the dendrites, spiral ganglion neurons and axons (Type I afferents) in a frequency specific-manner.

Cochlear implantation has been used successfully in individuals with sensory cochlear hearing loss for the past ~ 30 years. Individuals with cochlear sensory hearing loss, if implanted early enough (< 12 months of age), can attain communication skills equivalent to their normal hearing peers (Dettman et al., 2016; Geers et al., 2003; Leigh et al., 2013). Comparatively, outcomes are not as well defined in individuals with AN. The speech, language, and communication development of a significant proportion (~ 75%) of children with AN who receive a cochlear implant parallels that of implanted individuals with cochlear sensory hearing loss (Breneman et al., 2012; Ching et al., 2013; Madden et al., 2002; Miyamoto et al., 1999; Nassiri et al., 2018; Rance & Barker, 2009; Santarelli et al., 2015c; Teagle et al., 2010; Zeng & Liu, 2006). For the remainder (~25%), however, cochlear implant outcomes remain poor, with some individuals receiving minimal to no benefit from the implanted device (Gibson & Sanli, 2007; Roush et al., 2011; Teagle et al., 2010). Several systematic reviews on the topic have been conducted and although the findings are generally favourable in terms of cochlear implant outcomes, adequate evidence remains lacking (Fernandes et al., 2015; Humphriss et al., 2013; Roush et al., 2011). Furthermore, Attias and Raveh (2007) have reported on five infants diagnosed with AN who made a partial or full recovery 7 – 12 months later. The infants assessed showed absent ABRs at diagnosis, however, follow-up assessments showed recovery of ABR Waves I, III and V. Despite evidence indicating early implantation is crucial for optimum auditory performance, such findings suggest a more cautious approach needs to be adopted when considering infants with AN for cochlear implantation compared to their sensory hearing loss counterparts. Overall, further studies are still required to determine the efficacy of cochlear implantation in children with AN.

1.7 Why lesion site matters: explaining the variability in outcomes

The considerable range in lesion sites that produce the AN phenotype can explain the inconsistent cochlear implant outcomes reported in affected individuals. As mentioned, the electrode array of the cochlear implant bypasses a large portion of the peripheral auditory system, directly stimulating the SGN axons. Several studies have shown that cochlear implant performance is not dependent on the presence of the inner hair cells, inner hair cell-to-auditory afferent synapses, or terminal dendrites (Fayad et al., 1991; Fayad & Linthicum, 2006; Linthicum & Fayad, 2009). Therefore, it is unsurprising that cochlear implant outcomes in individuals with presynaptic/synaptic AN lesion sites typically parallel those of their sensory hearing loss peers (Jeong et al., 2007; Rance & Barker, 2009; Rodriguez-Ballesteros et al., 2003; Rouillon et al., 2006; Santarelli et al., 2011). Similarly, implant outcomes for individuals with OPA1 have, in general, been very promising, with the majority of recipients achieving good speech perception scores (Huang et al., 2009; Santarelli et al., 2015c; Santarelli et al., 2011). In the study conducted by Santarelli et al. (2015c), seven out of the eight participants with OPA1 showed significant improvements in speech perception ability after implantation. Demyelination and/or axonal loss, which occur in more advanced stages of the disease (Kjer et al., 1983), were cited as the possible reason for the lack of benefit observed in one of the participants in the study. Disease involvement of

these more proximal structures is likely to negatively impact the integrity of an implant's signal transmission.

Overall, there is a notable lack of research detailing implant outcomes for conditions affecting structures such as the SGNs, auditory nerve axons, myelin sheath and other central components. A few studies have reported poor implant outcomes for individuals with deafferenting and demyelinating conditions (Brookes et al., 2008; Goswamy et al., 2012; Miyamoto et al., 1999). These findings support the assumption that implant signal transmission is dependent on the integrity of structures such as the auditory nerve axons and myelin sheath. Similarly, the large variability in cochlear implant outcomes reported for individuals with perinatal hyperbilirubinaemia can also be attributed to the degree of disruption/deterioration in the structures necessary for signal transmission to occur. The poorest cochlear implant outcomes are reported for individuals who have auditory nerve hypoplasia, with the vast majority not receiving any benefit from their implanted device. The absence, or a significant reduction in auditory nerve afferents observed in these individuals typically prevents adequate electrical transmission from occurring. There have, however, been a few reported cases where the implant has provided some perceptual benefit, but no correlation between degree of malformation and cochlear implant performance has been established (Buchman et al., 2011; Young et al., 2012). Overall, the decision to proceed with cochlear implantation in post-synaptic conditions should be carefully considered.

The results of a meta-analysis, performed by Rance and Starr (2015), investigating the effect of predicted AN lesion sites, inferred from the aetiologies of affected individuals, on cochlear implant outcomes are shown in Figure 1.11. Altogether, the findings indicate far less variability in post-implant speech perception performance in individuals with lesion sites predicted to occur before the SGNs compared to what has generally been reported for cases involving the SGNs, axons, myelin or other more central structures (Brookes et al., 2008; Buchman et al., 2006; Mukherjee et al., 2013; Vermeire et al., 2003). The difficulty in predicting outcomes for individuals with these post-synaptic conditions stems from the lack of diagnostic specificity in identifying whether there are sufficient neural elements (SGNs, axons, myelin, and brainstem nuclei) present *and* intact for successful cochlear implant signal transmission to occur. To complicate the matter further, there is no consensus on the minimum number of neurons needed to facilitate this, with some studies reporting good post-implant speech perception performance in individuals with as few as 10% of their SGNs intact (Fayad et al., 1991; Fayad & Linthicum, 2006).

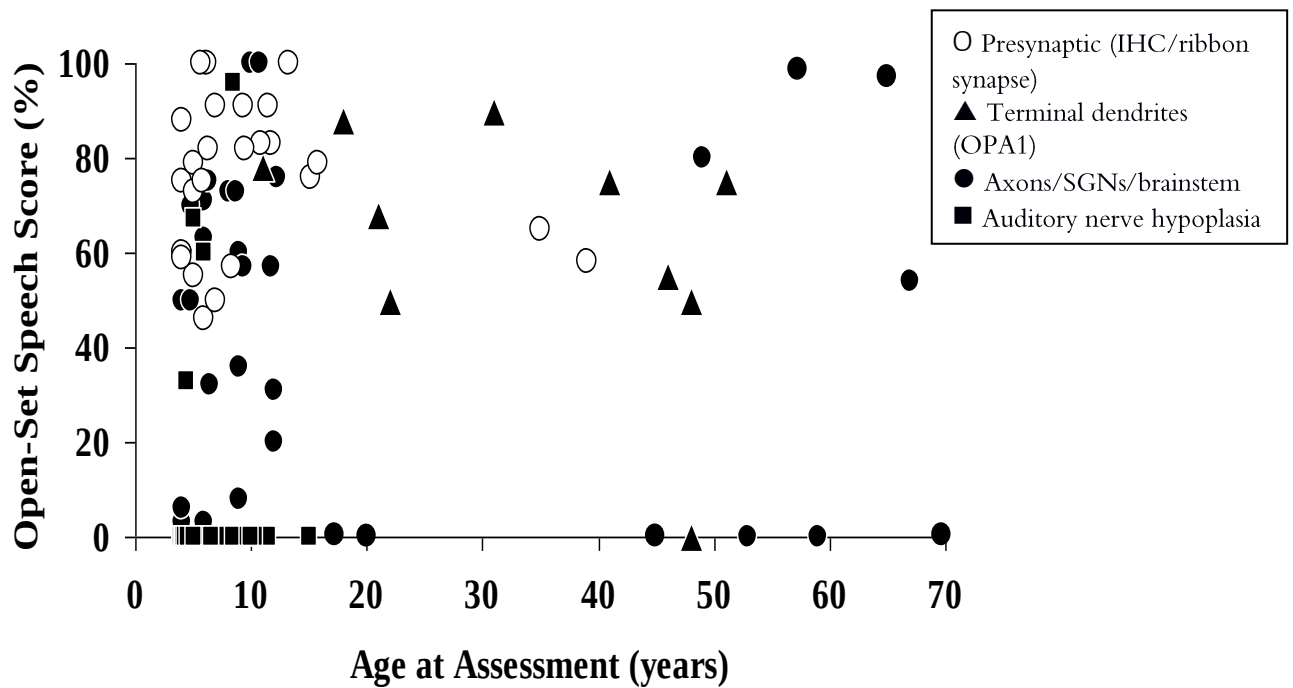


Figure 1.11 | Cochlear implant outcomes from individuals (n = 101) with auditory neuropathy as evidenced through post-operative open-set speech perception scores. Data shown includes individuals who received either unilateral or bilateral cochlear implants. Predicted pre-synaptic and post-synaptic lesion site(s) are indicated as shown in the legend. Presented data has combined information from several studies including, (Bradley et al., 2008; Breneman et al., 2012; Brookes et al., 2008; Colletti et al., 2013; Govaerts et al., 2003; He et al., 2014; Helbig et al., 2009; Huang et al., 2009; Jeong et al., 2007; Kang et al., 2010; Kutz et al., 2011; Mason et al., 2003; Miyamoto et al., 1999; Mukherjee et al., 2013; Oker et al., 2009; Rance & Barker, 2009; Rance et al., 1999; Rouillon et al., 2006; Santarelli et al., 2015c; Starr et al., 2004; Young et al., 2012). IHC: Inner hair cell; OPA1: optic atrophy 1; SGN: spiral ganglion neuron. Image modified from Rance and Starr (2015).

1.8 Magnetic resonance imaging (MRI)

A basic understanding of MRI is necessary to appreciate the techniques employed in this study. A brief introduction is provided here, for a more detailed review please refer to Westbrook (2016), McRobbie et al. (2017), or Weishaupt et al. (2008).

Magnetic resonance imaging is a non-invasive medical imaging technique that is used extensively in routine clinical practice and neuroscience research. It provides a way to investigate the anatomy and certain physiological processes of the body without the need for ionising radiation. Overall, MR imaging can be performed in several ways to investigate different aspects of biological tissue. For example, T1 and T2-weighted imaging sequences can be used to assess anatomical structures within the human body, whereas functional MRI (fMRI) provides a measure of blood oxygenation within the brain and can be used as an indirect assessment of brain activation. Another commonly used MRI sequence is dMRI which provides a measure of the diffusion of water molecules within the body and can be used to investigate white matter microstructure.

Since the discovery of MRI in the 1970's the field has developed rapidly, leading to a host of breakthroughs in the application and the precision of the technology. The fundamental principles, however, have remained relatively unchanged. Magnetic resonance imaging scanners use strong magnetic fields and radio frequency (RF) waves to generate images of biological tissue. This is made possible through three essential pieces of hardware: (i) a large superconducting magnetic coil, which generates a strong static magnetic field (B_0); (ii) transmitter and receiver coils, which allow RF fields to be generated and detected; and (iii) several magnetic gradient producing coils responsible for the production of gradient magnetic fields along three orthogonal directions (x, y, and z) (Liang & Lauterbur, 2000; McRobbie et al., 2017). The entire system is shielded using a Faraday cage to prevent any unwanted electrical interference.

1.8.1 Basic principles of MRI

Magnetic resonance imaging is grounded in the phenomenon of nuclear magnetic resonance, which in turn is derived from the fact that certain atomic nuclei possess magnetic moments like those of a simple bar magnet. These magnetic moments are generated from the interaction between the positive charge of an atom's nucleus and an intrinsic quantum mechanical property called nuclear spin, a type of angular momentum. A brief review of electromagnetism induction explains how this interaction can produce nuclear electromagnetism. According to Faraday's law of electromagnetic induction, it is known that an unbalanced moving electric charge, such as an atomic nucleus with nuclear spin, will produce a circulation of charge similar to a small current loop (see Figure 1.12). Current loops behave like a bar magnet, generating a magnetic field, which will experience torque in the presence of an externally applied magnetic field. It is this type of magnetic susceptibility that forms the foundation of nuclear magnetic resonance and how magnets can be used to generate a measurable signal from atomic nuclei within biological tissues.

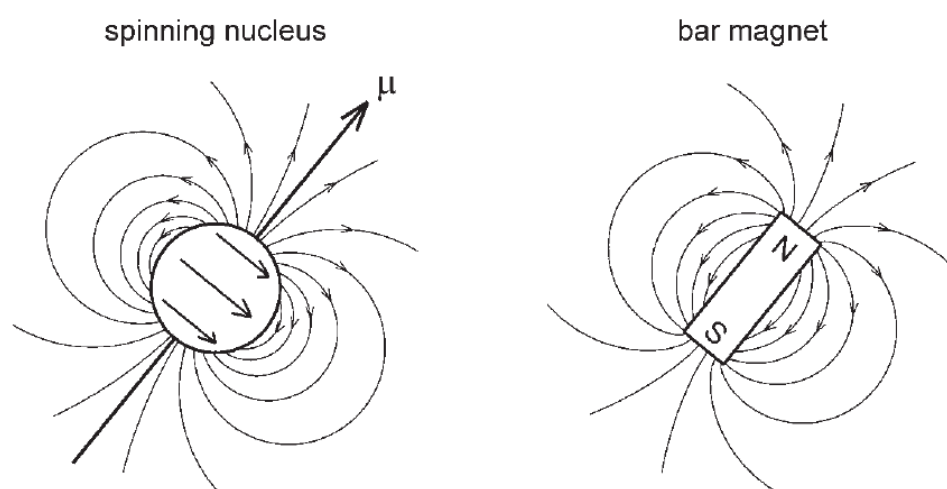


Figure 1.12 | Generation of nuclear magnetism. Atomic nuclei with odd numbers of protons and/or neutrons possess a magnetic moment produced through the interaction of nuclear spin and the overall positive charge of

the nucleus. This magnetic moment produces a small magnetic field which surrounds the nucleus of an atom and is analogous to the magnetic field of a simple bar-shaped magnet. The arrows on the nucleus indicate its direction of spin, whereas the curved lines surrounding the nucleus and bar magnet are called flux lines which serves as a graphical representation of the generated magnetic field (Prasad, 2006).

Only atoms with an odd number of protons and/or neutrons within their nucleus possess nuclear spin and therefore it is only these elements that possess a magnetic moment and are considered MR active. Although numerous elements are MR active, some of which include ^{31}P Phosphorous, ^{23}Na Sodium, ^{13}C Carbon and ^{19}F Fluorine, the most important for clinical uses of MRI is the common form of hydrogen: ^1H (Purcell et al., 1946). The reason for this is twofold: Firstly, ^1H possesses a particularly strong magnetic moment due to its single proton, which generates a larger MR signal than most other MR active elements. Secondly, ^1H is naturally abundant within biological tissue making it particularly suitable for imaging tissues of the human body, most of which contain a large quantity of H_2O .

1.8.2 How a measurable net magnetisation vector is generated from ^1H Hydrogen protons.

When imaging biological tissue it is not just a single ^1H proton that provides the source of the measured signal, but rather numerous ^1H protons present within the tissue. As such, all the magnetic moments of the protons within the tissue of interest need to be considered. In the absence of an external magnetic field (B_0), the magnetic dipole moments of the ^1H protons are randomly oriented within a sample, with no net magnetism being produced (see Figure 1.13A). In the presence of a B_0 field, however, the individual protons assume an alignment with the B_0 field. Like a compass needle in the presence of the earth's magnetic field, some of the ^1H protons align parallel to the direction of the B_0 field. A proportion, however, also align *anti*-parallel (see Figure 1.13B) (McRobbie et al., 2017; Westbrook, 2016). This difference in alignment is a result of the energy states of the ^1H proton. According to quantum theory, in the presence of an external magnetic field, ^1H protons can exist in only one of two energy states: either a high state or a low state. When protons are in the low energy state, they do not have enough energy to oppose the direction of the B_0 field. Hence, these protons take the path of least resistance and align themselves in the parallel direction. Conversely, those protons in the high energy state can oppose the direction of B_0 and align in the anti-parallel direction (Figure 1.13). The low energy state, however, is more favourable and therefore slightly more protons align in the parallel direction. Hence, the net magnetisation produced by the ^1H protons in the anti-parallel direction is cancelled out, leaving a small net magnetic effect from those ^1H protons in the parallel alignment. This net magnetisation occurs in the longitudinal direction or z-axis (M_z), parallel to the B_0 field, and is referred to as the net magnetisation vector (NMV) (Lauterbur, 1973; Weishaupt et al., 2008). At this stage, the NMV is not detectable because it occurs in the same direction as the B_0 field and is therefore indistinguishable from it.

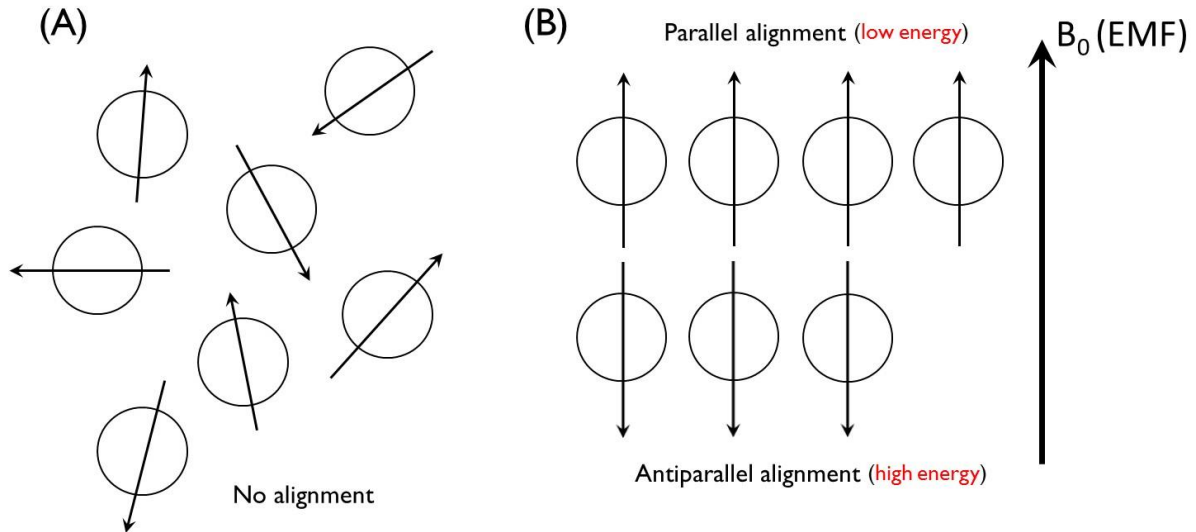


Figure 1.13 | The effect of an external magnetic field on the behaviour of ^1H protons. (A) The arrangement of ^1H protons in the absence of an external magnetic field (B_0). (B) The arrangement of ^1H protons in the presence of a static externally applied magnetic field (B_0). In the presence of a B_0 field a greater proportion of protons align in the parallel direction because less energy is required to oppose the main field. EMF: Electromagnetic field. Modified from Westbrook (2016)

It is important to note the parallel and anti-parallel alignments shown in Figure 1.13B are only 2-dimensional representations of how ^1H protons align in the presence of the B_0 field. Another feature of a proton's alignment within an external magnetic field must be considered to understand how the direction of the NMV can be manipulated away from the z-axis so that it can be measured. ^1H Hydrogen protons do not, in fact, align entirely parallel with the axis of the B_0 field. Rather, they rotate around its axis at a slight angle, tracing a circular path around B_0 . This deviation in their alignment is referred to as precession and is analogous to the 'wobble' of a spinning top around the axis of gravity; see Figure 1.14 for a diagrammatic representation. Precession occurs because of the interaction between the nuclear spin of ^1H (the spin of the proton) and the B_0 field, which alters the protons rotational axis causing it to spin around the axis of B_0 (Lauterbur, 1973).

The rate at which the ^1H protons precess around the B_0 field is known as the Larmor or resonant frequency and can be described by the Larmor equation:

$$\omega_0 = \gamma B_0$$

Where ω_0 is the Larmor resonance frequency, γ is the gyromagnetic ratio (a constant, specific to the nuclei being imaged), and B_0 is the main magnetic field strength (Larmor, 1900). In the presence of an external magnetic field the phase at which the ^1H protons precess is random and further energy is needed to alter this state.

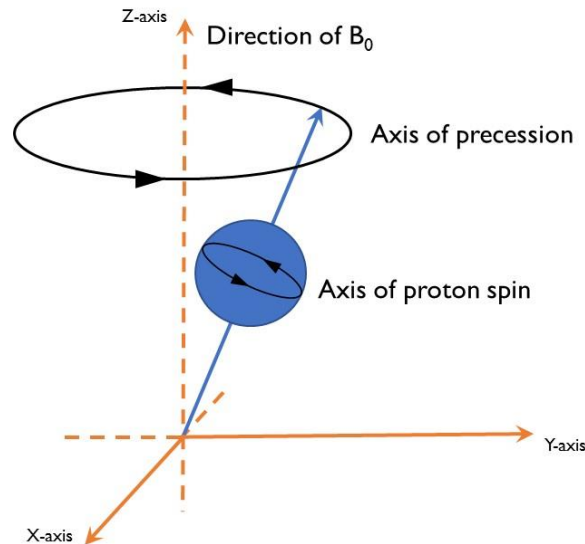


Figure 1.14 | Precession of a proton in the presence of a static, externally applied magnetic field (B_0). The Hydrogen proton has its own nuclear spin owing to the unbalanced charge of the proton. In the presence of a B_0 field the proton also exhibits a secondary spin (precession), in which it traces a circular path around B_0 . The proton in this figure is positioned using the coordinate system (x, y and z) to illustrate the direction of the magnetic field. Modified from Westbrook (2016).

1.8.3 Signal generation through resonance

In MRI, signal generation occurs through nuclear resonance. This is achieved by exposing the ^1H protons to electromagnetic RF pulses matching their Larmor resonance frequency, which provides the energy for the protons to resonate (Liang & Lauterbur, 2000). Two important events are achieved through resonance. Firstly, a proportion of the ^1H protons enter their higher energy state by absorbing energy from the RF pulse and thus their alignment with the B_0 field changes from parallel to anti-parallel. This in turn reduces the NMV in the longitudinal (M_z) direction. Secondly, when ^1H protons enter a resonant state, they begin to precess in-phase. This causes the NMV to shift away or ‘flip’ from the longitudinal plane (M_z) into the transverse plane (M_{xy}) (Figure 1.15). Once in the transverse plane, the NMV is no longer indistinguishable from the direction of the B_0 field and therefore it can be recorded in receiver coils situated within the MRI scanner (Westbrook, 2016). The protons, however, do not remain in this state permanently. Once the RF pulses are switched off, they come out of their resonant state and ‘relax’ back into a state of equilibrium (lower energy state), whereby transverse magnetisation (M_{xy}) is lost, and longitudinal magnetisation (M_z) is regained (Morris, 1986; Westbrook, 2016).

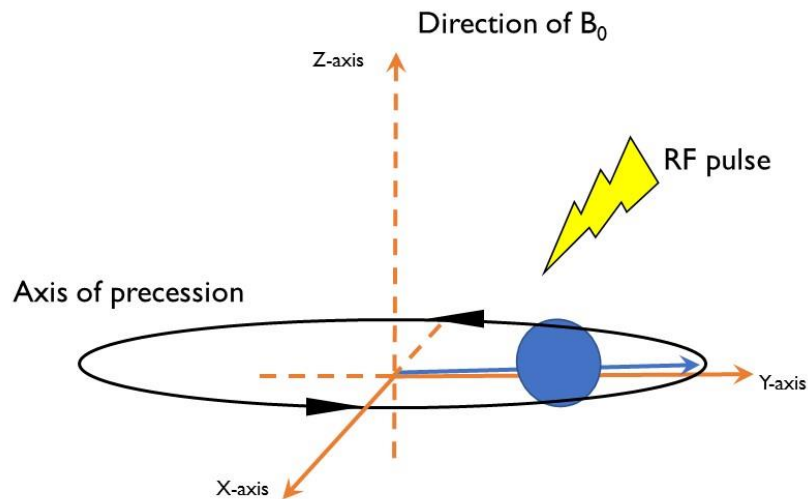


Figure 1.15 | Nuclear resonance. A resonant state is achieved by stimulating the ^1H protons with radio frequency pulses at their Larmor frequency. This causes the orientation of the nuclear magnetisation vector (blue arrow) to ‘flip’ from the longitudinal axis (M_z) into the transverse axis (M_{xy}) where it can be measured by receiver coils situated within the MRI scanner.

1.8.4 When protons relax

Measuring this relaxation time forms the basis of image production in MRI. The relaxation rate of ^1H protons varies depending on the tissue type. It is this information that is recorded and analysed to produce the final image contrasts seen in MR images. There are two main ways in which protons ‘relax’, named T1 (‘Time 1’) relaxation or longitudinal magnetisation (M_z) recovery and T2 (‘Time 2’) relaxation or transverse (M_{xy}) relaxation (Morris, 1986).

1.8.4.1 T1 relaxation

T1 relaxation, is the process whereby longitudinal magnetisation (M_z) returns to its original strength, prior to the introduction of RF pulses. T1 relaxation occurs as ^1H protons emit the energy absorbed from the RF pulses back into their surrounding environment. By doing so, a proportion of protons return to their low energy state and re-establish their parallel alignment with the B_0 field. This process is also commonly referred to as spin-lattice interaction, which is alluding to the transfer of energy between the spin-system of the proton to the surrounding environment or lattice. The recovery of longitudinal magnetisation over time follows an exponential curve and is defined by ‘T1’, a tissue-specific time constant. Specifically, T1 is the time taken for longitudinal magnetisation to return to ~63% of its original value (prior to the introduction of RF pulses) (Brown et al., 2015; McRobbie et al., 2017). As mentioned, T1 time varies according to the tissue being studied. For example, the T1 time of free water or cerebrospinal fluid is relatively long, whereas in bound/structured water or fat the T1 time is much shorter (Westbrook, 2016).

1.8.4.2 T2 Relaxation

In comparison, T2 relaxation is the process in which the ^1H protons lose their phase coherence and transverse magnetisation declines and eventually disappears (loss of magnetisation in the x-y plane) (Chavhan et al., 2009). The loss of phase coherence occurs from the interactions between the magnetic spins of protons, moving from the high energy state to the low energy state and vice-versa. In this process, the spin of protons is influenced by the minute magnetic fields from neighbouring atomic nuclei. Therefore, T2 relaxation is also commonly referred to as spin-spin interaction. The loss of transverse magnetisation over time can be described by an exponential curve and is defined as T2. Furthermore, T2 is defined as the time taken for transverse magnetisation to have lost ~63% of its maximal value. Like T1, T2 is also tissue-specific but typically the T2 relaxation time is much shorter (Brown et al., 2015; Westbrook, 2016).

1.8.4.3 Image contrasts

T1 and T2 relaxation occur simultaneously; however, images can be weighted differently by altering certain properties of how the RF pulses are applied. Parameters such as the echo time (TE), which is the time taken between an RF pulse and the collection of the signal, and the repetition time (TR), the time between RF pulses can affect the weighting and contrast of an image (Brown et al., 2015; Morris, 1986). Different weightings can elucidate different structural features depending on the chemical and structural make-up of the biological tissue under investigation. For example, fluids will appear dark in T1-weighted images, whereas fluids in T2-weighted images will appear bright (Van Hecke et al., 2015). This information can be utilised by radiographers/specialists to differentiate between pathologic and normal tissue. Neuroanatomists also use the information provided by the different contrast images to discern boundaries between brain structures and nuclei. Figure 1.16 shows two axial images at the level of the cochlea obtained using different acquisition parameters.

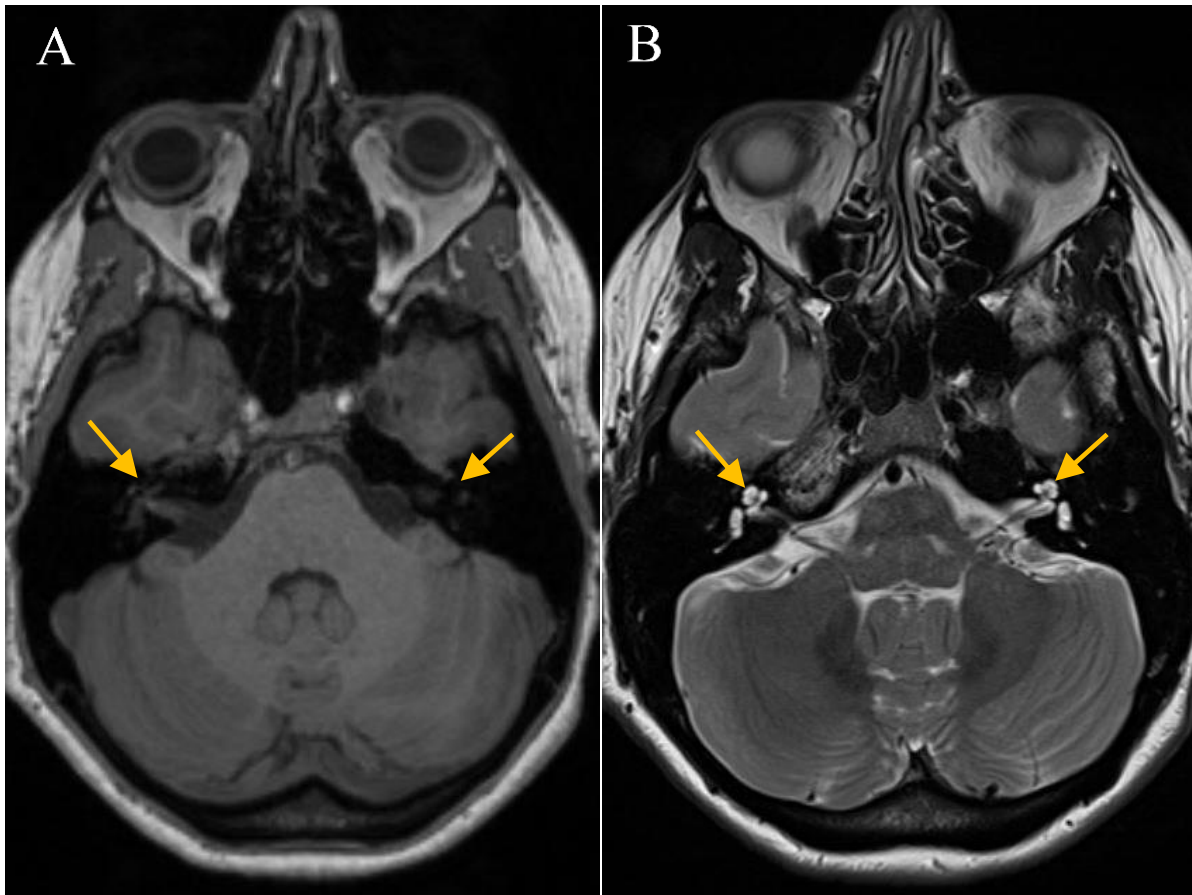


Figure 1.16 | Example of T1 and T2-weighted structural MR images. (A) T1-weighted image showing an axial slice of the brainstem at the level of the cochlea (B) T2-weighted image of the same slice shown in (A). By altering certain acquisition parameters different structures can be visualised. Note that the fluid filled cochlea (shown by the orange arrows) is much easier to identify in the T2-weighted image (B).

1.8.5 Image production

The acquired digitised MR signal is initially mapped and stored in a spatial frequency domain, called k-space. The data within k-space is deposited in a matrix and once the entire k-space matrix has been acquired it can be transformed into the final image using the Fast Fourier transform (Liang & Lauterbur, 2000).

The final MR image consists of a field of view (FoV) which contains the selected anatomical region that have been imaged. The FoV is composed of a matrix of pixels, the smallest 2-dimensional elements that make up the complete image. Pixels represent the MR signal intensity acquired from a very small volume of tissue within an individual's body, called a volume element (voxel) (Figure 1.17). The MR signal intensity from each voxel carries the information pertaining to the relaxation times of the protons within it. As discussed, it is this information that is used to distinguish tissue types and structures within MR images. Decoding the RF signal data using the Fast Fourier Transform therefore produces the grey scale image of each pixel and the image contrast is a direct representation of the amplitude of the RF signal at the same location (Figure 1.17).

The overall resolution of an image is dependent on the size of each voxel. All the anatomical structures within a given voxel contribute to the signal intensity measured. Hence, if a large voxel size is used in the imaging process the final averaged signal intensity will be a reflection of many different tissue types (e.g. white matter (WM), grey matter (GM), cerebrospinal fluid (CSF) and vascular tissue) each with its own cellular structure and fluid content (Van Hecke et al., 2015). This means that the resultant image contrast of the corresponding pixel lacks the specificity to show a completely accurate representation of the underlying tissue structure. This type of ‘contamination’ is known as the partial volume effect and it poses a significant problem as it has the potential to cause the difference between tissue types and structures to be indistinguishable in the final MR image. The main strategy for minimising partial volume effects is through restriction of voxel size, thus increasing the spatial resolution of an image.

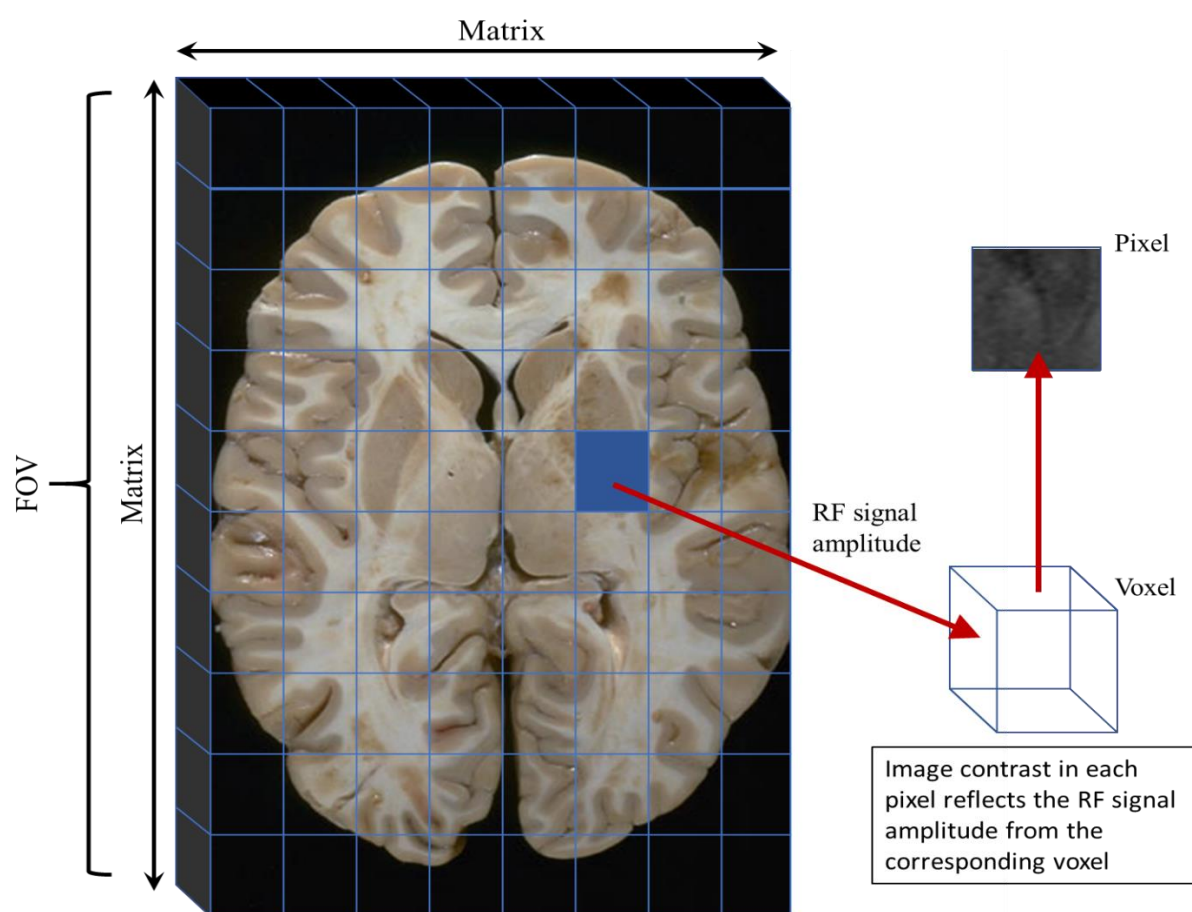


Figure 1.17 | Spatial characteristics of MR images; reconstruction of an MR image involves decoding recorded radio frequency (RF) information using the Fast Fourier Transform. The RF signal carries the details regarding its location which corresponds to a specific volume element (voxel). A voxel is the smallest 3-dimensional unit of the MR image, the RF signal corresponding to each voxel provides information about the behaviour of the water molecules within that space. This information is decoded and translated into image intensities, which are displayed on the corresponding pixels. For this illustration, the matrix size is shown to be significantly larger than what is normally used for an MR image. FoV: Field of view.

1.9 Diffusion magnetic resonance imaging (dMRI)

Diffusion MRI is one of the most commonly used MRI contrasts, especially in the study of brain changes due to stroke. It also has a wide range of applications in neuroscience research. Fundamentally, the image contrasts produced by dMRI encapsulate the rate of water diffusion within biological tissue (Van Hecke et al., 2015). This has allowed for a much more detailed examination of the sophisticated white matter and neural fibre architecture *in vivo* than is possible with anatomical contrast MR images alone (Beaulieu, 2002; Mori et al., 2005). Furthermore, dMRI can be performed using existing MRI technology making it readily available in many clinical settings. It is also non-invasive, does not rely on any contrasting agents, ionising radiation or chemical tracers, and it is generally well tolerated by patients.

1.9.1 Basic principles of molecular diffusion

To understand how MRI can be used to ‘capture’ the diffusion process of water molecules within the human body, an appreciation for molecular diffusion is necessary. Molecular diffusion within a solution, also commonly referred to as random motion or Brownian motion, was first observed by Brown (1827) and further studied and mathematically defined by Einstein (1905) and Sutherland (1905). Due to the dissipation of thermal energy and local concentration fluctuations, molecules in a solution are in constant motion, exchanging energy and colliding with other molecules or atoms that are present. As a result of these collisions, a molecule is constantly changing direction. This dynamic process is too complex to predict and therefore, from a practical perspective, a molecule’s direction changes effectively at random and hence, the motion of molecules is often described as a ‘random walk’ (Figure 1.18) (Jones, 2010; Van Hecke et al., 2015). From this random walk it is impossible to predict the distance that any given molecule will diffuse over a period of time, however, it is possible to acquire a statistical value on how a group of molecules will diffuse. This is given by the mean square displacement $\langle r^2 \rangle$, which reflects the mean of the Gaussian distribution of displacements, that a given group of molecules accomplishes over an interval, and is stated by the Einstein equation of diffusion:

$$\langle r^2 \rangle = 2Dt$$

Whereby, the mean square displacement increases with a given period of time (t), and the diffusion coefficient (D), which is the proportionality constant of the medium, with a physical unit of mm^2/s (Einstein, 1905).

1.9.1.1 Isotropic and anisotropic diffusion

While all this may sound complex, the process of diffusion is actually quite simple and can be easily conceptualised by considering the behaviour of a drop of (red) ink in a glass of still water. The ink is observed to spread out slowly over time, while its centre remains in the same location. After quite some time has elapsed, the entire glass’ contents would appear red. If this process was examined on a

molecular scale, it would be observed that all the molecules in the glass are ‘performing’ their random walk i.e. both the ink and water molecules are colliding and pushing each other around within the glass. While the diffusion of ink is being considered in this example, the same goes for water molecules in a glass of water. That is, if a group of water molecules in a glass of water could be tagged and observed over time, these molecules would be diffusing among the other water molecules in the same way the ink’s molecules were diffusing. Since, the water molecules in this example are able to diffuse freely in any direction, without being constrained by any obstructions or obstacles, this type of diffusion is termed ‘isotropic diffusion’ (Figure 1.18).

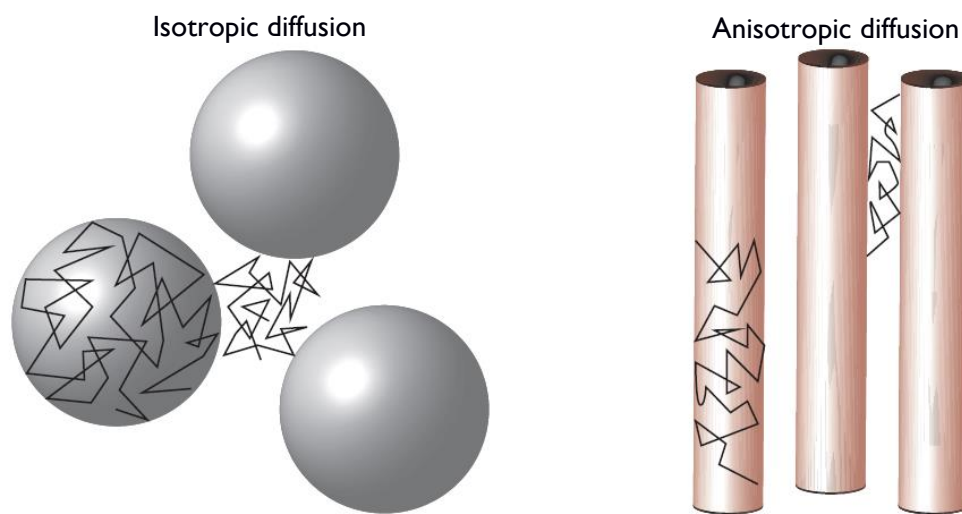


Figure 1.18 | Overview of the diffusion process within two different types of tissue architecture. The diffusion process or ‘random walk’ of the water molecules is depicted here as the black lines. *Left:* Although the water molecules are restricted/hindered by boundaries their displacement is equal in all directions and therefore this is considered to be isotropic diffusion. *Right:* The diffusion of water is hindered in the direction perpendicular to the cylinders (cylinders represent the structure of white matter), and therefore the diffusion is greater along these structures. This is considered to be anisotropic diffusion. Figure adapted from Beaulieu (2002).

In the human body, the type of unhindered isotropic diffusion that has been discussed up to this point would likely only be observed in the CSF of the ventricles (Jones, 2010; Van Hecke et al., 2015). Everywhere else, the presence of boundaries, such as cell membranes, macromolecules, and fibres, alter the diffusion process of water molecules, causing a deviation from the isotropic diffusion seen in a glass of water. This departure from isotropic diffusion is most marked in the myelinated axons within the brain. Axons are typically coherently orientated structures, which can be thought of as tightly packed cylinders (Figure 1.19). If these structures were placed within a glass of water, the water molecules would no longer be free to move in any direction and instead, their ability to diffuse would be hindered by the walls of the cylinders (Figure 1.18). Hence, if a drop of ink was observed diffusing among such cylinders, the displacement of its molecules would be seen to be hindered in the direction perpendicular to the orientation of the cylinders (due to the walls of the cylinders acting as a barrier), while in the direction parallel to the cylinders the molecules would be free to move. After some time, the ink would

appear to take on an ellipsoid shape reflecting the orientation of these cylinders. This type of diffusion, whereby diffusion is directionally dependent, is termed anisotropic diffusion.

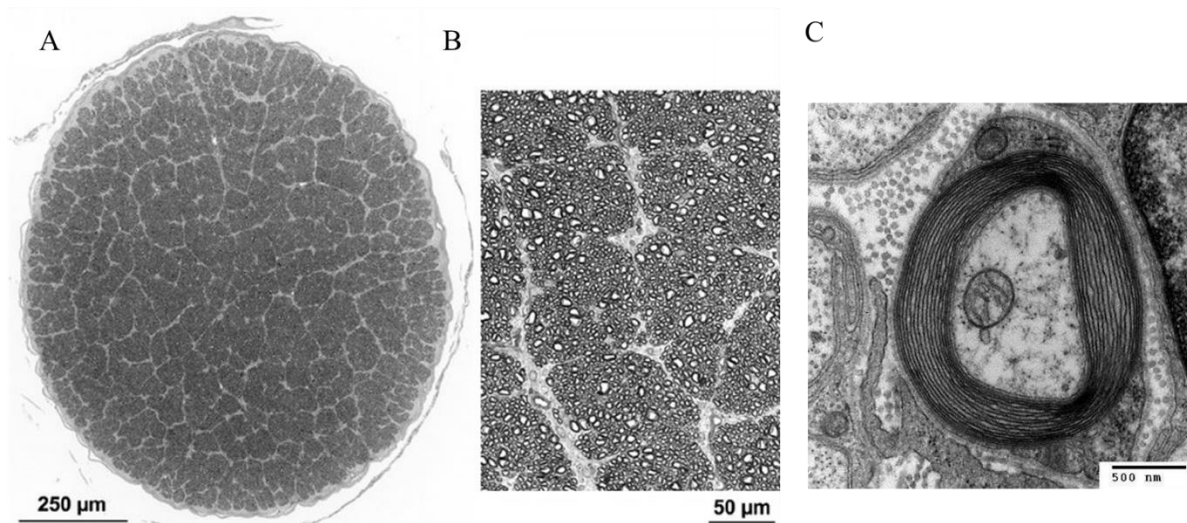


Figure 1.19 | Structure of myelinated axons. (A) Cross-section of an optic nerve showing the tightly packed and coherently oriented myelinated axons. (B) Magnified section of (A), showing individual axons. (C) Transmission electron micrograph showing a slice perpendicular to a single axon. The image illustrates the structure of the axon at a magnification of 500 nm. The concentric rings observed in the image are the axon's myelin sheath (Britannica, 1993; Whiting et al., 2014).

In the brain, anisotropic diffusion is most commonly observed in white matter structures due to their coherently oriented axons/fibres (Beaulieu & Allen, 1994). Comparatively, grey matter contains fewer organised fibre structures, it is predominantly composed of soma, dendrites and supporting cells including astrocytes and microglia. As such, grey matter has an overall higher degree of complexity, and while diffusion is restricted/hindered by cell membranes, the lack of coherently oriented structures means that the diffusion process altogether appears less constrained and a more isotropic diffusion pattern is generally observed (Beaulieu, 2002). In CSF, diffusion is considered isotropic due to the absence of obstacles. Isotropic and anisotropic diffusion patterns are illustrated in Figure 1.18. The type of diffusion patterns observed in the different tissue types of the brain (i.e. GM, WM and CSF) forms the basis of how dMRI can be used as a probe to examine the tissue architecture within the human body *in-vivo*.

1.9.2 How diffusion is measured

To capture the diffusion process of water molecules within a sample the MRI signal intensity must be sensitised to the amount of diffusion that is occurring. This is achieved using ‘pulsed magnetic field gradients’ (Stejskal & Tanner, 1965) (most commonly referred to as ‘diffusion gradients’), which induce predictable spatial and temporal fluctuations in the main B_0 magnetic field, causing the resonant frequency of the ^1H protons of water molecules to vary along the direction of the applied gradient (Le Bihan, 2003). ^1H Hydrogen protons that undergo no displacement will remain in-phase, whereas the phase

of those that have been displaced will change according to the extent of displacement that has occurred. As a result, the RF waves emitted by the magnetised ^1H protons (i.e. the measured MR signal) will also vary according to the degree of displacement, compared to static protons. In particular, the MR signal obtained from the static ^1H protons remains robust, whereas the acquired signal is *attenuated* by the degree of displacement for those protons that have undergone diffusion (i.e. the signal becomes more attenuated the further the protons travel). It is also important to note that gradients are directionally dependent and only diffusion along the applied direction is detectable.

In addition to introducing pulsed magnetic field gradients, Stejskal and Tanner also mathematically defined the reduction of MR signal due to the extent of diffusion occurring in the presence of an applied diffusion gradient. This is known as the Stejskal and Tanner equation:

$$S = S_0 e^{-bD}$$

Several important aspects of dMRI can be derived from this equation. Firstly, to measure the extent of diffusion occurring in a sample, two MR images (at a minimum) need to be acquired. The first is a non-diffusion weighted image, similar to a T2-weighted image described earlier, which is defined as S_0 . The second image is the diffusion sensitised image, given by S . It is the relative difference between the two images that provides information about the *extent* of diffusion occurring. The S in this equation should always be smaller than S_0 , because the process of diffusion will have caused attenuation in S . Furthermore, the b -value (s/mm^2) corresponds to all the relevant *scanning parameters* used to acquire the diffusion-weighted image i.e. diffusion-weighted images can be made more sensitive to diffusion by increasing the duration and the strength of the diffusion gradients applied and these aspects are captured by the b -value (Le Bihan & Breton, 1985). An image obtained with a higher b -value will be more sensitive to diffusion than one obtained with a lower b -value (i.e. a b -value of 0 s/mm^2 will not provide a diffusion weighting in the acquired image). Finally, also present in the equation is the diffusion coefficient (D), which was introduced in Einstein's diffusion equation in the previous section. While this diffusion coefficient (D) holds true for the experiments conducted by Stejskal and Tanner (1965), in which diffusion was measured in an unhindered isotropic environment, the same does not apply for the *hindered/restricted* diffusion patterns observed in the highly complex tissues of the brain. Instead, the diffusion coefficient varies according to the type of diffusion occurring, and the b -value used in a given scan. As such, Le Bihan et al. (1986) introduced the concept of the apparent diffusion coefficient (ADC), which is a more accurate term to describe the diffusion observed in such complex environments. Hence, solving the Stejskal and Tanner (1965) equation for the ADC will provide a representation of the total amount of diffusion occurring in any given voxel, along the direction of the applied diffusion gradient. This information can be visualised on an ADC map, whereby areas in the brain where unhindered diffusion is occurring, like in CSF, appear hyperintense, while areas of restricted/hindered diffusion, like in WM and to a lesser extent GM, appear more hypointense (Figure

1.20A) (Huisman, 2010). It is important to note that the contrasts described here for the *ADC* map are the opposite for dMR images (i.e. diffusion sensitised images (*S*) are inversely related to their *ADC* values; Figure 1.20B).

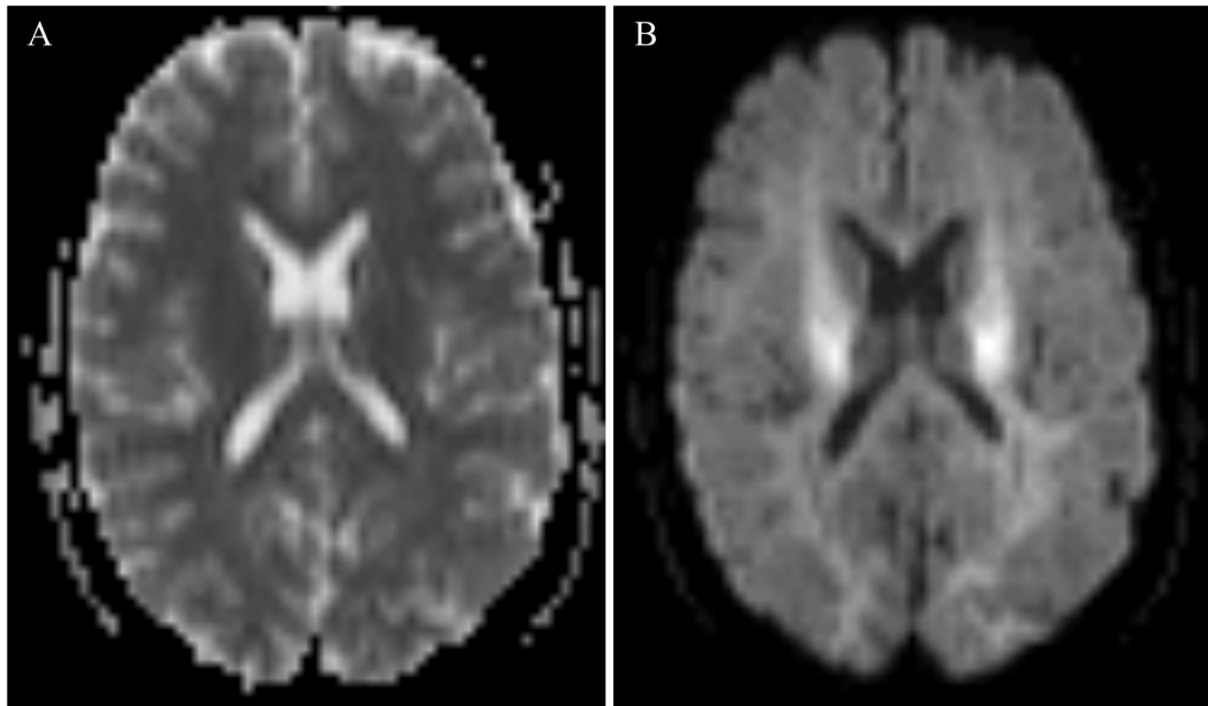


Figure 1.20 | Example of an axial averaged *ADC* map and dMR image of the brain. (A) On the *ADC* map the CSF is hyperintense, whereas the grey and white matter show up as hypointense. (B) On the dMR image (also commonly referred to as the trace-DW image or diffusion-weighted image (DWI)) the intensities are opposite to those of the *ADC* map. Note that both the *ADC* map and the dMR image shown here are an *average* of 60 individual images obtained using different diffusion gradient directions.

1.9.3 Putting it all together

When this information is considered in the context of the different types of diffusion that occur within tissue structures of the brain (WM, GM and CSF), it becomes evident how multiple diffusion gradients, along different directions, can be applied to obtain the diffusion ‘profile’ within a given voxel. In turn, the diffusion profile can then be used to gain a better understanding of the underlying tissue architecture *in-vivo*. For example, if the predominant direction of white matter tracts (the direction in which diffusion is unhindered) matches the direction of the applied diffusion-encoding gradient, the MR signal recorded will be attenuated and hence, the dMR image will appear darker in this region. Comparatively, if the applied gradients are applied perpendicular to the direction of the tracts (the direction in which diffusion is hindered by the walls of the tracts), less MR signal is attenuated, and the image will appear brighter in this region (Huisman, 2010; Jones, 2010). In essence, the MRI scanner takes a snapshot of all the water molecules present at two-time intervals. In the brief period between the two snapshots, the water molecules diffuse. The extent of this diffusion is dependent on the tissue architecture and is measured by the extent of attenuation of the recorded signals. The larger the attenuation of the signal the larger

the amount of displacement of the water molecules within a given voxel (Van Hecke et al., 2015). Finally, it is important to note that when diffusion is measured using multiple gradients, no single *ADC* value can adequately capture the orientation-dependent water mobility observed in highly anisotropic tissue such as white matter (Jones, 2010). Hence, diffusion models, designed to interpret such directional information, have been developed and will be discussed in the subsequent sections on diffusion tensor imaging and constrained spherical deconvolution.

1.9.4 Diffusion MRI artefacts

The imaging sequence typically used to acquire dMRI data (spin-echo echo-planar imaging) is susceptible to a range of artefacts, including thermal noise, subject motion, Gibbs ringing, magnetic susceptibility-induced distortions, and eddy current-induced distortions, all of which can have a detrimental impact on the signal-to-noise ratio of the acquired images (Basser & Jones, 2002; Van Hecke et al., 2015). Hence, these artefacts can subsequently reduce the accuracy of both qualitative and quantitative downstream analysis methods. As such, it is recommended (and often necessary) to correct for some or all these artefacts after the images have been acquired, to increase the signal-to-noise ratio, thus improving the quality of the images. In this section, some of the most clinically relevant artefacts will be discussed, including possible methods and considerations for correction.

1.9.4.1 Thermal noise

Random fluctuations of the MR signal can be caused by the motion of electrons and/or ions within the sample of interest (Johnson, 1928; Nyquist, 1928). This is typically referred to as thermal or Johnson-Nyquist noise and it can significantly reduce the signal-to-noise ratio of the acquired dMRI data. Removing thermal noise, aptly termed ‘denoising’, has been a long-standing issue in dMR image processing. Numerous algorithms exist for removing such noise from acquired images, some of which are based on concepts such as, the maximum likelihood estimation method (Rajan et al., 2011; Sijbers & Den Dekker, 2004), adaptive non-local means (Manjón et al., 2010), and random matrix theory (Veraart et al., 2016b; Veraart et al., 2016c). Some of the available algorithms, however, result in a loss of spatial resolution in the acquired imaging data, and introduce additional partial volume effects, to the detriment of downstream analysis methods (Van Hecke et al., 2015). As such, care should be taken when selecting the most appropriate method(s) for implementation in the dMRI pre-processing pipeline. In general, ideal methods should correct for MR signal fluctuations occurring from thermal noise and not from other origins, such as physiological noise, Gibbs ringing, and fine anatomical detail (Rajan et al., 2011; Veraart et al., 2016b). Furthermore, most denoising methods should be implemented at the beginning of pre-processing pipelines. This is because the effectiveness of denoising algorithms can be reduced by downstream processing steps using interpolation and smoothing, which change the characteristics of the thermal noise present.

1.9.4.2 Gibbs ringing artefact

Gibbs ringing artefact, also known as truncation, and spectral leakage artefact, is one of the most common artefacts in MRI (Stadler et al., 2007). Gibbs ringing occurs as a consequence of using the Fast Fourier transform when reconstructing the acquired MR signal into the final image. Theoretically, any complex signal, such as the signals recorded in MRI, can be reduced to an infinite number of sine waves of different phases, frequencies and amplitudes. In MRI, however, constraints on acquisition time and signal-to-noise limitations, means that only a finite number of frequencies can be sampled, and therefore the image contrasts are approximated from a smaller subset of the entire harmonic series that is present (i.e. the Fast Fourier series is truncated, with the high frequency information of the image often not recorded) (Veraart et al., 2016a). This induces overshoots or undershoots in the approximation of the final image contrasts in each voxel, which appears as a series of bright or dark lines in the acquired image that run parallel to edges where there is a steep transition in MR signal intensity (i.e. areas in the brain where there is CSF next to white matter). Since any diffusion leads to a decay in the MR signal intensity, Gibbs ringing is most apparent in non-diffusion weighted images as these have a larger intensity in each voxel (Figure 1.21). While less easily observed on dMRI data, Gibbs ringing has a significant adverse impact on diffusion estimates, and therefore it is generally recommended that appropriate algorithms are employed to correct for these artefacts (Perrone et al., 2015; Veraart et al., 2016a). Fortunately, numerous approaches are available that can remove Gibbs ringing in the pre-processing steps of the dMRI analysis pipelines (Archibald & Gelb, 2002; Kellner et al., 2016; Perrone et al., 2015; Sarra, 2006).

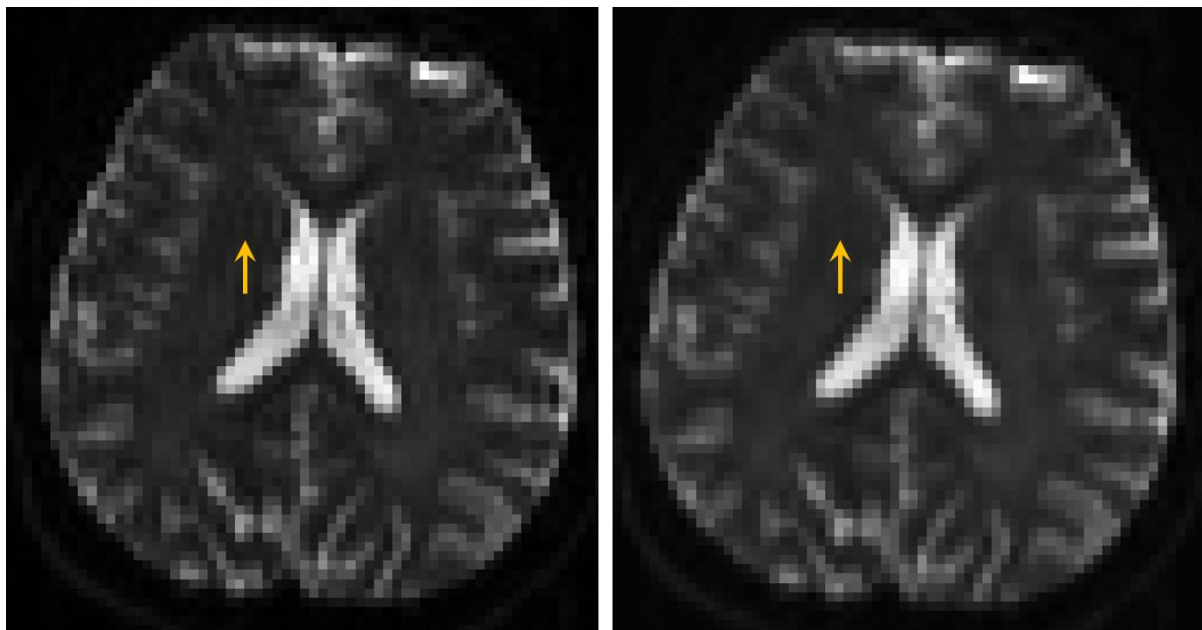


Figure 1.21 | Gibbs ringing artefact. *Left:* Non-diffusion weighted image (i.e. image acquired with a b -value of 0 s/mm^2) without any Gibbs ringing artefact correction applied. The artefact is most easily identified in regions of high intensity gradients, such as the transition between the CSF of the ventricles and the surrounding white/grey matter). The orange arrow indicates an area where the characteristic bright and dark lines of Gibbs ringing is easily identifiable. *Right:* The corrected image.

1.9.4.3 Subject motion

Gross subject motion can severely reduce the quality of the acquired dMRI data, causing data mis-registration or mis-alignment, especially when the motion of the subject exceeds that of the chosen pixel size (typically between 2 – 2.5 mm for dMRI) (Anderson & Gore, 1994). Since it is often necessary to acquire numerous dMR images (with varying diffusion gradient directions) to accurately estimate diffusion properties, mis-alignment between dMR images can introduce significant errors in these estimates (Jones, 2010). Diffusion MRI data is especially susceptible to subject motion because of lengthy scan times. For instance, acquiring dMRI data for clinical purposes can take between 5 – 10 mins, and research scans typically take even longer. Although subjects are instructed to lie as still as possible during the scanning procedure, such long scanning times means that body or head motion can be difficult to avoid.

The mis-registration caused by subject motion can visually affect the acquired image in several ways. In some cases, it may appear as a rim of high anisotropy with oriental bias around the periphery of the brain (Van Hecke et al., 2015). In other cases, more subtle visual changes in maps of certain diffusion metrics, such as mean diffusivity and fractional anisotropy, can be observed. In general, subject motion can be most easily recognised by manually looping through all the acquired dMR images at a rate of ~10 frames per second (Van Hecke et al., 2015). In the event that subject motion is identified in the acquired imaging data, image registration tools can be used in the pre-processing stage of the image analysis pipeline that can correct for most artefacts caused by mis-registration (Andersson & Sotiropoulos, 2016; Jones, 2010; Jones & Cercignani, 2010).

1.9.4.4 Magnetic susceptibility-induced distortions

Magnetic susceptibility-induced distortions (also commonly referred to as echo planar imaging (EPI) susceptibility-induced distortions) arise due to the diamagnetic nature of biological tissue. This means that in the presence of an externally applied magnetic field, biological tissue will become magnetised and exert a magnetic field opposing that of the externally applied B_0 field. As a result, the magnetic field experienced by the biological tissue will be slightly lower than that of the scanner B_0 magnetic field, which can cause a localised loss of the MR signal and potential geometric distortions in adjacent structures (Morelli et al., 2011; Schenck, 1996). These distortions typically appear as compressed regions, whereby the signal from numerous voxels is compressed into one voxel (Figure 1.22A), and/or regions of smearing, where the signal from one voxel is stretched over several voxels (Figure 1.22B) (Tax et al., 2016). The magnetic susceptibility of different tissues, however, is not equal and therefore the B_0 field is dependent on the tissue composition of the body part(s) being imaged. Areas where air-filled cavities interface with bone or tissue, such as the frontal sinus and the temporal lobe, have greater magnetic susceptibility and are therefore more prone to geometric distortions (Andersson et al., 2003).

Several techniques can be implemented during image acquisition to reduce susceptibility-induced geometric distortions. These include the use of shim coils, which introduce additional magnetic fields that can improve the homogeneity of the B_0 field in areas known to be prone to magnetic susceptibility-induced distortions (Roméo & Hoult, 1984). Parallel imaging methods, such as SMASH, SENSE, or GRAPPA, designed to decrease the time taken to acquire dMR images can also reduce the effects of B_0 inhomogeneities produced by diamagnetic biological tissue (Griswold et al., 2002; Sodickson & Manning, 1997; Tax et al., 2016). Furthermore, there are also several methods that can correct for these distortions in the pre-processing stage of the dMR image analysis pipeline. One of the most common is the reverse polarity gradient method, which requires the acquisition of two additional images, with opposite phase encoding directions (and therefore with distortions occurring in opposite directions) (Andersson et al., 2003; Chang & Fitzpatrick, 1992). The additional images enable the undistorted image to be reconstructed. While corrections can be made post-acquisition using this method, it requires additional images to be acquired and therefore it is necessary to decide whether it will be implemented prior to acquiring the imaging data. Another method, based on non-rigid image registration, introduced by Irfanoglu et al. (2012) shows that an undistorted image, such as an anatomical image (i.e. T1 or T2-weighted image), which is less susceptible to magnetic susceptibility-induced distortions, can also be used to unwarp geometric distortions present in dMRI data.

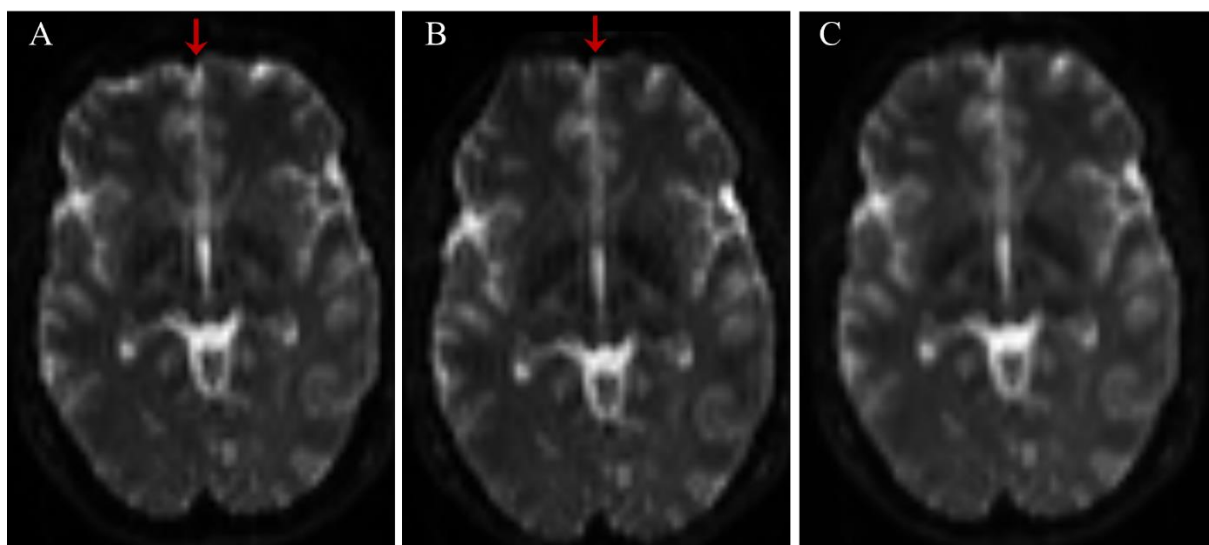


Figure 1.22 | Magnetic susceptibility-induced distortions. (A) Susceptibility-induced distortions when using posterior-anterior phase encoding results in displacements towards the back (red arrow). (B) Anterior-posterior phase encoding results in displacements towards the front (red arrow). (C) Corrected image.

1.9.4.5 Eddy current-induced distortions

Magnetic field gradients of high amplitude are necessary to acquire dMR images, and the gradient coils situated within the MRI scanner bore that produce these diffusion gradients have to be rapidly switched on and off. This induces electrical distortions, referred to as eddy currents, in the conductors present within the MRI equipment, which can disrupt the spatial encoding process of image acquisition (Rohde

et al., 2004). The result is that the acquired dMR images can be shifted, compressed, stretched, and or sheared. Such geometric distortions are most obvious in the raw dMR images in the phase encoding-direction (most commonly the anterior-posterior direction; Figure 1.23), and they can have a significant impact on diffusion-derived metrics calculated on a voxel-by-voxel basis (i.e. voxel-based morphometry) (Leite & Castillo, 2016; Van Hecke et al., 2015). Furthermore, eddy current distortions are particularly problematic when dMRI data is acquired with more gradient directions, such as in high angular resolution diffusion-weighted imaging data (HARDI), and at higher b -values (Jones & Cercignani, 2010).

Fortunately, there are several methods of minimising the effects of eddy currents that can be implemented in the image acquisition process as well as the pre-processing stage of the dMRI analysis pipeline. For example, using a *twice-refocused* spin-echo diffusion imaging sequence rather than a single-refocused spin-echo sequence can reduce eddy currents and subsequent geometric image distortions (Reese et al., 2003). Numerous algorithms, using various image registration-based techniques, have also been developed that can correct for eddy current distortions in the image pre-processing stage (Andersson & Sotiropoulos, 2016; Haselgrove & Moore, 1996; Rohde et al., 2004). Typically, these techniques will register all the dMR images to the non-diffusion weighted image ($b = 0 \text{ s/mm}^2$), which does not have any eddy current induced distortions (Van Hecke et al., 2015).

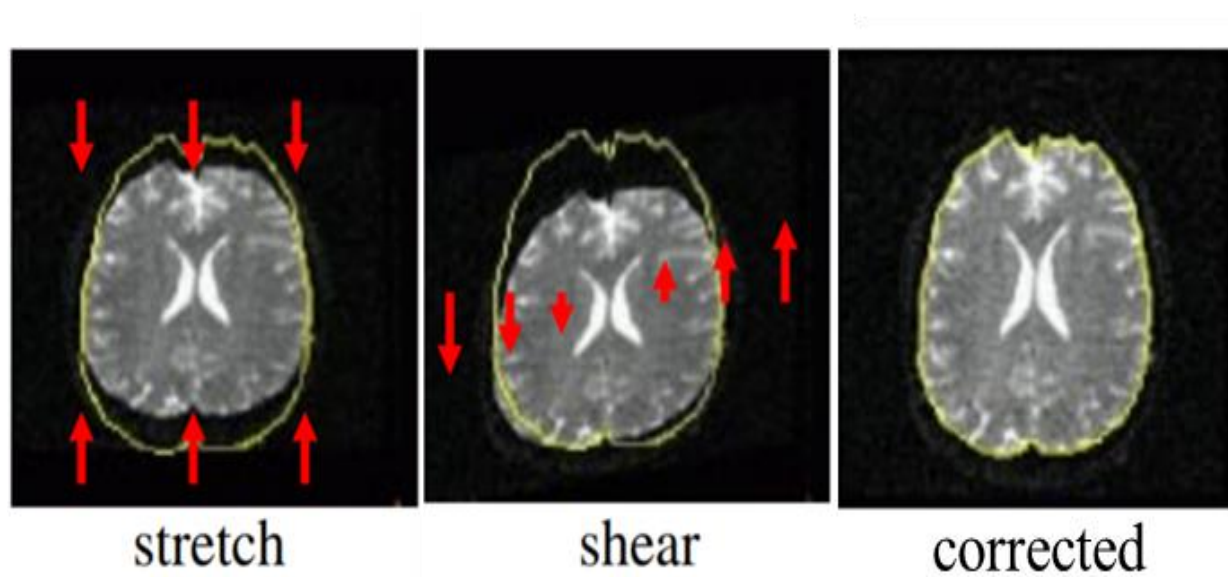


Figure 1.23 | Eddy current-induced distortions. An example of some of the different types of eddy current-induced distortions that can occur in the phase encoded anterior-posterior direction (indicated by the arrows). The yellow outline in each image provides an indication of the shape of the undistorted $b = 0$ image. A corrected image is also included to show how registration-based methods can mitigate the effects of eddy current distortions. Modified from Mangin et al. (2001).

1.9.5 Diffusion tensor imaging (DTI)

Whereas dMRI refers to the specific image contrasts obtained through the application of directional gradients, DTI is a type of modelling technique that utilises dMRI datasets. DTI provides an analysis

of the 3-dimensional diffusion process, offering a way to map the orientation of this process within each voxel. The diffusion tensor itself is a mathematical concept, which can be used to describe the direction and magnitude of the diffusion of water molecules. In order to obtain a suitable 3-dimensional tensor, the diffusion direction of water molecules (MRI signal) must be measured in at least six different directions or axes (Basser & Jones, 2002). More measures will typically yield a more accurate model of the diffusion process; however, the standard tensor model is limited in its ability to display this information. Implications of this significant constraint are discussed later.

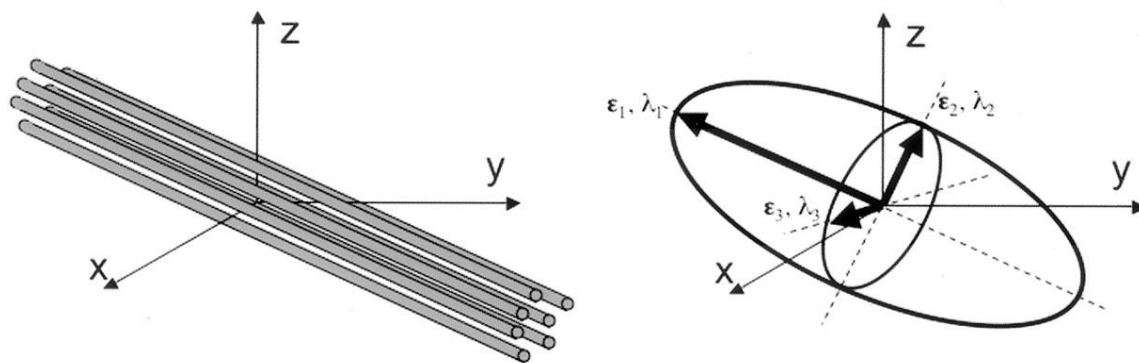


Figure 1.24 | Principles of diffusion tensor imaging. *Left:* White matter fibre tracts within the brain and brainstem are arbitrarily situated in respect to the MRI scanner geometry (x, y, and z). *Right:* The diffusion profile obtained from the tracts shown on the left would be modelled as an ellipsoid using diffusion tensor imaging. The orientation of the ellipsoid is defined by three eigenvectors ($\epsilon_1, \epsilon_2, \epsilon_3$), and the ellipsoid's shape is characterised by three eigenvalues ($\lambda_1, \lambda_2, \lambda_3$). Modified from Jellison et al. (2004).

Mathematical decomposition of the diffusion tensor yields both scalar (eigenvalue) and vector (eigenvector) components. The diffusion tensor consists of three eigenvalues (principal diffusivities - $\lambda_1, \lambda_2, \lambda_3$), representing the magnitude of diffusion. The tensor also consists of three eigenvectors, in three mutually perpendicular directions, corresponding to the direction of diffusion ($\epsilon_1, \epsilon_2, \epsilon_3$). Hence, the tensor can be characterised geometrically as a 3-dimensional symmetric ellipsoid (Figure 1.24). The shape of this ellipsoid will vary depending on the magnitude and direction of the diffusion process measured. For example, in white matter tracts, diffusion is most unhindered in the direction the tract is running and most constrained in the direction perpendicular to this. Therefore, the ellipsoid will appear elongated in the direction of the tracts and 'squashed' in the perpendicular direction (Figure 1.24). The longest axis of the ellipsoid is the principal eigenvector, which is a representation of the maximal diffusion direction. Comparatively, diffusion in cerebrospinal fluid is unhindered in all directions and can be graphically represented as a sphere. In this way DTI, can be used to provide valuable information regarding tissue microstructure (Figure 1.25). Furthermore, analysis of the tensor can provide important information about the extent of diffusion that is occurring in a given region.

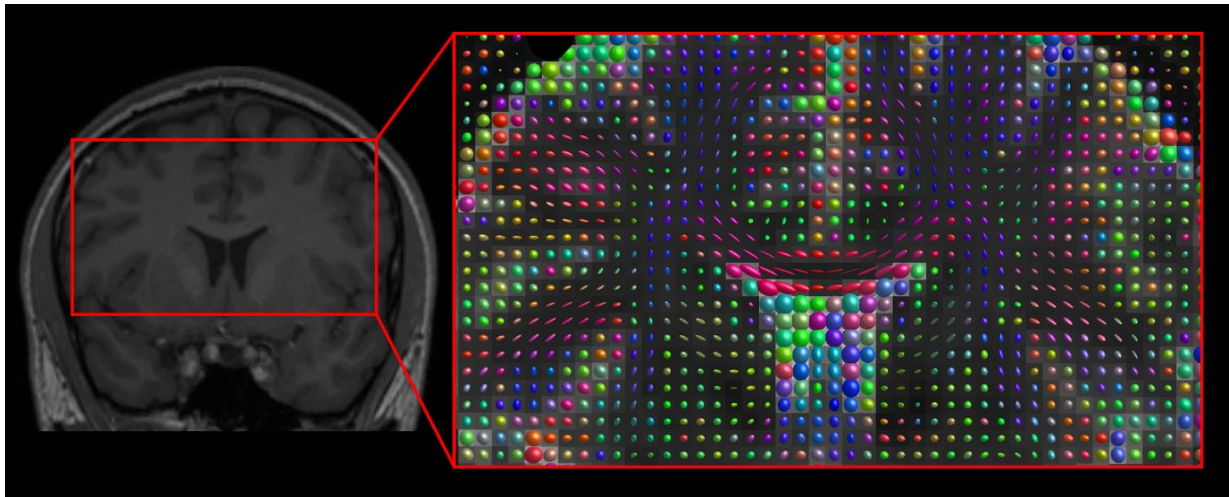


Figure 1.25 | Diffusion tensor imaging in practice. *Left:* T1-weighted image showing a coronal cross-section of the brain of a healthy volunteer. *Right:* Magnified region showing a corresponding dMRI image overlaid with diffusion tensor ellipsoids. The fibres of the corpus callosum, which connects the left side of the brain to the right, are clearly captured by the diffusion tensors (red elongated lobes in the centre).

1.9.5.1 Quantitative and qualitative analyses based on the diffusion tensor model

Several quantitative metrics can also be derived from the diffusion tensor. These provide important information about different aspects of the diffusion process. Diffusion tensor based quantitative measures can be used to statistically compare differences in tissue architecture based on diffusion processes between patients and patient cohorts. The available analyses can provide information on several properties. These include, the total amount of diffusion within a given voxel (mean diffusivity, which is equivalent to the *ADC* averaged over all diffusion directions acquired – i.e. the mean *ADC*), the direction(s) which exhibit the greatest diffusion (fractional anisotropy (*FA*)), the degree of anisotropic diffusion (axial diffusion), and the rate of diffusion in the transverse plane (radial diffusion) (Soares et al., 2013). In neuroscience research, *FA* is the most commonly applied metric and is used to explore underlying tissue architecture. For example, a change in the orientation, structural integrity of tracts or an increase in water content within tissues will result in recordable changes within the *FA*.

The diffusion tensor model can also be used to ‘reconstruct’ the white matter pathways of the brain using tractography algorithms, which can enable a qualitative analysis of brain connectivity and white matter pathways of interest. As discussed, the white matter within the brain is composed predominantly of axons or ‘nerve fibres’, which are coherently organised into bundles. Axonal cell membranes act as barriers to water molecules and therefore restrict their diffusion process in the direction perpendicular to the orientation of the axons (Figure 1.18). Using the information provided by the raw dMRI signal, diffusion models, such as the diffusion tensor, attempt to derive the geometry of the nerve fibres. The reconstructed models of the underlying tissue architecture provide information regarding the local fibre orientation (within a given voxel), however, they do not provide any indication as to the connectivity of axons over a distance. This information, however, can be obtained by joining local fibre orientations

to generate continuous pathways or ‘streamlines’ which connect distant brain regions. This ‘piecing’ together of local fibre orientations is performed using tractography algorithms.

1.9.5.2 Limitations of diffusion tensor imaging

Diffusion tensor imaging can provide unique insights into tissue architecture of the brain, and as such, it has become an increasingly popular exploratory technique. Despite its widespread applicability and usefulness, several important limitations exist with DTI. The most significant of which is its inability to characterise multiple fibre tracts that are crossing, fanning, touching or bending within a single voxel (Behrens et al., 2007; Tournier et al., 2011). This is a result of the inherent constraints in the tensor ellipsoid model, which allows the rendering of only a single principle diffusion direction (Alexander et al., 2002; Frank, 2001). The significance of this issue becomes apparent when considering that at the image resolutions currently attainable with dMRI (typically voxel sizes of $\sim 2 \times 2 \times 2$ mm), a vast portion of voxels will contain different nerve fibres of varying orientations (Alexander et al., 2001; Mori et al., 2005). In fact, Jeurissen et al. (2013) have found multiple fibre populations present in excess of 90% of voxels containing white matter. Hence, the inability for DTI to resolve crossing fibres has significant repercussions for DTI-based tractography methods.

In the presence of multiple fibre directions, the tensor model will no longer reflect any of the fibre tract orientations that are present within a voxel, and instead produce an estimation of the mean fibre direction (Alexander et al., 2001; Assaf et al., 2004; Tuch et al., 2002). Incorrect rendering of multiple fibre directions can therefore result in false negative or false positive white matter tract reconstructions by tracking algorithms (Behrens et al., 2007; Jeurissen et al., 2013). The demonstrable limitations of DTI-based tractography are apparent throughout the literature and can be particularly detrimental when applying tractography to improve neurosurgical procedures (Behrens et al., 2007; Berman, 2009; Farquharson et al., 2013a; Kinoshita et al., 2005). Consequently, considerable effort has gone into developing more sophisticated and robust techniques that overcome the inherent shortcomings of DTI. Constrained spherical deconvolution in conjunction with high angular resolution diffusion-weighted imaging (HARDI) is one such technique designed to deliver more precise fibre orientation estimates and as a result is an invaluable tool for improving the accuracy of diffusion tractography studies (Van Hecke et al., 2015).

1.9.6 High angular resolution diffusion-weighted imaging (HARDI)

The term HARDI was first introduced by Tuch et al. (2002) and refers to an acquisition strategy which uses dense sampling on the sphere (i.e. many unique diffusion directions are measured in a given voxel), using only one b -value (see Figure 1.26). The rationale behind applying this diffusion gradient sampling scheme was to resolve multiple fibre directions in a voxel which may otherwise not be captured with less dense sampling. In regards to acquisition parameters, HARDI is similar to standard dMRI, however with HARDI a significantly larger number of diffusion-weighted gradient orientations and a larger b -

value is utilised (Van Hecke et al., 2015). Currently, the minimum ideal number of diffusion directions used to perform a robust diffusion tensor reconstruction is approximately 30 (Jones, 2004). Comparatively, the acquisition strategy will generally be considered HARDI if data is acquired in greater than 40 directions (Van Hecke et al., 2015). Consequently, HARDI has extensive applications in improving neural fibre tracking and reconstruction methods as it provides a much more detailed diffusion profile within each voxel.

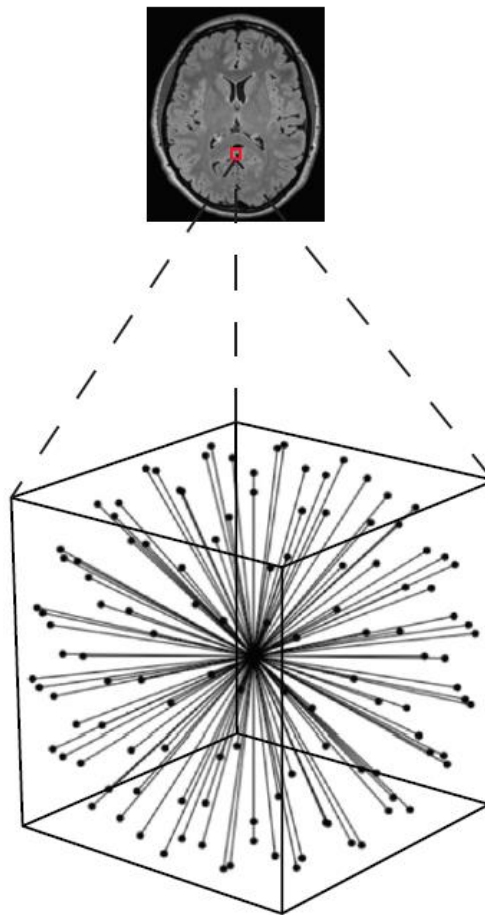


Figure 1.26 | High angular diffusion weighted imaging. Illustration showing the large number of diffusion weighted directions that are acquired within a single voxel of the diffusion-weighted image. High angular diffusion weighted imaging attempts to provide a detailed diffusion profile by sampling as densely as is practicable. Figure adapted from Van Hecke et al. (2015).

1.9.7 Constrained Spherical Deconvolution (CSD)

In the advent of HARDI, constrained spherical deconvolution, a so called ‘higher-order’ diffusion model, attempts to provide a more tangible solution to the ‘fibre crossing’ problem of DTI. Constrained spherical deconvolution offers a way to overcome this issue by providing a direct way to map the distribution of fibre orientations from the diffusion-weighted signal attenuation measured over the surface of a sphere (Tournier et al., 2004). The diffusion-weighted signal obtained from the HARDI data can be expressed as the convolution over the sphere (using spherical coordinates) of a response function (which is a representation of the attenuated diffusion signal of a single coherently orientated

population of fibres) with the *fibre orientation distribution function* (FOD; Figure 1.27; Tournier et al. (2004)). Hence, CSD assumes that the diffusion-weighted signal of any given fibre bundle can be described by a single response function. The inverse operation, spherical deconvolution, can then be applied to identify the orientations of distinct fibre populations within a given voxel (the FOD). Furthermore, the deconvolution step is constrained in order to increase the precision of the FODs, thus reducing noise contamination and limiting physically impossible fibre orientations (Tournier et al., 2007).

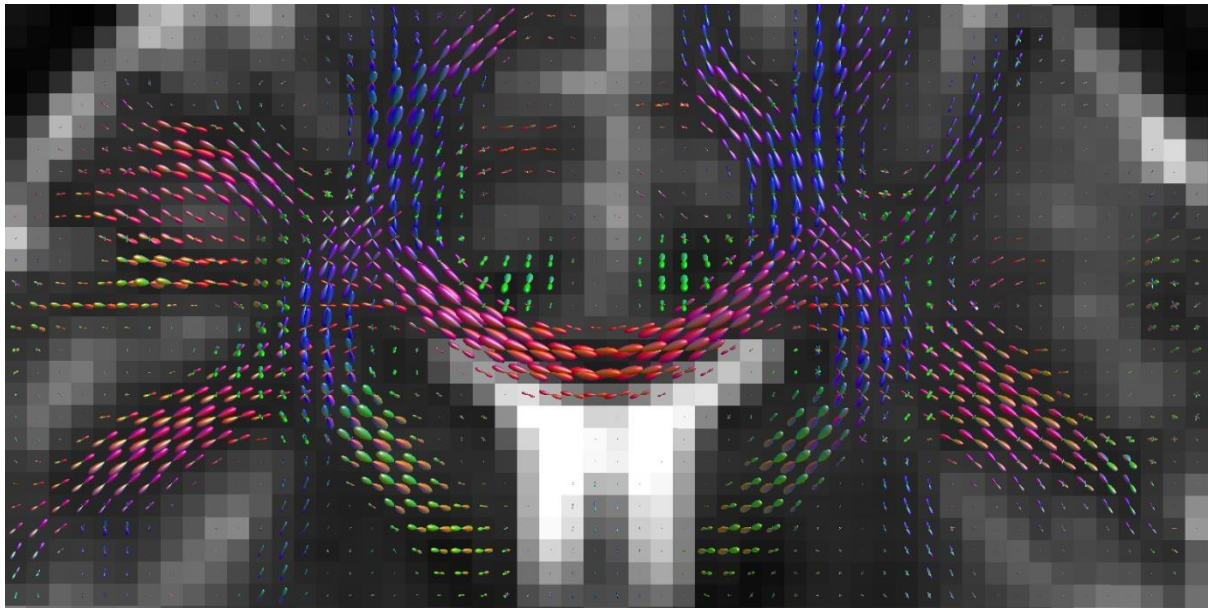


Figure 1.27 | Fibre orientation distributions from a healthy volunteer subject. Coronal cross-section of a dMR image of the brain overlaid with the fibre orientation distributions (FODs) obtained from constrained spherical deconvolution (Tournier et al., 2007). The corpus callosum is readily identifiable in the centre of the image with the red FOD lobes indicating the direction of the fibres in this region (left-right). The FODs also capture the lateral projections of the corpus callosum, which cross over the corona radiata (inferior-superior; represented by the blue FOD lobes). The cross-section of the brain shown here is the same as the one shown in Figure 1.25.

The mathematical basis of CSD is reliant on several assumptions regarding the diffusion process within the human body and how the diffusion data is acquired. Firstly, the time-scale at which the diffusion images are acquired is extremely short, as such CSD relies on the assumption that spatially distinct fibres have virtually no exchange of water molecules. This means that the diffusion signal measured from spatially distinct regions can be added independently to produce the total signal measured. The second assumption applies to curved fibres. It is assumed that the radius of curvature of white matter fibres is larger than the measured displacement of water molecules (a few tens of microns), meaning that there is effectively no exchange between regions of a fibre bundle that are orientationally distinct. Finally, it is assumed that the diffusion characteristics and the signal profiles of different white matter fibre bundles are indistinguishable and therefore can be described by the same fibre response function (Tournier et al., 2004).

Compared to the diffusion tensor model and other techniques available (q-space approach, diffusion spectrum imaging etc.), CSD provides several advantages. Firstly, because CSD is a direct translation of the diffusion-weighted signal it is not reliant on any model of diffusion and therefore it provides a much more detailed and accurate description of the diffusion process. Secondly, for the same reason, no a priori information is required about the number of distinct fibre orientations present in each voxel. Lastly, it is relatively simple in regard to its computational requirements and therefore CSD-based reconstruction can occur relatively fast without the use of specialised computing resources (Tournier et al., 2004). Like DTI, constrained spherical deconvolution and the other higher-order diffusion models can provide other, arguably more relevant quantitative metrics than those obtained with DTI (Raffelt et al., 2017). Furthermore, it has been shown that tractography algorithms based on higher-order diffusion models are better able to reconstruct white matter connections within the brain and brainstem, generating more anatomically plausible and accurate streamlines, particularly in areas of complex fibre arrangements (Farquharson et al., 2013; Mormina et al., 2015). This has obvious benefits for improved accuracy of neurosurgical procedures, but also in the study of human white matter tracts. An example of whole-brain tractography based on CSD is shown in Figure 1.28. Furthermore, a recent review describing previous research that has used dMRI-based techniques to study the white matter tracts of the auditory brainstem is provided in Appendix 1.

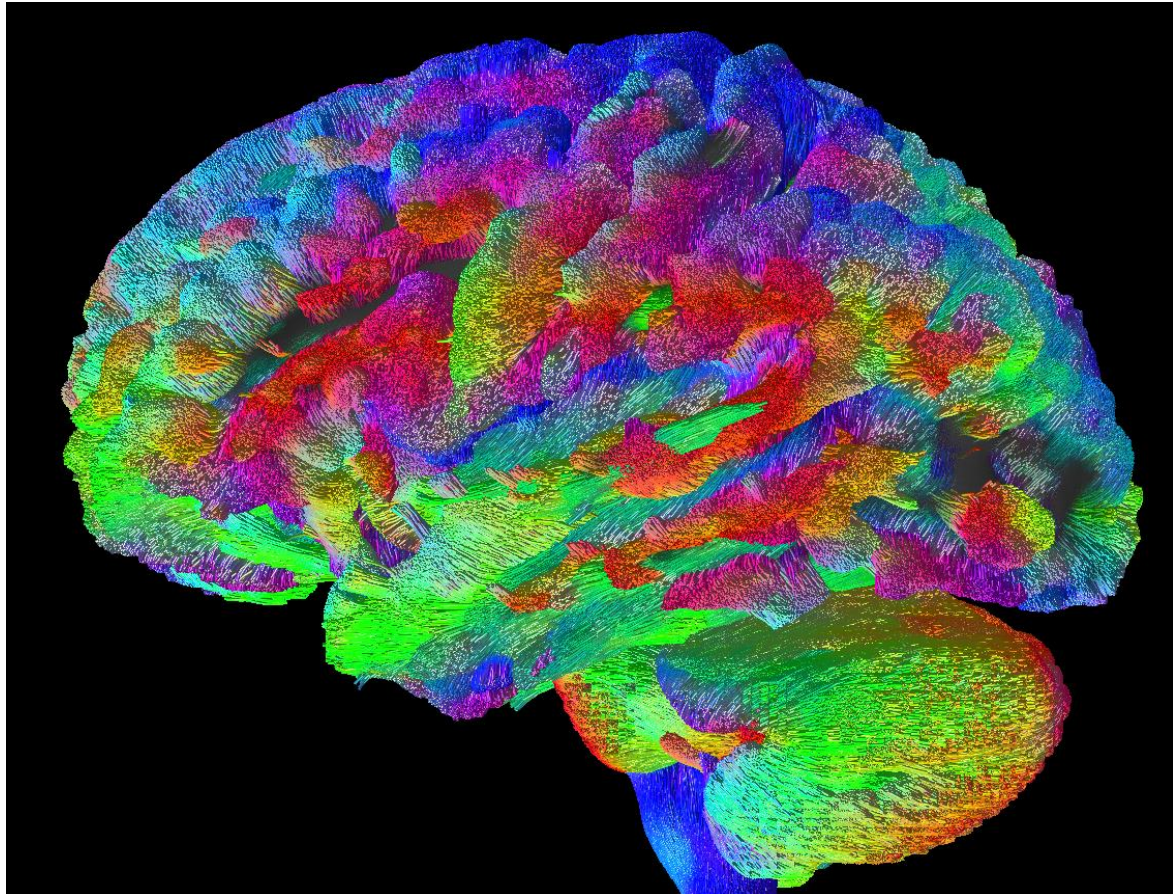


Figure 1.28 | An example of tractography. A 3-dimensional render showing a sagittal view of constrained spherical deconvolution-based whole-brain tractography. Tract orientation is coloured according to the directional encoded colour (DEC) convention, whereby: Left-right = red, anterior-posterior = green, and superior-inferior is blue. Image was generated using MRtrix3 (Tournier et al., 2019).

1.9.8 Fixel-based analysis

Fixel-based analysis is a recently developed quantitative analysis framework, which is based on the analysis of HARDI data using an FOD-based higher order diffusion model, such as CSD. The term ‘fixel’ refers to a specific *fibre* population in a given *voxel*. Hence, fixel-based analysis enables fibre tract-specific statistical analyses to be carried out between patient populations, while leveraging the benefits of more complex diffusion models in regions with complex white matter fibre arrangements (Raffelt et al., 2015; Raffelt et al., 2017). As part of the fixel-based analysis framework, microstructural changes relating to fibre-bundle morphology can be investigated with the Apparent Fibre Density (AFD) metric. In particular, the fixel-based analysis framework can provide specific information about the differences in the AFD metric between populations by identifying both the location and the specific fibre orientations in which differences exist (Raffelt et al., 2012b; Raffelt et al., 2017).

1.9.8.1 Apparent Fibre Density (AFD)

The AFD metric provides a way to measure the intra-axonal restricted volume compartment at each fixel, making it sensitive to white matter pathologies affecting the number of axons, at a

microstructural, within-voxel level (Raffelt et al., 2012b). The AFD metric is derived from the FOD amplitudes, that can be computed using higher-order diffusion models and is based on the widely held assumption that the diffusion of water is restricted in the radial direction within axons (Assaf & Bassler, 2005; Barazany et al., 2009; Raffelt et al., 2012b). Since FOD amplitudes are approximately proportional to the radial diffusion-weighted signal, particularly at high b -values (i.e. $b \geq 3000$ s/mm²), the AFD metric can be used to determine the fibre/axonal density of a given fibre bundle (Figure 1.29). It can therefore also be applied to determine differences in axonal density within the white matter tracts of the brain between different groups (Gajamange et al., 2018; Mito et al., 2018; Raffelt et al., 2012b).

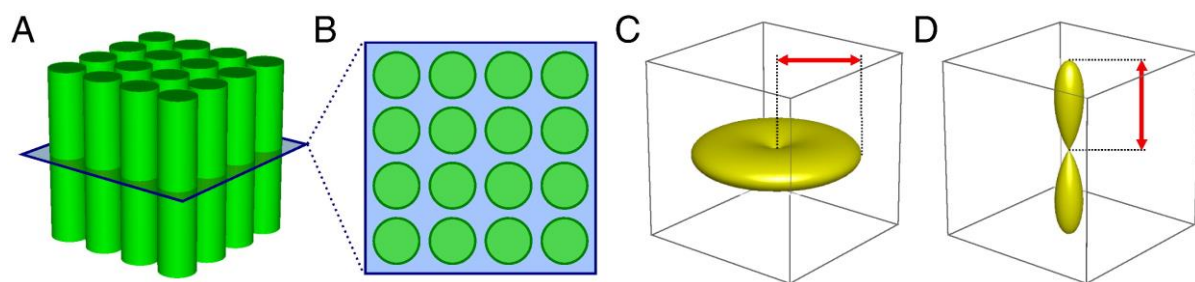


Figure 1.29 | Apparent Fibre Density metric. (A) The green tubes represent coherently arranged axons within a given voxel. (B) The perpendicular plane of the axons shown in A. This cross-section depicts the intra-axonal compartment (area within the axons) and the extra-cellular compartment (shown in blue). (C) The expected diffusion-weighted signal profile of the axons depicted in A. The diffusion-weighted signal is maximally attenuated in the direction parallel to the axon fibres, in which water diffusion is relatively unconstrained. In comparison, the diffusion signal in the perpendicular (radial) direction, in the direction that water diffusion is constrained by cell membranes, is attenuated the least. Hence, the magnitude of the diffusion weighted signal (shown by the red arrow) is approximately proportional to the intra-axonal compartment (i.e. the area within the green circles shown in B). The amplitude of the FOD (shown by the red arrow), along a given orientation is directly proportional to the size of the diffusion-weighted signal obtained in the radial plane (arrow in C), and therefore it also represents the intra-axonal volume along that same orientation. Figure republished with permission from NeuroImage via the Copyright Clearance Centre, Inc. (Raffelt et al., 2012b).

1.10 Study aims and hypotheses

1.10.1 Nature and scope of this study

This thesis incorporates the disciplines of Audiology, Neurology, Auditory Neuroscience, Neuroradiology, MR Physics and Image Analysis to apply advances in neuroimaging to better understand, visualise and quantify the auditory brainstem in normal hearing and affected populations.

1.10.2 Aims

1. To visualise the major white matter fibre tracts in the auditory brainstem (tracts occurring between the VIII nerve/cochlear nucleus and inferior colliculus) in normal hearing individuals using diffusion-based fibre tracking algorithms.

2. To compare the accuracy of different diffusion models (diffusion tensor imaging vs. constrained spherical deconvolution) and associated tractography algorithms in delineating the auditory brainstem white matter tracts.
3. To investigate the site of lesion and pathophysiological mechanism(s) underpinning the hearing deficits in individuals with a genetically characterised type of AN known as AUNX1 using a fixel-based analysis.
4. To compare dMRI-based quantitative metrics, such as the Apparent Fibre Density, with functional hearing outcomes in individuals who have auditory neuropathy to establish if auditory perception is associated with discernible/specific neuro-anatomical changes.
5. To investigate the site of lesion and pathophysiological mechanisms underpinning the hearing deficits in individuals with the auditory neuropathy phenotype as a result of different aetiologies, such as CMT, RTD, hypoxia, and FRDA, using the Apparent Fibre Density metric.

1.10.3 Hypotheses

1. The major fibre tracts of the auditory brainstem can be visualised in individuals with normal hearing and auditory neuropathy using HARDI data and Single-Shell 3-Tissue CSD-based probabilistic tractography.
2. Single-Shell 3-Tissue CSD and the associated probabilistic tractography algorithm (iFOD2) is better able to reconstruct the white matter tracts of the auditory brainstem than the widely adopted diffusion tensor model.
3. Individuals with AUNX1 will show reduction in Apparent Fibre Density within the auditory brainstem tracts consistent with the assumed pathophysiological mechanism of focal axonal degeneration of the vestibulocochlear VIII cranial nerve.
4. The degree of neuro-anatomical dysfunction quantified using the Apparent Fibre Density metric will correlate with the degree of dysfunction on behavioural, psychoacoustic and electrophysiological auditory test measures.
5. The AFD metric will vary according to aetiology. Individuals who exhibit the auditory neuropathy phenotype due to neurodegenerative conditions will have the lowest AFD values within the auditory system.

Part II

Chapter 2: Using fibre tractography to map the white matter tracts of the auditory brainstem *in-vivo*: a feasibility study

2.1 Introduction

As discussed in detail in section 1.9, dMRI is an image acquisition technique capable of measuring the amount and directionality of the diffusion of water molecules within biological tissue *in-vivo*. While image contrasts obtained using dMRI provide useful information regarding tissue microstructure, mathematical models of the diffusion process enable the diffusion profile of a given voxel to be visually examined in 3-dimensions and therefore provide a much more comprehensive overview of the underlying tissue architecture. Since the diffusion of water molecules is dependent on the tissue microstructure (i.e. anisotropic diffusion is exhibited in white matter bundles, whereas in grey matter and cerebrospinal fluid diffusion is more isotropic) these diffusion models can be used to investigate microstructural features of brain tissue, including potential neurodegenerative changes in white matter fibre tracts (Basser & Pierpaoli, 1996; Mito et al., 2018; Raffelt et al., 2012b).

The diffusion tensor was the first of these models to be developed. Up until recently it was one of the most commonly used methods of characterising and studying the underlying tissue microstructure from the measured diffusion-weighted signal (Basser et al., 1994). Diffusion tensor imaging, however, is not capable of resolving multiple white matter fibre arrangements, such as bending, crossing or kissing fibres, within a given voxel and as a result it represents such complex fibre arrangements erroneously (Behrens et al., 2007; Mori & Tournier, 2013). As such, there are considerable limitations to the accuracy of DTI, especially given that approximately 90% of white matter voxels within the brain and brainstem contain these complex fibre configurations (Behrens et al., 2007; Farquharson et al., 2013; Mori & Tournier, 2013). Hence, any metrics or further downstream processing reliant on the accuracy of the diffusion tensor model are likely to be compromised. This is particularly apparent in tractography experiments, which use the directional information provided by the diffusion model to produce virtual maps or ‘streamlines’ of continuous white matter pathways in the brain and brainstem (for a review of tractography methods please refer to Jeurissen et al. (2017)). Several studies have shown that white matter tract reconstructions performed with DTI-based tractography algorithms are unreliable and often contain misleading information (Auriat et al., 2015; Farquharson et al., 2013; Thomas et al., 2014). This has potentially serious clinical repercussions as such techniques are routinely used in neurosurgical planning and for intraoperative navigation (Bozzao et al., 2010; Potgieser et al., 2014).

To overcome the limitations of DTI, many so-called ‘higher-order’ diffusion models have been developed that are better able to deal with complex fibre arrangements, some of which include CSD

(Dhollander & Connelly, 2016; Jeurissen et al., 2014; Tournier et al., 2007), Q-ball imaging (Tuch, 2004), and the diffusion orientation transform (Ozarslan et al., 2006). Each of these models uses a different approach and mathematical framework to interpret the diffusion-weighted signal. Additionally, multi-compartmental models, including multi-shell, multi-tissue CSD (Jeurissen et al., 2014) and single-shell 3-tissue CSD (SS3T-CSD) (Dhollander & Connelly, 2016) are also better able to deal with the effects of partial voluming, therefore improving tractography-based reconstructions in WM voxels containing other tissues and CSF. These models typically achieve this by obtaining unique response functions for the different tissue types (i.e. WM, GM, and CSF) and using this additional information to model the constituent tissue components within a given voxel. Recent studies have shown that tractography algorithms based on such higher-order diffusion models provide fibre trajectories that are biologically more plausible than those obtained from DTI-based tractography algorithms, even when the same dMRI data is analysed (Calamante, 2019; Farquharson et al., 2013; Jeurissen et al., 2011; Tournier et al., 2012).

The algorithms used for tractography also vary considerably in the way they interpret the diffusion models and estimate fibre trajectories. Irrespective of the diffusion model used for data analysis, tractography algorithms can be divided into two main classes, *deterministic* and *probabilistic*. Deterministic tractography algorithms reconstruct white matter tracts by generating fibre trajectories or ‘streamlines’ based on the most appropriate fit of the fibre orientation within each voxel. Typically, this ‘best fit’ is the direction of maximal diffusivity, as defined by the diffusion model (Jones, 2010). Deterministic algorithms, however, do not take into account uncertainties in fibre orientations. As a result, any noise that is present in the data (i.e. due to imaging noise and artefacts) can interfere with the ability of these algorithms to reconstruct connections between voxels. Comparatively, probabilistic tractography algorithms do consider the uncertainty in the fibre orientations depending on the quality of the data. As fibre trajectories are constructed, probabilistic algorithms statistically sample from the distribution of possible fibre orientations to determine the connections with the highest probability of existing (Jeurissen et al., 2017). Overall, deterministic approaches provide conservative estimates of possible fibre connections and are prone to false-negatives, whereas probabilistic approaches produce a greater number of false-positives.

In addition to these tractography approaches, other processing methods have also been developed which can improve the overall accuracy of fibre tracking algorithms. Anatomically-constrained tractography (ACT; Smith et al. (2012)), for example, incorporates the information obtained from the tissue segmentation of an anatomical contrast image (i.e. T1- or T2-weighted image) to guide the fibre-tracking process. The anatomical detail provided by additional anatomical images has been shown to improve the delineation of white matter tracts within the brain and reduce the likelihood that biologically implausible streamlines are reconstructed (Maffei et al., 2018; Martinez-Heras et al., 2015;

Smith et al., 2012). This method, however, has not been used to study smaller white matter pathways within the brain and brainstem and therefore its efficacy in such regions is not known.

The auditory brainstem tracts form a major component of the central auditory nervous system and are involved in processing, filtering, and analysing of signals arriving from the cochlea. Overall, these tracts form a complex multi-synaptic network with many commissural and contralateral connections (Figure 2.1). Afferent fibres from the cochlear nucleus carry auditory information to the SOC, the lateral lemniscus, the nuclei of the IC and the medial geniculate nucleus. The medial geniculate nucleus is the last nucleus of the auditory brainstem, after which the auditory radiations convey auditory information to the primary auditory cortex. Each of the brainstem nuclei are vital for the processing and integration of acoustic signals and any disruption to their ability to function normally can seriously impact many aspects of audition, including the ability to integrate temporal cues, discriminate frequencies, and resolve intensity differences (Pickles, 2015). As such, the subcortical auditory nuclei have been shown to be involved in the pathogenesis of several hearing disorders, including auditory neuropathy, auditory processing disorder, auditory agnosia, and tinnitus (Celesia & Hickok, 2015; Crippa et al., 2010). Despite their role in such conditions, there exists no stereotaxic structural atlas, and very few studies have used dMRI tractography methods to study the white matter tracts of the auditory brainstem *in-vivo*. Such a paucity of research limits the ability to further investigate potential structural and connectivity-related abnormalities in different conditions.

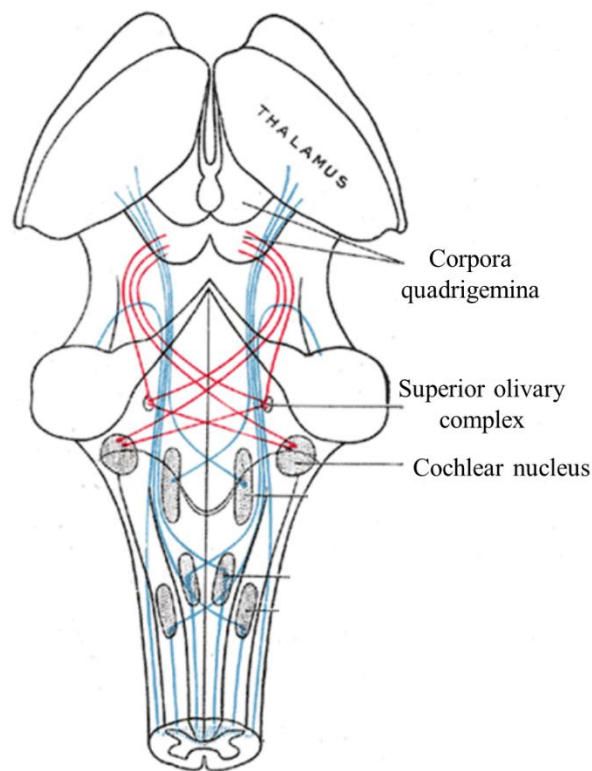


Figure 2.1 | Schematic of the auditory brainstem pathways (shown in red) from Gray's Anatomy of the Human Body (Gray, 2009). The corpora quadrigemina encompasses both the inferior and superior colliculi.

2.2 Aims

The aim of this study was to compare the performance of the traditionally most commonly used diffusion model, the diffusion tensor, with a higher-order model (CSD) to determine which method is most accurate at delineating the white matter tracts of the auditory brainstem. Furthermore, this study aims to introduce a tractography approach to reconstruct the white matter fibre tracts occurring between the vestibulocochlear nerve and the IC in three normal hearing individuals at different developmental stages of life: infancy, childhood and adulthood.

2.3 Methods

2.3.1 Participants

Three normal hearing individuals participated in this study. Participant clinical and demographic information is shown in Table 2.1. The participants did not have any known neurological or psychiatric conditions. All the participants were recruited by word of mouth and the study was approved by the Ethics Committee of the Royal Victorian Eye and Ear Hospital (HREC 15/1254H; see Appendix 2).

Table 2.1 | Participant clinical and demographic information.

Subject	Age at assessment	Gender	Audiometry L/R		
			4FA (dB HL)	OAEs	ABR wave V latency (ms)
1	5 months	F	*	+/+	6.6/6.6
2	10 years	M	10/11.25	+/+	5.7/5.6
3	31 years	M	0/-1.25	+/+	5.4/5.5

* = behavioural audiometry not performed due to participants age. OAEs: otoacoustic emissions; ABR: Auditory brainstem response. Values displayed for the ABR wave V latency were in response to a stimulus presentation intensity of 90 dB nHL

2.3.2 Human research ethics committee approval

Approval to carry out the following study was obtained through the Human Ethics and Research Ethics Committee of the Royal Victorian Eye and Ear Hospital, Melbourne, Victoria (project reference number: 15/1254H; see Appendix 2). The study protocol was undertaken in strict accordance to the ethical standards defined in the 1964 Declaration of Helsinki. All participants received information regarding the nature and purpose of the study after which they provided written informed consent and were eligible to withdraw at any time.

2.3.3 Image acquisition

MRI data was acquired on a 3 Tesla Siemens Magnetom Skyra scanner (Siemens Medical Solutions, Erlangen, Germany) using a 32-channel head coil receiver. A twice refocused, spin-echo, EPI sequence was employed to acquire 64 axial diffusion-weighted images (with a $b = 3000 \text{ s/mm}^2$) equally spaced

over a hemisphere, and 8 non-diffusion weighted images ($b = 0 \text{ s/mm}^2$) for all three of the participants in this study. Diffusion MRI acquisition took approximately 10 mins in total.

To account for the young age of subject #1 image acquisition parameters were different compared to the two other participants in this study. That is, because subject #1 was imaged under natural sleep (due to their age), multiband imaging was employed to speed up the acquisition time, with only a slight reduction in data quality. Moreover, due to the comparatively small head size of subject #1, images were acquired using a higher spatial resolution. The following acquisition parameters were used for subject #1: repetition/echo time = 8400/110 ms, 2.3 mm isotropic voxels, FoV = 240 x 240 mm, matrix size = 96 x 96, and multiband factor 3. This protocol was repeated twice, and all acquired data was combined into a single dMRI-dataset. For subject #2 and #3 the following parameters were used for image acquisition: repetition/echo time = 8400/110 ms, 2.5 mm isotropic voxels, FoV = 240 x 240 mm, matrix size = 96 x 96, and acceleration factor = 2.

For all the participants a high resolution T2-weighted image (voxel size = 0.5 x 0.5 x 2.0 mm, repetition/echo time = 4750/101 ms, flip angle = 150°, slice thickness = 2 mm) and a T2-weighted fluid attenuated inversion recovery (FLAIR) image (voxel size = 0.9 mm³, repetition/echo time = 6000/387 ms, slice thickness = 0.9 mm) were also acquired and co-registered to the diffusion-weighted scan of each subject with the rigid body co-registration algorithm available through the Statistical Parametric Mapping software package version 12 (SPM12; <https://www.fil.ion.ucl.ac.uk/spm/>; Penny et al. (2011)). These structural images were used to aid in the identification of the ROIs for subsequent fibre tracking, and for improved anatomical visualisation of the tractography results, which were overlaid on the high resolution T2-weighted images.

2.3.4 Diffusion magnetic resonance imaging processing

Diffusion MRI processing was performed using the MRtrix3Tissue software package (<https://3Tissue.github.io>), a fork of MRtrix3 (Tournier et al., 2019). While most of the processing steps were conducted using MRtrix3Tissue, some of the scripts interfaced with external image-processing software packages, including FSL (Jenkinson et al., 2012) and ANTs (Avants et al., 2009). Pre-processing steps included denoising (Veraart et al., 2016b; Veraart et al., 2016c), correcting for Gibbs-ringing artefact (Kellner et al., 2016), field map correction for EPI distortions, motion correction (Andersson et al., 2003; Andersson & Sotiropoulos, 2016; Smith et al., 2004), and bias field correction (N4, <http://stnava.github.io/ANTs/>; Avants et al. (2009)). The spatial resolution of the dMRI data was also regridded using cubic b-spline interpolation, to generate an isotropic voxel size of 1.3 mm (Raffelt et al., 2012b).

After the completion of these pre-processing steps, white matter FODs were calculated using SS3T-CSD based on tissue specific response functions for WM, GM and CSF (Dhollander & Connelly, 2016;

Dhollander et al., 2016). For comparison, the diffusion tensor was also fitted to the dMRI data using the least squares method as described in detail by Veraart et al. (2013).

2.3.5 Defining the regions of interest and tractography

Target and seed regions for fibre tracking were manually demarcated using co-registered T2-weighted images for guidance. The left vestibulocochlear nerve was designated as the seed region and the inferior colliculi were selected as the target regions for each individual (Figure 2.2). These regions were selected based on their ease of identification on co-registered T2-weighted images. For instance, the vestibulocochlear nerve could be identified as a distinct protrusion on the axial and coronal slices, joining the cochlea to the brainstem. Moreover, the inferior colliculi could be identified as distinct circular regions on axial and coronal slices, and as protrusions when viewed in the sagittal plane. Exclusion regions were also defined in order to constrain the tractography results to the brainstem area. These included slices immediately below the vestibulocochlear nerve and above the inferior colliculi. Moreover, the cerebellum was also excluded from the tractography analysis.

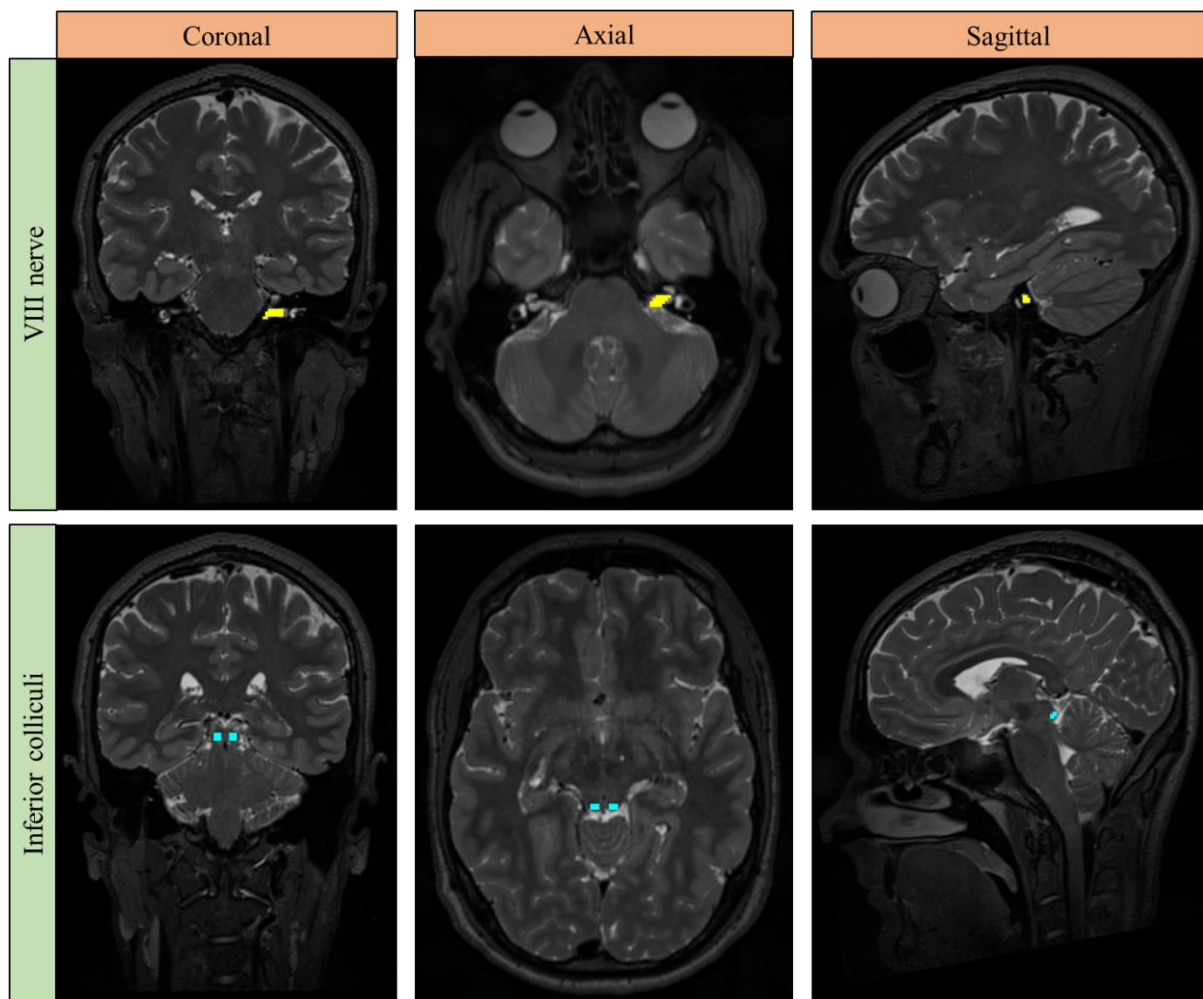


Figure 2.2 | Seed and target regions of interest. Coronal, axial and sagittal T2-weighted structural MRI slices overlaid with seed regions (left VIII nerve (top)) shown in yellow and target regions (inferior colliculi (bottom))

shown in blue. Images shown are all from subject 2, however, the same regions were identified for all three participants within this study. All images are shown in radiological view.

The diffusion models, SS3T-CSD and DTI, were combined with appropriate tractography algorithms to generate tractograms of the auditory brainstem tracts. The approaches used included: (i) Diffusion tensor imaging in combination with a deterministic tractography algorithm (Basser et al., 2000); (ii) Diffusion tensor imaging in combination with a probabilistic tractography algorithm (Jones, 2008); (iii) SS3T-CSD combined with a probabilistic tractography algorithm (iFOD2; Tournier et al. (2010)) with manually demarcated exclusion regions, and; (iv) SS3T-CSD in combination with probabilistic tractography and anatomical information obtained from a co-registered T1-weighted image (Anatomically-Constrained Tractography (ACT); Smith et al. (2012)) (Figure 2.3). Anatomically-Constrained Tractography was performed using the FSL Automated Segmentation Tool (FAST; Zhang et al. (2001)), which provides an estimation of the partial volume fractions of white matter, grey matter, and CSF for all voxels within the brain region. Using the information provided by the automated tissue segmentation tool, the ACT algorithm places several constraints on the generation and termination of tractography streamlines. These include: (i) streamlines that enter any structures or regions identified to consist of CSF are automatically rejected; (ii) streamlines that enter cortical grey matter regions are considered to have reached an appropriate termination point and therefore they are accepted; (iii) if streamlines extend beyond the field of view of the dMR image or the anatomical contrast image, or they leave a pre-defined mask, the streamline is terminated and accepted; and, (iv) streamlines that have passed from white matter into subcortical structures are not permitted to exit, and streamlines will be truncated to prevent this from occurring (Smith et al., 2012). Some of these additional constraints, especially those relating to grey matter termination, are better suited to tractography studies of the cortex or whole-brain and may not provide any benefit in mapping subcortical structures such as the auditory brainstem pathways (Smith et al., 2012).

For each of the analyses the same manually demarcated seed and target ROIs were used. Furthermore, fibre tracking was performed in each subject's native image space from seed to target ROIs and vice versa. Tractography results consisted only of those streamlines that contacted the target ROIs. Fibre tracking was terminated when 10,000 tracks were generated between the ROIs or when 100,000 tracks in total had been initiated from the seed point. Default parameters were used in MRtrix3Tissue for all tractography algorithms discussed.

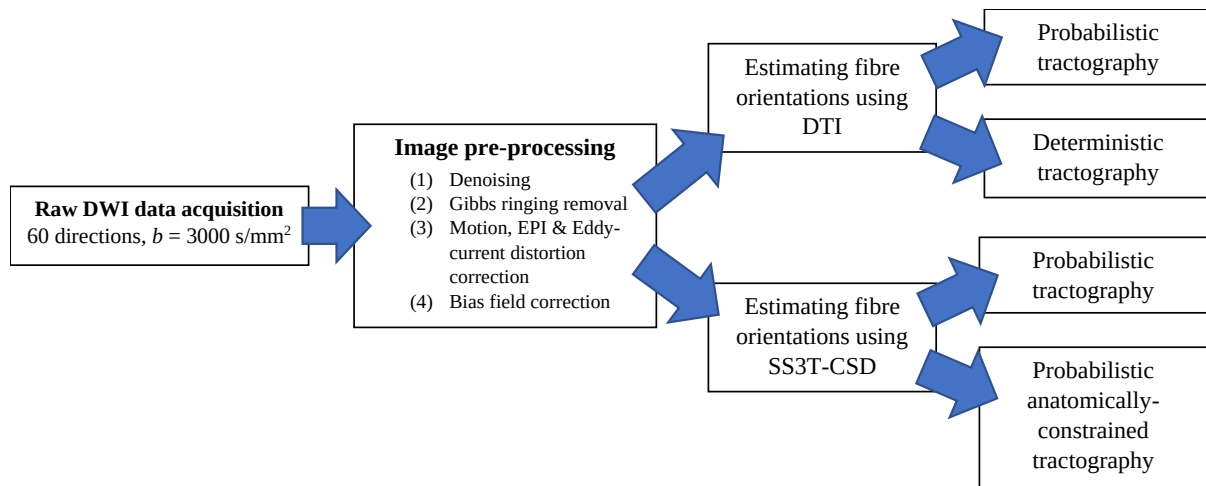


Figure 2.3 | Schematic showing the pipeline used to produce the different tractography results. Image acquisition and pre-processing steps were the same for all tractography approaches used in the study. DWI: diffusion-weighted image; EPI: echo planar imaging; DTI: diffusion tensor imaging; SS3T-CSD: Single-Shell 3-Tissue Constrained Spherical Deconvolution.

2.3.6 Audiological assessment

Pure tone audiometry, OAE testing and ABRs were recorded to confirm all three participants had normal auditory function.

2.3.6.1 Pure tone audiometry

Pure tone audiometry was carried out using an Interacoustics AD226 audiometer with ER-3A insert earphones. Hearing thresholds were measured at octave frequencies from 0.250 to 8 kHz in subject #2 and #3. Subject #1 was too young to be tested using PTA and hearing acuity was assessed using objective tests. Both subject #2 and #3 had normal hearing thresholds (thresholds were < 20 dB HL) at each frequency tested.

2.3.6.2 Otoacoustic emissions

Distortion product otoacoustic emissions were recorded using a GSI Audera evoked-potential system. Data for each participant was recorded for both ears at 0.5-, 1-, 2-, 3-, and 4 kHz. The stimuli were presented at an intensity of 65 dB SPL for f1 and 55 dB SPL for f2 (f1:f2 test ratio of 1.2). A response was considered present only if the response amplitude was ≥ 6 dB for three consecutive frequency bands. All three participants had extant emissions, a finding indicative of normal outer hair cell function.

2.3.6.3 Auditory brainstem response

Auditory brainstem response recordings were conducted using a GSI Audera evoked-potential system. The stimulus, rarefaction 100 μ s broadband clicks, were presented to each participant through ER-3A insert earphones at a rate of 33.1 Hz. A total of two waveforms were obtained with 1200 post stimulus sweeps for each response. The ABR was recorded using AMBU Neuroline 720 silver/silver chloride surface electrodes with the following electrode configuration: the inverting electrode was situated on

the ipsilateral mastoid, the ground electrode was attached to the brow, and the non-inverting reference electrode was attached on the forehead just below the hairline. Physiologic filtering was used to pass electrical activity between 30 – 1500 Hz for subject #1, and 150 – 3000 Hz for subject #2 and #3. The artefact rejection level was set at 25 μ V. ABR tracings were recorded at 90 dB nHL for subject #2 and #3. Waveform morphology and latencies were normal for each participant. For subject #1 the ABR was obtained down to threshold (25 dB nHL) in both ears and hearing was confirmed to be within normal limits.

2.4 Results

2.4.1 SS3T-CSD vs. Diffusion Tensor Imaging

Deterministic and probabilistic DTI-based tractography methods generated no streamlines between the seed (left VIII nerve) and target regions (inferior colliculi) and vice versa in any of the three participants. As shown in Figure 2.4, the diffusion tensor was not able to resolve areas of crossing fibres, which occur throughout the auditory brainstem tracts, whereas the SS3T-CSD model produced FOD estimates that captured the complex fibre architecture in these regions. Additionally, DTI was also not capable of dealing with the low anisotropy that occurred in the single-fibre regions (areas with no crossing fibres) of the VIII nerve. As a result, the tensors produced by DTI in these regions provided a poor representation of the diffusion profile (i.e. the tensors appeared more spherical, indicating low anisotropy) (Figure 2.4). This is likely explained by the fact that the VIII nerve is very thin and surrounded by non-white matter tissue, which makes it especially susceptible to the effects of partial voluming. That is, the voxels in these regions appear to have a low degree of anisotropy due to being partial volumed with surrounding structures/tissues that have a more isotropic diffusion profile, such as the fluid. Comparatively, since the SS3T-CSD model is better able to account for the effects of partial voluming with non-white matter structures (Dhollander & Connelly, 2016; Dhollander et al., 2016), the FOD diffusion estimates provided a much more accurate representation in these single-fibre regions as well. Hence, the SS3T-CSD-based probabilistic tractography method consistently produced robust streamlines and tracts in all three participants of this study (Figure 2.5).

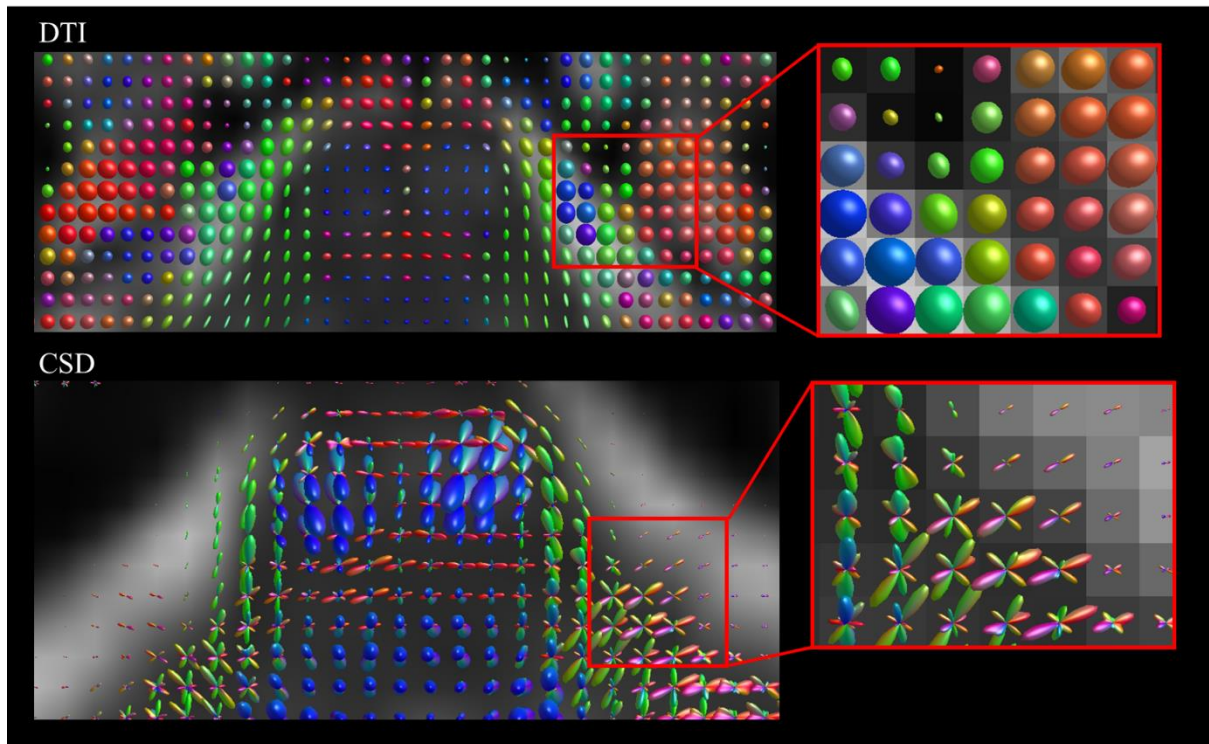


Figure 2.4 | Comparison of diffusion tensor imaging and constrained spherical deconvolution. Axial slice of the pre-processed diffusion-weighted image from subject #3 showing the ponto-medullary junction with tensor-derived orientations (*top*) and CSD fibre orientation distribution estimates (*bottom*) overlaid. The magnified regions in the red boxes (*right*) show the fibre orientation estimates within individual voxels (shown as grey squares) in the region where the VIII nerve afferent fibres synapse in the cochlear nucleus. In both the single-fibre regions of the VIII nerve and the crossing fibre regions, DTI erroneously interprets the MR signal to be of low anisotropy, incorrectly rendering the tensors as spheres.

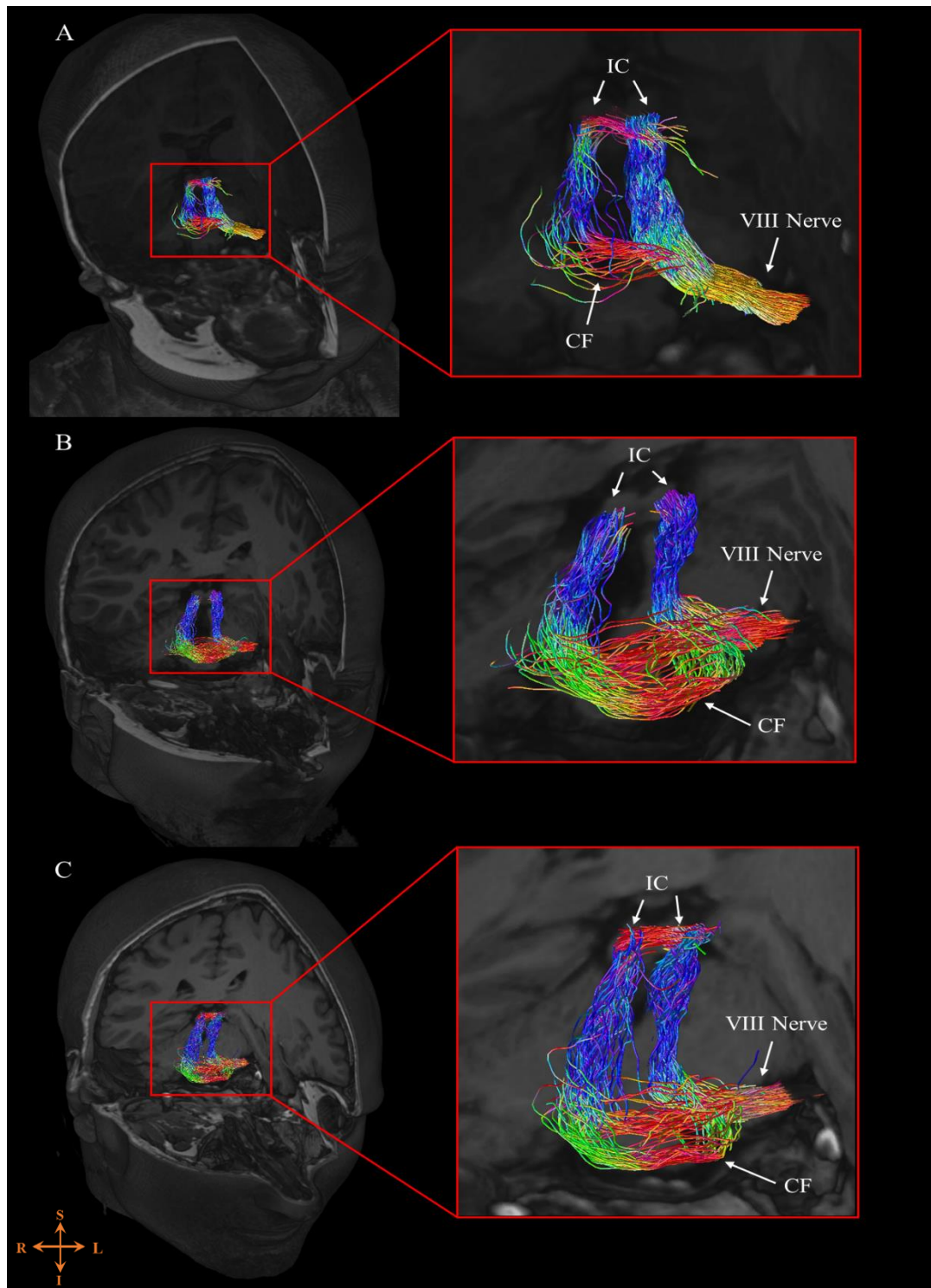


Figure 2.5 | Single Shell 3-Tissue Constrained Spherical Deconvolution-based probabilistic tractography of the auditory brainstem. *Left:* 3-dimensional render of each participant's head with axial, coronal, and sagittal slices removed to show the location of the auditory brainstem fibres in the context of surrounding brain/brainstem structures. *Right:* Magnification of auditory brainstem tracts for each subject. Tractography was seeded from the left VIII nerve to the inferior colliculus on both sides. All ROIs were delineated in each subject's native space. Tract orientation is represented by the different colours and follows the directionally encoded colour convention; Left-right is red, anterior-posterior is green, and superior-inferior is blue. (A) Tractography results obtained from an infant aged 5 months (subject #1). (B) Tractography results obtained from a child aged 10 years (subject #2), and (C) Tractography results obtained from an adult, aged 31 years (subject #3). IC: inferior colliculus; CF: crossing fibres.

2.4.2 Qualitative evaluation of the SS3T-CSD-based probabilistic tractography method

Single-Shell 3-Tissue CSD-based probabilistic tractography showed similar results for all three subjects that participated in the study (Figure 2.5). Tract reconstructions showed the presence of the left VIII nerve, ipsilateral projections from the left CN to the left IC, crossing fibres between the left and right CN, and projections from the left VIII nerve to the right contralateral IC in all three participants. In subject #1 and subject #3 commissural connections between both inferior colliculi were also present.

2.4.3 Anatomically-constrained SS3T-CSD-based tractography

Tissue segmentation of the brainstem structures using the ACT algorithm was not capable of correctly identifying the white matter VIII nerve regions entering the brainstem (Figure 2.6). As such the VIII nerve was incorrectly identified as an exclusion region (non-white matter structure), and therefore it was not included in the subsequent tractography analysis. Hence, ACT failed to adequately generate streamlines to the VIII nerve for each subject and as a result many of the crossing fibres and contralateral connections were also not correctly reproduced (Figure 2.7). While only the results for subject #3 are shown in Figure 2.7 are shown, similar results were obtained for subject #1 and #2.

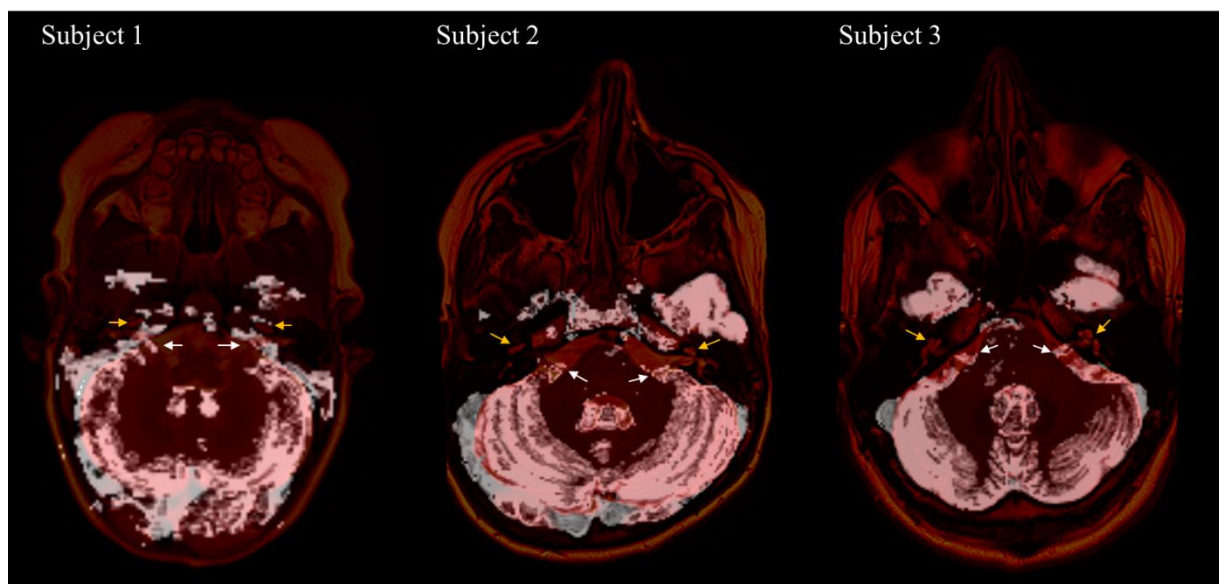


Figure 2.6 | Axial slice showing the anatomically-constrained regions (white/pink) and an overlaid T2-weighted image (in red) for each subject at the level of the cochlea. The white/pink regions are structures that the ACT image segmentation algorithm determined to be exclusion regions (i.e. non-white matter regions). The white arrows indicate the regions where the VIII nerve enters the brainstem. As shown, these areas are incorrectly identified on the segmented image as non-white matter regions, which subsequently prevented these structures from being included in the tractography analysis. The yellow arrows show the location of the cochlea for each subject.

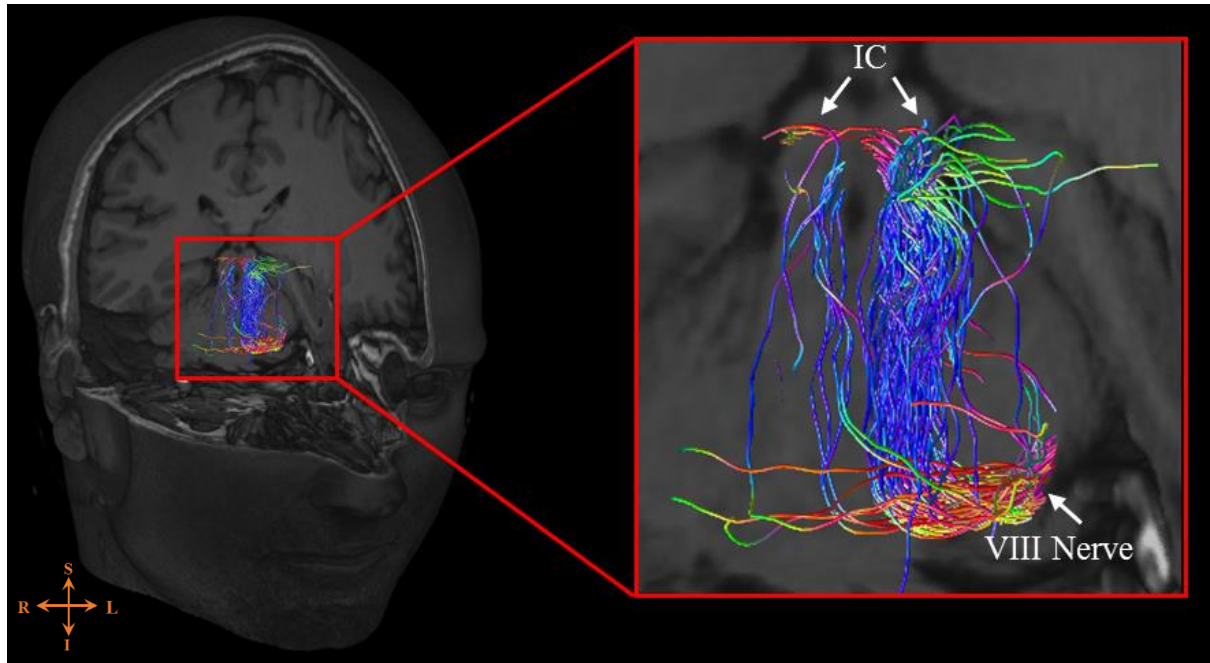


Figure 2.7 | Results of Anatomically-constrained CSD-based probabilistic tractography in reconstructing the white matter tracts of the auditory brainstem. Only the results for subject #3 are shown. The same seed and target regions were used when performing ACT as those used to produce the tracts in Figure 2.5.

2.5 Discussion

This study: (i) investigated the feasibility of reconstructing the auditory brainstem white matter tracts between the VIII nerve and IC *in-vivo*, (ii) investigated the limitations of DTI-based tractography and advantages of SS3T-CSD-based tractography in delineating these tracts, and (iii) examined the performance of the ACT framework in conjunction with SS3T-CSD-based tractography.

Overall, the major auditory tract connections occurring between the VIII nerve and inferior colliculus were successfully mapped using SS3T-CSD-based tractography in three participants of considerably different ages. Comparatively, DTI-based tractography, consistently failed to produce any viable tracts between the ROIs in all three participants. As shown in Figure 2.4, the inability of the diffusion tensor model to resolve complex fibre configurations in each voxel was the likely cause of this failure, although the low anisotropy of single fibre regions in the VIII nerve, due to partial volume effects, was also a probable contributor. Our findings are consistent with other studies that have investigated the accuracy of DTI-based tractography algorithms to delineate white matter tracts in different sensory and motor systems, including the corticospinal tracts (Farquharson et al., 2013; Ford et al., 2013), arcuate fasciculus (Mormina et al., 2015), and optic radiations (Mandelstam, 2012).

While in many instances ACT has been shown to provide more anatomically plausible tractography results than tractography algorithms not ‘informed’ by anatomical information obtained from structural MR images (Girard et al., 2014; Horbruegger et al., 2019; Mancini et al., 2019; Smith et al., 2012), this was not the case for the auditory brainstem tracts. In fact, when the ACT framework was applied in the

current study, the majority of VIII nerve tracts were excluded, and only a few of those tracts were rendered in the final image (Figure 2.7). As a result, many of the commissural and contralateral connections generated without using ACT were not present in the final ACT analysis. This finding is explained by the fact that the segmentation of anatomical T1-weighted images in this instance excluded the regions of the internal auditory canal, which contain the white matter tracts of the VIII nerve.

The tracts generated using SS3T-CSD-based probabilistic tractography (Figure 2.5) were largely consistent with those that have been reported in post-mortem dissections, animal models, and tracer studies (Cant & Benson, 2003; Moore, 1987; Pickles, 2012). Similar results have also recently been published using *in-vivo* dMRI data obtained on a 7T scanner (Figure 2.8; Sitek et al. (2019)). Notably, in our study, the left VIII nerve was clearly present in all three participants, as were commissural connections between the cochlear nuclei. In subject #1 and subject #3 the commissure of the IC was also clearly visible, however, in subject #2 this connection did not appear to be present. Furthermore, direct tracts connecting the CN to the contralateral IC, which have been relatively well characterised throughout the literature (Adams, 1997; Cant, 2005; Irvine, 2012; Oliver, 1984), were also clearly produced in the results obtained in the current study. While tracer experiments have shown that this contralateral connection has a greater fibre density than the ipsilateral pathway (Coleman & Clerici, 1987), this was also not replicated using tractography methods.

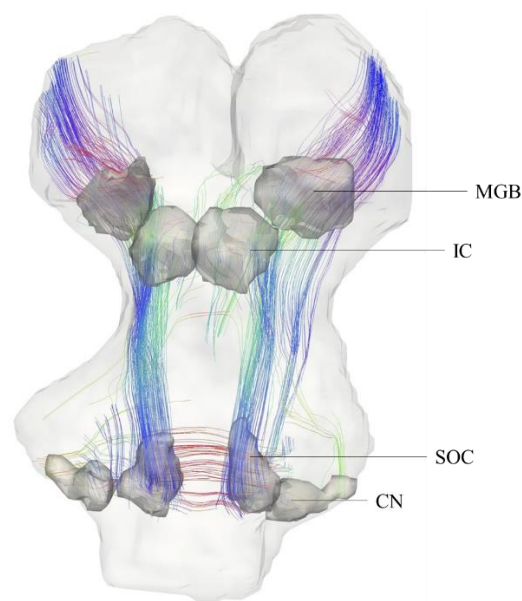


Figure 2.8 | Auditory brainstem pathways obtained using deterministic tractography on 7T dMRI *in-vivo* data. Tracts shown were obtained from a single subject and traverse each of the functionally defined auditory subcortical ROIs, including the cochlear nucleus (CN), superior olivary complex (SOC), inferior colliculus (IC), and medial geniculate body (MGB) (each ROI is shown in dark grey). Tract orientation is represented by the different colours and follows the directional encoded colour (DEC) convention; Left-right is red, anterior-posterior is green, and superior-inferior is blue. Figure modified to include labels of the prominent auditory nuclei. Republished with permission from eLIFE via the Copyright Clearance Centre, Inc. (Sitek et al., 2019).

Although SS3T-CSD-based tractography successfully generated some of the major tracts running between the left VIII nerve and bilateral inferior colliculi there are several significant limitations to using tractography algorithms to study cortical and subcortical white matter tracts *in-vivo*. Some of these limitations apply to tractography algorithms in general, whereas others are likely to be unique to tracking the auditory brainstem tracts or other small subcortical pathways.

Firstly, while the VIII nerve and inferior colliculi were relatively easy to identify on the co-registered T1 and T2-weighted images, other subcortical nuclei of the auditory system, such as the SOC, CN and lateral lemniscus, are virtually indistinguishable at lower magnetic field strengths (Hawley et al., 2005; Sigalovsky & Melcher, 2006; Zanin et al., 2019). In part, this issue stems from the low contrast of the anatomical images obtained using conventional scanners (i.e. with magnetic field strengths of 1.5 – 3T), which prevents these small subcortical nuclei from being readily distinguishable from other subcortical structures. Diffusion MR images can provide additional image contrast that can aid in the identification of these nuclei, however, such images typically have a low spatial resolution (i.e. voxel sizes in dMR images obtained using conventional magnetic field strengths are typically $\sim 2.5 \times 2.5 \times 2.5$ mm), which reduces the likelihood that small anatomical structures are adequately captured (Ford et al., 2013; Maffei et al., 2015) (Figure 2.9). Overall, these issues can reduce the accuracy of manual and automated ROI delineation, directly impacting the replicability and accuracy of tract reconstructions using tractography algorithms. While MRI scanners with higher field strengths, in the range of 7 – 11T, have been shown to produce anatomical images with more clearly identifiable subcortical nuclei (Ford et al., 2013; García-Gomar et al., 2019; Prats-Galino et al., 2012; Sitek et al., 2019), these are not currently readily available for clinical use and are therefore limited in their applicability. Moreover, functional MRI (fMRI) activation of the auditory subcortical nuclei has also been shown to be a useful adjunct to anatomical and diffusion MRI data in the identification of these structures (Javad et al., 2014; Sitek et al., 2019). Higher magnetic field strengths have also improved the precision of fMRI analysis for this purpose (De Martino et al., 2013; Moerel et al., 2015).

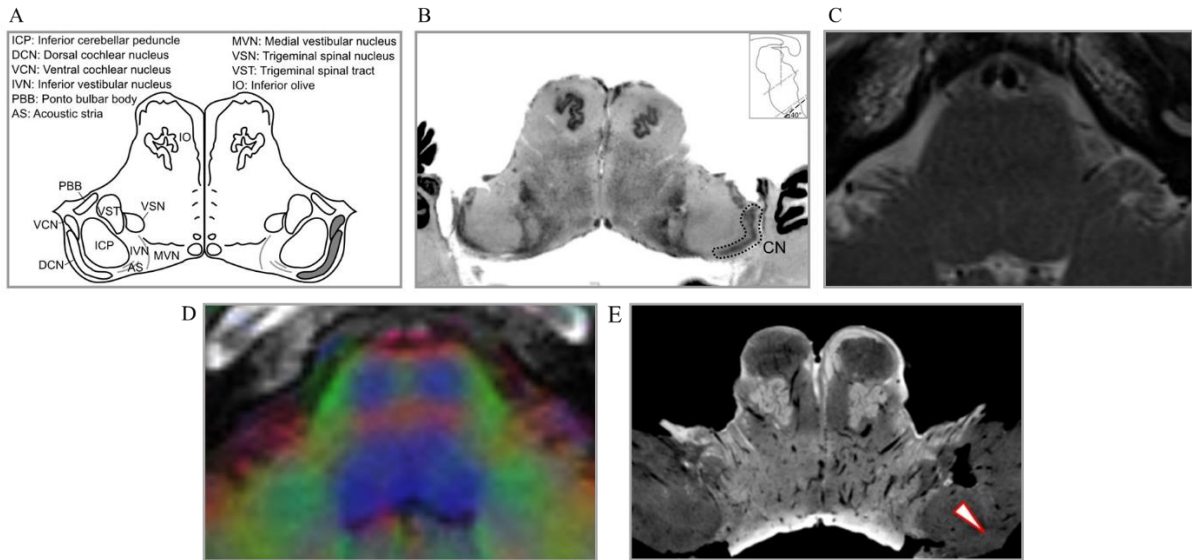


Figure 2.9 | Contrast of subcortical nuclei obtained using different MRI techniques and field strengths. (A) Diagram of axial slice at the level of the pontomedullary junction showing the location of the dorsal cochlear nucleus (DCN) and ventral cochlear nucleus (VCN) (Moore, 1987; Sitek et al., 2019). (B) Histology sample of axial slice showing the same region depicted in (A) (Hawrylycz et al., 2012). The cochlear nucleus (CN) is clearly visible and easily identified. (C) Anatomical contrast provided by a T2-weighted image obtained from a 3T Siemens scanner (voxel size = $0.5 \times 0.5 \times 2.0 \text{ mm}^3$). The cochlear nucleus is not readily identifiable at this resolution. (D) Panchromatic sharpened T1-weighted image (Dhollander et al., 2015), which shows the additional contrast information obtained from the dMRI image. Colours represent the local orientation of the subcortical pathways shown in this slice: Left-right = red, anterior-posterior = green, and superior-inferior = blue. (E) Superior anatomical contrast provided by a T2-weighted image obtained from a 7T scanner ($100\mu\text{m}$ resolution). Data was acquired from an *ex-vivo* brainstem sample. Arrow indicates the location of the cochlear nucleus.

Secondly, limitations in the spatial resolution of the dMRI data is another factor that affects the ability to reconstruct the auditory tracts of the brainstem. In the clinical diagnostic setting, the ability to acquire high spatial resolution data is limited by the available scanning time. As discussed, dMR images are therefore typically acquired with an isotropic voxel size of $\sim 2.5 \text{ mm}$. At this resolution, tract reconstructions of brainstem fascicles can be artificially inflated and contain more false positives compared to data obtained with higher spatial resolutions i.e. $\sim 0.3 \text{ mm}^3$ (Ford et al., 2013). This issue arises, in part, because tractography algorithms are not able to identify between different tracts that traverse a single voxel (i.e. with dMRI data obtained at a lower spatial resolution a single voxel is more likely to contain fibres from several different tracts; (Alexander, 2005; Mori & Tournier, 2013)). Therefore, tracking algorithms can easily deviate from the correct course and end up mapping a neighbouring tract. To complicate the matter further, other acquisition parameters such as the signal-to-noise ratio, the angular resolution, and the diffusion weighting(s) of the acquired imaging data are also likely to have a significant impact in the accuracy of the tract reconstruction (Alexander et al., 2002; Lin et al., 2001). For a detailed review of the literature including methodological considerations and limitations of diffusion-based tractography methods for the study of the auditory brainstem please refer to Zanin et al. (2019); Appendix 1.

Finally, there is a notable paucity of reference standards (also known as ground truth data) that can be used to validate tractography experiments of the auditory brainstem. Typically, tracer studies are considered the “gold standard” to determine white matter connectivity, however, while tracer experiments have been used to study the accuracy of tractography algorithms for other sensory and motor systems (Dauguet et al., 2007; Dyrby et al., 2007; Schaeffer et al., 2018), no such studies currently exist for the auditory brainstem pathways. Furthermore, tracer experiments are not without their limitations. For instance, studies have shown that the accuracy of tracer methods are highly dependent on factors such as staining sensitivity, tracer uptake, and distortions generated during image processing (Schilling et al., 2019). Moreover, due to their invasive nature, all tracer experiments are based on animal models, which are generally close approximations to human anatomy, however, significant differences can exist depending on the animals being studied (Moore, 1987; Thomas et al., 2014). Since tracer experiments are virtually impossible for human subjects, validating the accuracy of tractography algorithms against post-mortem histologic or dissected specimens has significant potential. For example, Maffei et al. (2018) used micro-dissection of post-mortem *ex-vivo* human brain specimens to investigate the validity of CSD-based probabilistic tractography in reconstructing the tracts of the acoustic radiations. Results showed that while CSD-based tractography captured the general profile of the acoustic radiations the algorithm also produced many false-positive tract reconstructions not observed in the dissected specimens. Similar comparisons for the subcortical auditory tracts are necessary to ensure tractography algorithms are mapping tracts accurately. Overall, however, the paucity of tractography validation studies is not only an issue for studying the auditory brainstem but remains a significant limitation for tractography-based research in general (Aydogan et al., 2018; Maier-Hein et al., 2017; Thomas et al., 2014).

2.6 Conclusion

The SS3T-CSD-based probabilistic tractography algorithm, with manually delineated ROIs and exclusion regions, provided the most plausible and anatomically accurate tract reconstruction of the auditory brainstem pathways compared to DTI-based tractography and ACT. Furthermore, tract reconstructions were consistent for all three participants in this study, indicating relatively good replicability of results regardless of age. The ability to reconstruct the tracts running between the VIII nerve and inferior colliculi enables these structures to be studied qualitatively and also potentially quantitatively. This in turn, may provide unique insights into possible changes occurring in these pathways in different clinical populations. As such, future studies will benefit from tracer studies and/or *ex-vivo* human specimens to validate the ability and accuracy of tractography algorithms in mapping the many connections of the subcortical auditory nuclei.

Chapter 3: Reduced fibre density in the auditory brainstem white matter tracts in X-linked auditory neuropathy

3.1 Preface

This manuscript has been submitted to *Ear and Hearing* and is currently under review. This chapter includes both the journal specific headings of the manuscript and its references.

Zanin, J., Dhollander, T., Rance, G., Yu, L., Lan, L., Wang, H., Lou, X., Connelly, A., Nayagam, B., Wang, Q. (Under review). Fibre specific changes in white matter microstructure in individuals with X-linked auditory neuropathy. *Ear and Hearing*.

3.2 Abstract

Objectives: Auditory neuropathy is the term used to describe a group of hearing disorders, in which the hearing impairment occurs as a result of abnormal auditory nerve function. While our understanding of this condition has advanced significantly over recent years, the ability to determine the site of lesion and the extent of dysfunction in affected individuals remains a challenge. To this end, we investigated potential axonal degeneration in the white matter tracts of the brainstem in individuals with X-linked auditory neuropathy. We hypothesised that individuals with X-linked auditory neuropathy would show focal degeneration within the VIII nerve and/or auditory brainstem tracts, and the degree of degeneration would correlate with the extent of auditory perceptual impairment.

Design: This was achieved using a higher-order diffusion MRI-based quantitative measure called Apparent Fibre Density as obtained from a technique called Single-Shell 3-Tissue Constrained Spherical Deconvolution and analysed with the fixel-based analysis framework. Eleven subjects with genetically confirmed X-linked auditory neuropathy and eleven normal hearing controls were assessed using behavioural and objective auditory measures. Diffusion MRI data was also collected for each participant.

Results: Fixel-based analysis of the brainstem region showed that subjects with X-linked auditory neuropathy had significantly lower Apparent Fibre Density in the VIII nerve compared to controls, consistent with axonal degeneration in this region. Subsequent analysis of the auditory brainstem tracts specifically showed that degeneration was also significant in these structures overall. The Apparent Fibre Density findings were supported by objective measures of auditory function, such as auditory brainstem responses, electrocochleography, and otoacoustic emissions, which showed VIII nerve activity was severely disrupted in X-linked auditory neuropathy subjects while cochlear sensory hair cell function was relatively unaffected. Moreover, Apparent Fibre Density results were significantly correlated with temporal processing ability (gap detection task) in affected subjects, suggesting that the

degree of VIII nerve degeneration directly impacts the ability to resolve temporal aspects of an acoustic signal. Auditory assessments of sound detection, speech perception, and the processing of binaural cues were also significantly poorer in the X-linked auditory neuropathy group compared to the normal hearing controls.

Conclusions: The results of this study suggest that the diffusion MRI-based measure of Apparent Fibre Density may provide a useful adjunct to existing auditory assessments in the characterisation of the site of lesion and extent of dysfunction in individuals with auditory neuropathy. Additionally, the ability to determine the degree of degeneration has the potential to guide rehabilitation strategies in the future.

3.3 Introduction

The term ‘auditory neuropathy’ refers to a group of hearing disorders, which are characterised by a disruption in the neural firing patterns of the auditory nerve (absent or severely disordered auditory brainstem responses (ABRs)) in the presence of normal cochlear outer hair cell function, which is confirmed by extant otoacoustic emissions and/or cochlear microphonics (Starr *et al.*, 1996; Starr *et al.*, 2010). The inability to record an ABR in affected individuals is attributed either to a disruption in the synchrony of neural firing (dyssynchrony) and/or a lack of neural elements within the auditory nerve and brainstem (deafferentation) (Starr *et al.*, 1996; Zeng *et al.*, 2005). Suggested pathophysiological mechanisms involve both pre-synaptic and post-synaptic structures of the auditory system (Rance and Starr, 2015). Perceptual consequences of impaired auditory neural firing patterns include deficits in the ability to encode temporal (timing) cues, discriminate speech, localise sounds, and identify auditory signals in the presence of background noise (Starr *et al.*, 1991; Starr *et al.*, 1996; Zeng *et al.*, 2005; Rance and Starr, 2015). Although such deficits can also occur in cochlear sensory hearing loss, individuals with auditory neuropathy perform disproportionately poorer compared to hearing-matched peers (Zeng *et al.*, 2005; Zeng and Liu, 2006b; Rance *et al.*, 2008; Rance *et al.*, 2012b).

There is a considerable range of aetiologies responsible for auditory neuropathy, including congenital and acquired conditions. Many have a genetic basis and the identification of the genes involved has helped to identify lesion sites and contribute to a better understanding of the underlying mechanisms involved in pathogenesis (Santarelli, 2010; Moser and Starr, 2016). Additionally, many of the identified genetic variants have been linked to syndromic and non-syndromic neurodegenerative disorders. One such condition is X-linked auditory neuropathy (AUNX1), a non-syndromic hereditary condition, characterised by an onset of hearing difficulties during adolescence (Wang *et al.*, 2003). To date, no prevalence studies have been conducted and the condition has only been reported in individuals of Chinese descent, however, more cases may be discovered as the use of genetic testing in the diagnosis of auditory neuropathy becomes more widespread (Wang *et al.*, 2003; Santarelli, 2010; Manchaiah *et al.*, 2011). Individuals with the condition present with audiological test results consistent with the auditory neuropathy phenotype. That is, affected individuals have absent or severely disordered ABRs,

normal otoacoustic emissions, absent acoustic reflexes, impaired speech perception ability, and elevated hearing thresholds (Wang *et al.*, 2003; Wang *et al.*, 2006; Zong *et al.*, 2015). The neural potentials of the inner hair cells and auditory nerve have also been studied using electrocochleography and findings are indicative of a lesion site affecting the auditory nerve afferent fibres, while inner hair cell function is spared (Zong *et al.*, 2015). Furthermore, auditory findings are supported by structural MRI results of the internal auditory canal showing that a proportion of affected individuals develop cochlear nerve hypoplasia (Zong *et al.*, 2015).

In-line with the developing trend to characterise the genetic basis of the auditory neuropathy phenotype, Zong *et al.* (2015) have identified mutations in the *AIFM1* gene to be responsible for AUNX1. The gene *AIFM1* has been mapped to chromosome 23q27.3 and codes for the protein apoptosis-inducing factor 1, which is involved in maintaining normal mitochondrial function (Susin *et al.*, 1999; Hangen *et al.*, 2010; Hangen *et al.*, 2015). The connection between mitochondrial dysfunction and auditory neuropathy is not unique to AUNX1 (Cacace and Pinheiro, 2011). In numerous other neurodegenerative conditions, such as Friedreich's ataxia (Cacace *et al.*, 1983; Rance *et al.*, 2008; Santarelli *et al.*, 2015a), Mohr-Tranebjaerg syndrome (Merchant *et al.*, 2001, Wang *et al.*, 2019), and CMT2 (Starr *et al.*, 2003; Züchner *et al.*, 2004; Rance *et al.*, 2012b), mitochondrial dysfunction has been shown to cause axonal degeneration and associated demyelination of the auditory nerve, manifesting in the auditory neuropathy phenotype. Furthermore, peripheral sensory structures of the auditory system, such as the outer and inner hair cells, have been shown to remain largely intact in these conditions. Supporting evidence, however, is not easily obtainable, with post-mortem histopathological studies of human temporal bones being the main source of validation (Spoendlin, 1974; Hallpike *et al.*, 1980; Merchant *et al.*, 2001; Starr *et al.*, 2003; Bahmad *et al.*, 2007).

Recently, Rance and Starr (2015) have proposed the use of diffusion-weighted magnetic resonance imaging (dMRI) analysis techniques to gain insights into the microstructure of the auditory tracts *in-vivo* in individuals with auditory neuropathy. To date, no studies have attempted to use such techniques to characterise changes in white matter tract architecture in affected individuals. The paucity of research is likely explained by the complexity of the auditory tracts, which are relatively thin (small tract dimensions) and run a circuitous path from the cochlea to the auditory cortex with many adjacent and crossing tracts from other neural networks (Pickles, 2012). Until recently many of the available dMRI analysis techniques could not cope with such complex fibre arrangements rendering the ability to study such tracts problematic (Maffei *et al.*, 2018; Zanin *et al.*, 2019). Although significant issues remain (refer to Zanin *et al.* (2019) for review), new dMRI acquisition methods in conjunction with higher-order diffusion models of microstructure, such as constrained spherical deconvolution techniques, provide more accurate representations of underlying white matter architecture than the widely adopted diffusion tensor model (Tournier *et al.*, 2007; Farquharson *et al.*, 2013; Jones *et al.*, 2013). Furthermore, the advent of these higher-order models has enabled the development of algorithms capable of

quantifying and comparing degenerative changes in specific fibre populations. One such technique is a fixel-based analysis ('fixel' refers to a specific fibre population within a voxel) introduced by Raffelt *et al.* (2017). The fixel-based analysis framework can be used to investigate tract-specific changes in microstructure and macrostructure, which are typically assessed by a metric called "Apparent Fibre Density" (AFD; Raffelt *et al.* (2012b)). This technique has been used to study several neurodegenerative conditions, including motor neuron disease (Raffelt *et al.*, 2012b), multiple sclerosis (Gajamange *et al.*, 2018), and Alzheimer's disease (Mito *et al.*, 2018), in which tract-specific deteriorations within the brain have been successfully characterised.

In this work, we apply a fixel-based analysis to study the tracts of the auditory brainstem. The aim of this study was to investigate the site of lesion and pathophysiological mechanism(s) underpinning the hearing deficits in individuals with a genetically characterised type of auditory neuropathy known as AUNX1. The white matter microstructure of the auditory brainstem tracts in affected individuals was assessed using measures of AFD as part of the fixel-based analysis framework (Raffelt *et al.*, 2012b; Raffelt *et al.*, 2017). It was hypothesised that individuals with AUNX1 would show a reduction in AFD within the auditory brainstem tracts consistent with the assumed pathophysiological mechanism of focal axonal degeneration of the vestibulocochlear VIII cranial nerve. Furthermore, it was also hypothesised that the degree of axonal degeneration would correlate with the auditory perceptual impairment in affected individuals.

3.4 Materials and methods

3.4.1 Participants

Eleven AUNX1 subjects and eleven normal hearing controls participated in the study. Age at assessment ranged from 17 to 55 years. A two-sample *t*-test did not show a significant difference in the ages of the AUNX1 subjects compared to controls (AUNX1: 33.8 ± 12.8 years; controls: 28.4 ± 6.7 , $P = 0.23$). Demographic, clinical features and audiological details for the AUNX1 subjects are presented in Table 3.1. All subjects with AUNX1 showed no signs of speech production disorders and each participant reported the onset of their hearing difficulties occurred post-lingually. Linkage analysis mapped the AUNX1 locus to chromosome Xq23-q27.3, and further identified the causal AIFM1 gene by whole exome sequencing (Wang *et al.*, 2006; Zong *et al.*, 2015). Furthermore, none of the subjects with AUNX1 reported unsteadiness, areflexia or numbness of their extremities, which are common symptoms of peripheral sensory neuropathy and have reportedly occurred in later stages of AUNX1 disease progression (Zong *et al.*, 2015). All participants were recruited through the Department of Otolaryngology, Head and Neck Surgery at the People's Liberation Army General Hospital (PLAGH 301; Beijing, China). Ethics approval was obtained through the Institutional Review Board of the Ethics Committee of PLAGH. The study protocol was undertaken in strict accordance to the ethical standards defined in the 1964 Declaration of Helsinki. All participants received information regarding the nature

and purpose of the study after which they provided written informed consent and were eligible to withdraw at any time.

Table 3.1 | Clinical, demographic and audiological data from the AUNX1 group

Subjects	Gender	Age at assessment (years)	Age at onset (years)	Genetic details	Vestibular dysfunction*	Audiometry (left/right)				Ear tested	Gap (ms)
						4FA (dB HL)	Acoustic reflexes	OAEs	ABR Wave V Latency (ms)		
1	M	18	11	c.1394C>T (p.A465V)	No	43/33	Abs/Abs	+/+	Abs/Abs	Right	30
2	M	18	14	c.1678T>C (p.Y560H)	No	46/41	Abs/Abs	+/+	Abs/Abs	Right	11
3	M	23	14	c.1264C>T (p.R422W)	Yes	34/36	Abs/Abs	+/+	Abs/Abs	Left	22
4	M	25	11	c.1773C>G (p.I591M)	Yes	63/48	Abs/Abs	+/+	Abs/Abs	Right	12
5	M	24	9	c.1492G>A (p.V498M)	No	50/63	Abs/Abs	+/+	Abs/Abs	Left	19
6	M	39	17	c.1030C>T (p.L344F)	No	38/50	Abs/Abs	+/+	Abs/Abs	Left	29
7	M	40	11	c.1265G>A (p.R422Q)	Yes	55/38	Abs/Abs	+/+	Abs/Abs	Right	30
8	M	43	16	c.1030C>T (p.L344F)	No	34/30	Abs/Abs	Abs/Abs	Abs/Abs	Right	5
9	M	42	18	c.1264C>T (p.R422W)	No	49/39	Abs/Abs	+/+	Abs/Abs	Right	18
10	M	47	14	c.1265G>A (p.R422Q)	Yes	49/51	Abs/Abs	+/+	Abs/Abs	Left	31
11	M	56	16	c.1265G>A (p.R422Q)	Yes	58/59	Abs/Abs	+/+	Abs/Abs	Left	23

*Vestibular dysfunction as determined by assessments of semicircular canal and/or otolith function (i.e. calorics, video head impulse testing, and vestibular evoked myogenic potentials). Abs = absent; 4FA = four frequency average (average thresholds at 0.5, 1, 2, and 4 kHz); ABR = auditory brainstem response.

3.4.2 Standard audiological assessments

Hearing thresholds were assessed at octave frequencies from 250 Hz – 8 kHz (Madsen Astera audiometer). Testing was performed in a sound-attenuating booth. A four-frequency average hearing level (4FA; 0.5, 1, 2, and 4 kHz) was subsequently calculated, which was used for further analyses. Tympanometry and acoustic reflex thresholds were assessed using a Gradson-Stadler TympStar impedance audiometer. Tympanograms were categorised according to the classification system developed by Jerger (1970). Middle ear muscle acoustic reflex thresholds were measured ipsilaterally and contralaterally to the stimulated ear at octave frequencies from 500 Hz – 4 kHz. Thresholds were considered in the normal range if they were present at ≤ 100 dB hearing level (HL) (Silman and Gelfand, 1981; Gelfand *et al.*, 1990), and absent if there was no repeatable response at an intensity > 110 dB HL.

3.4.3 Otoacoustic emissions

Distortion product otoacoustic emissions were recorded from each participant at 1, 2, 3, 4, 6, and 8 kHz using a Madsen Capella Cochlear Emissions analyser. Stimulus tones, f1 and f2, were presented at intensity levels of 65 dB SPL and 55 dB SPL, respectively. The f2/f1 ratio was maintained at 1.21. An otoacoustic emissions response amplitude of ≥ 6 dB for three consecutive frequency bands was required for a response to be considered present.

3.5 Speech perception

3.5.1 Speech perception in quiet

Open-set speech perception testing in quiet was performed using the Chinese Definition Testing Monosyllables list (Wang *et al.*, 2003; Ji *et al.*, 2011a; Ji *et al.*, 2011b). Each list consists of 50 monosyllabic words, which were presented monaurally, 30 dB above each participant's 1- and 2 kHz hearing threshold average. Participants were asked to repeat each word after they heard it and only responses that contained the consonant, vowel and tone of each word were recorded as correct. Words were presented via ER-3A insert phones and scored in real-time by a Chinese Mandarin-native speaking audiologist. Subsequently, a percentage score was calculated based on the number of words correctly identified.

3.5.2 Binaural speech perception

A Chinese Mandarin binaural speech perception assessment was developed to investigate the listener's ability to make use of binaural cues (differences in interaural timing and intensity) to perceive a target sentence in the presence of spatially separated babble. Similar assessments have been developed in English (Dirks and Wilson, 1969; Gelfand *et al.*, 1988; Cameron and Dillon, 2007), however, there is a paucity of available tests in Mandarin.

3.5.2.1 Stimuli

The Mandarin speech perception (MSP) sentence material was used as target speech (Fu *et al.* (2011); <http://www.tigerspeech.com/msp/msp.html>). Each MSP list comprises of ten phonemically balanced sentences, which consist of seven words typically encountered in day-to-day conversations. Chinese Mandarin 4-talker babble, developed by Xi *et al.* (2012), was used as competing noise.

Stimuli were presented through two Behringer MS16 active studio monitor loudspeakers in the free-field. Testing was conducted in a sound attenuating room. The stimuli were played through the on-board soundcard (Conexant ISST) of a Hewlett Packard laptop using the audio software Audacity (version 2.2.2; <https://sourceforge.net/projects/audacity>).

3.5.2.2 Assessment

This assessment compared the ability to accurately perceive speech in noise when the listener had no binaural difference cues available to them (i.e. the target speech and 4-talker babble noise were both presented from the same direction) to when binaural cues were available (target speech was spatially separated from the noise source by an azimuth of $\pm 90^\circ$). Binaural speech perception testing was completed using two test conditions in which the loudspeaker configurations varied (Figure 3.1). A sound level meter was used to ensure the 4-talker babble reached the participant at 65 dB SPL. Both the target speech and 4-talker babble were presented to the participant simultaneously for both test conditions.

The procedure for the assessment is shown in Figure 3.1. (A) The target speech (MSP sentence material) was initially adjusted to reach the participant at 75 dB SPL, while the babble was maintained at 65 dB SPL (+10 dB signal-to-noise ratio). The participant was asked to repeat each sentence as accurately as possible and a native Mandarin-speaking audiologist scored each word. Words were marked as correct only if they were repeated without any errors. The signal-to-noise ratio was adjusted by reducing the target speech until the participant scored $\leq 50\%$ for an entire list. (B) The target speech loudspeaker was then moved to an azimuth of $\pm 90^\circ$ to the participant's better hearing ear (based on their 4FA). A sound level meter was used to confirm the signal-to-noise ratio had not changed from the previous speaker configuration. The participant was then asked to repeat another MSP list in the presence of the 4-talker babble. As before, the percentage of words correctly repeated was calculated and recorded. Performance was measured as the difference in speech perception scores between the two test conditions. This percentage difference score was used to evaluate the advantage gained from spatially manipulating the direction of the target speech.

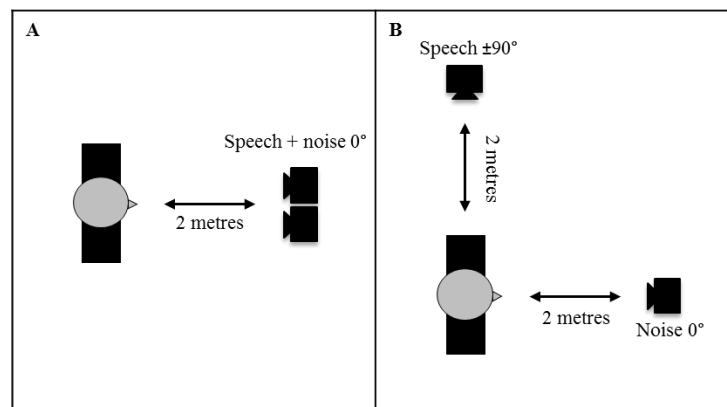


Figure 3.1 | Binaural speech perception assessment test setup. For both loudspeaker configurations the participant remained seated. (A) Two loudspeakers were positioned at head height, two metres from the participant at an azimuth of 0° . One loudspeaker presented the target speech (Mandarin Speech Perception sentence material (Fu *et al.*, 2011)) and the other presented the competing noise, 4-talker babble (Ai *et al.*, 2016). (B) After the participant completed the assessment with the setup shown in (A), the loudspeaker presenting the target speech was positioned at an azimuth of $\pm 90^\circ$ to the participant's better hearing ear (based on the 4-frequency average hearing threshold). The distance between the participant and the loudspeakers remained unchanged as did the participant's position and head orientation. The position of the loudspeaker presenting the 4-talker babble also remained in the same position, at 0° azimuth to the participant.

3.5.3 Electrophysiology

3.5.3.1 Auditory Brainstem Responses

Auditory brainstem responses were recorded using the Intelligent Hearing System (IHS) SmartEP system (Intelligent Hearing Systems, Inc., Miami, FL, U.S.A). Alternating polarity 100 μ s clicks were presented to each ear individually at 100 dB above normal hearing level (nHL) through ER-3A insert earphones. A stimulus presentation rate of 8 Hz was used, and two waveforms were obtained from each ear with 2000 post-stimulus sweeps for each recording. Potentials were recorded using silver/silver

chloride surface electrodes (high forehead to earlobe, ipsilateral to the stimulated ear). Two experienced audiologists carried out the *post hoc* analysis of the waveforms, which involved the identification of ABR waves I, III and V. Furthermore, for those individuals with absent ABRs the waveforms were separated into rarefaction and condensation clicks and the cochlear microphonic was identified where present, as per Rance *et al.* (1998).

3.5.3.2 Electrocochleography

Extratympanic electrocochleography (ECochG) was performed on ten out of the 11 subjects with AUNX1 and seven out of the 11 normal hearing controls. Responses were recorded using the IHS SmartEP equipment. The active ball-electrode was covered in conductive paste and placed onto the tympanic membrane. The reference and ground silver/silver chloride surface electrodes were placed on the ipsilateral earlobe and forehead, respectively. An ER-3A insert earphone was used to deliver the stimulus, a 100 dB nHL alternating polarity 100 μ s click, which was presented at a rate of 7.1 Hz. Potentials were averaged over 500 trials. Two experienced audiologists carried out the *post hoc* analysis of the waveforms, which involved the identification of the cochlear microphonic, summing potential and compound action potential.

3.5.4 Auditory temporal processing

Temporal processing ability was evaluated using a gap detection in noise task. The assessment sought to establish the shortest silent period a participant could identify in a burst of broadband noise on 70.7% of occasions. An adaptive, three-alternative, forced-choice procedure was used, with a two-down and one-up approach described in detail by Levitt (1971). The background stimuli were bursts of filtered broadband noise (0-6 kHz) and the target stimuli were identical except they contained a short period of silence which lasted between 1 and 60 ms (Rance *et al.*, 2009). Stimuli were delivered via ER-4 insert earphones to the better hearing ear (based on each participant's 4FA) at a sensation level ≥ 30 dB above their 4FA threshold.

3.5.5 Image acquisition and pre-processing

MRI data was acquired using a 3 Tesla Siemens Magnetom Skyra system, with a 32-channel head coil receiver. Diffusion-weighted imaging was performed using a twice refocused, spin-echo, echo planar imaging sequence with the following parameters: repetition/echo time = 8400/110 ms, 2.5 mm isotropic voxels, field of view = 240 x 240 mm, matrix size = 96 x 96, and acceleration factor = 2. A total of 64 diffusion-weighted images ($b = 3000$ s/mm²) and 8 non-diffusion weighted images ($b = 0$ s/mm²) were acquired with a total acquisition time of ~10 minutes. Diffusion-weighted images were visually assessed for motion artefacts (assessment was based on the presence of 'venetian blind artefacts') and datasets were excluded if excessive motion was identified. Additionally, a high resolution T2-weighted image (voxel size = 0.5 x 0.5 x 2.0 mm, repetition/echo time = 4750/101 ms, flip angle = 150°, slice thickness = 2 mm) was also acquired for each participant.

Pre-processing of dMRI data included denoising (Veraart *et al.*, 2016a; Veraart *et al.*, 2016b), removal of Gibbs-ringing artefact (Kellner *et al.*, 2016), eddy-current correction (Andersson and Sotiropoulos, 2016), and motion correction (Smith *et al.*, 2004). The spatial resolution of the dMRI data was upsampled using cubic b-spline interpolation to a voxel size of 2 mm³ (Raffelt *et al.*, 2012b).

Subsequently, single-shell 3-tissue constrained spherical deconvolution (SS3T-CSD; Dhollander and Connelly (2016)) was used to compute fibre orientation distributions (FODs) based on the 3-tissue (white matter, grey matter, and cerebrospinal fluid) group averaged response functions (Dhollander *et al.*, 2016). All 3 tissue compartments were then used to estimate a spatially smoothed varying multiplicative field for global and local (bias field) intensity normalisation. Computed FOD images from each participant were used to generate a study-specific, unbiased FOD template. Both linear and non-linear registration techniques were employed to facilitate this as described previously by Raffelt *et al.* (2012a) and Raffelt *et al.* (2011). Whole-brain probabilistic tractography (iFOD2 fibre tracking algorithm; Tournier *et al.* (2010)) was performed on the study-specific unbiased FOD template producing 20 million streamlines. To reduce reconstruction biases these 20 million streamlines were filtered down to 2 million using the spherical-deconvolution informed filtering of tractograms algorithm (Smith *et al.*, 2013).

Overall, pre-processing and processing steps were carried out using commands implemented within MRtrix3Tissue (<https://3tissue.github.io/>), a fork of Mrtrix3 (Tournier *et al.*, 2019). Some of the MRtrix3Tissue scripts, including commands used for eddy-current and motion correction, interfaced with an external software package: The Functional Magnetic Resonance Imaging of the Brain Software Library (FSL; Jenkinson *et al.* (2012)).

3.5.6 Fixel-based diffusion-weighted magnetic resonance imaging analysis:

Apparent Fibre Density

The fixel-based analysis was limited to the brainstem and corresponding cranial nerves for all participants. The brainstem region was manually demarcated on the population template. The metric of AFD was subsequently computed for each subject for all fixels contained within this brainstem mask. The AFD metric is computed from the FOD amplitude, which is proportional to the radial diffusion-weighted signal originating from the intra-axonal compartment of a fibre-bundle (Raffelt *et al.*, 2012b). As such, the fibre density measurement is sensitive to within-voxel microstructural changes and can be used to investigate group differences in the density of white matter tracts.

3.5.6.1 Auditory brainstem tract analysis

Segmentation of tracts connecting the auditory nerve and inferior colliculus, a major auditory nucleus of the midbrain, was achieved by manually delineating these regions on the study-specific template and tracking between them using the iFOD2 probabilistic tracking algorithm. This technique has been used

previously to delineate some of the auditory brainstem tracts in a single subject, as described by Zanin *et al.* (2019). In the current study generated tracts were subsequently converted into a fixel mask to enable AFD values to be computed for each participant.

3.5.7 Statistical analysis

Minitab (version 18.1 for Windows) was used to perform the statistical analyses, except those related to fixel-based morphometry. Two-tailed *t*-tests were used to determine statistical differences between the two groups for all auditory assessments, unless otherwise stated. The fixel-based statistical analysis was performed using the default parameters in the `fixelcfstats` command available within the MRtrix3Tissue software package. This command uses *Connectivity-based fixel enhancement*, which performs connectivity-based smoothing and statistical inference, as described in Raffelt *et al.* (2015). To determine which fixels were significantly different between cohorts, non-parametric permutation testing over 5000 permutations was employed and family-wise error-corrected (FWE) *p*-values were assigned to each fixel (Nichols & Holmes, 2002). Fixels which were significantly different between the cohorts (FWE-corrected *p*-value < 0.05) were visualised using the *mrview* tool in MRtrix3Tissue. Statistically significant fixels were colour-coded by significance level with $P_{\text{FWE}} > 0.05$ in black, $P_{\text{FWE}} \leq 0.01$ in orange/yellow, $P_{\text{FWE}} \leq 0.001$ in yellow/white.

3.6 Results

3.6.1 Fixel-based analysis of the diffusion magnetic resonance imaging data

Fixel-based analysis of the brainstem showed significantly lower AFD values within the fixels of the VIII nerve in the AUNX1 group compared to controls ($P_{\text{FWE}} < 0.001$; Figure 3.2). A quantitative comparison of the significant region identified using the fixel-based analysis, including data points for individual participants, is shown in Figure 3.3. No other fixels within the brainstem region were significantly different between the control and affected groups once results were corrected for multiple comparisons.

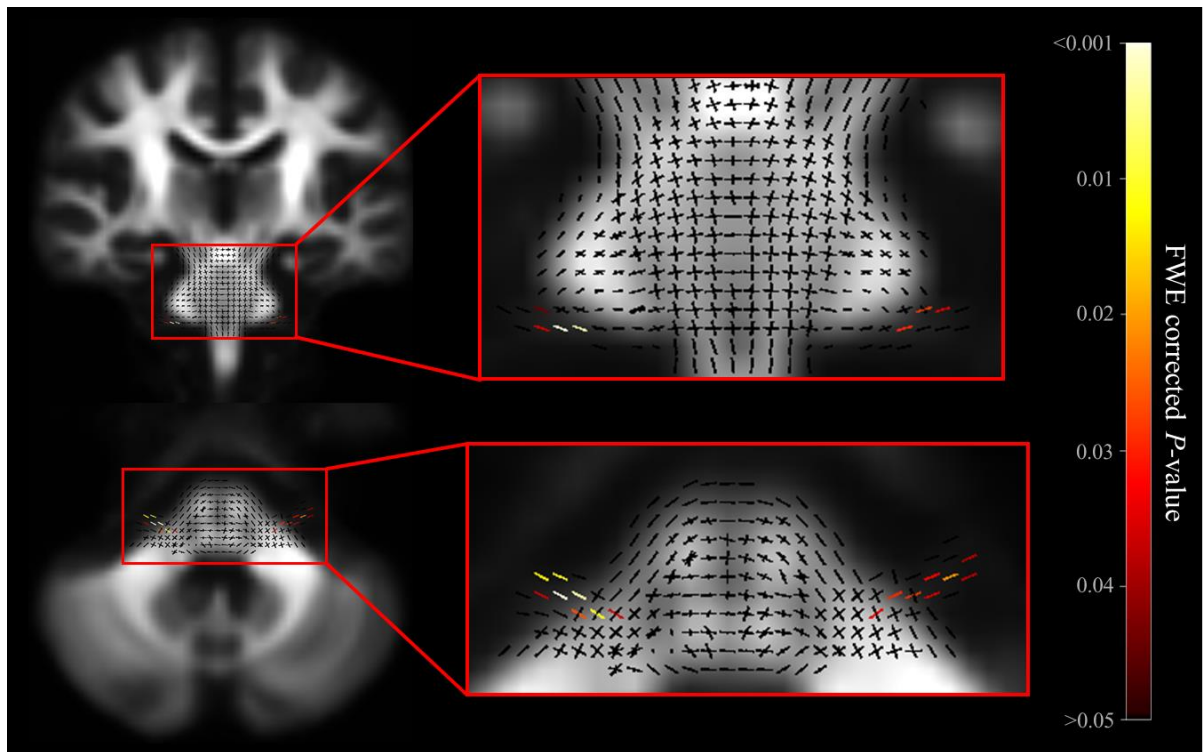


Figure 3.2 | Fixel-based analysis showing the white matter tract regions with significantly lower AFD in the AUNX1 subjects compared to controls. *Left:* Coronal (top) and axial (bottom) slice of the whole brain showing the region in which the fixel-based analysis was performed. *Right:* Magnification of brainstem region showing the location of the fixels which had significantly lower AFD values in the AUNX1 group compared to controls. These significant fixels are colour-coded based on family-wise error-corrected P -values and colours correspond to the bar shown on the right. The most significant fixels are coloured yellow/white ($P_{\text{FWE}} < 0.001$).

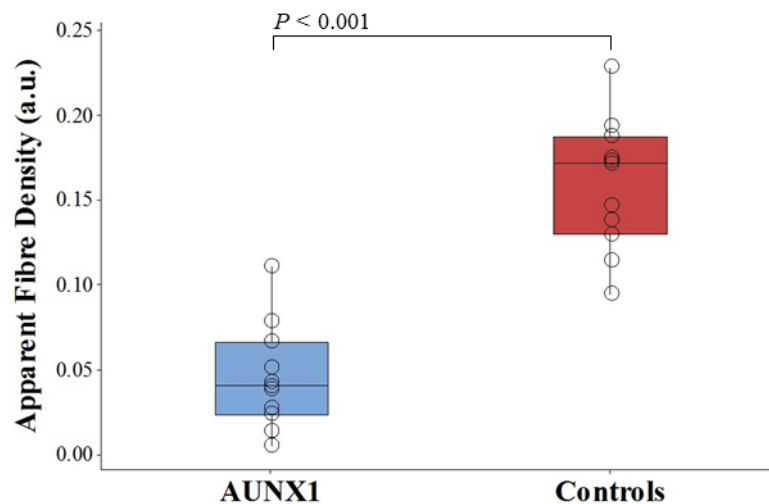


Figure 3.3 | AFD results. Quantification of the spread of AFD results in the regions that were identified as significant through the fixel-based analysis of the brainstem as illustrated in Figure 3.2. Unfilled circles represent AFD values for each subject. Apparent Fibre Density values are represented in arbitrary units (a.u.).

3.6.2 Auditory brainstem tract analysis

To determine whether axonal degeneration in AUNX1 extended beyond the auditory nerve, AFD values of the auditory brainstem tracts were also examined. To achieve this, probabilistic tractography was used to identify the tracts occurring between the auditory nerve and inferior colliculus (Figure 3.4). Apparent Fibre Density values were significantly reduced in these white matter tracts in the AUNX1 participants compared to the normal hearing controls (AUNX1: $0.33 \pm .03$; controls: 0.38 ± 0.04 , $P \leq 0.002$; Figure 3.4D).

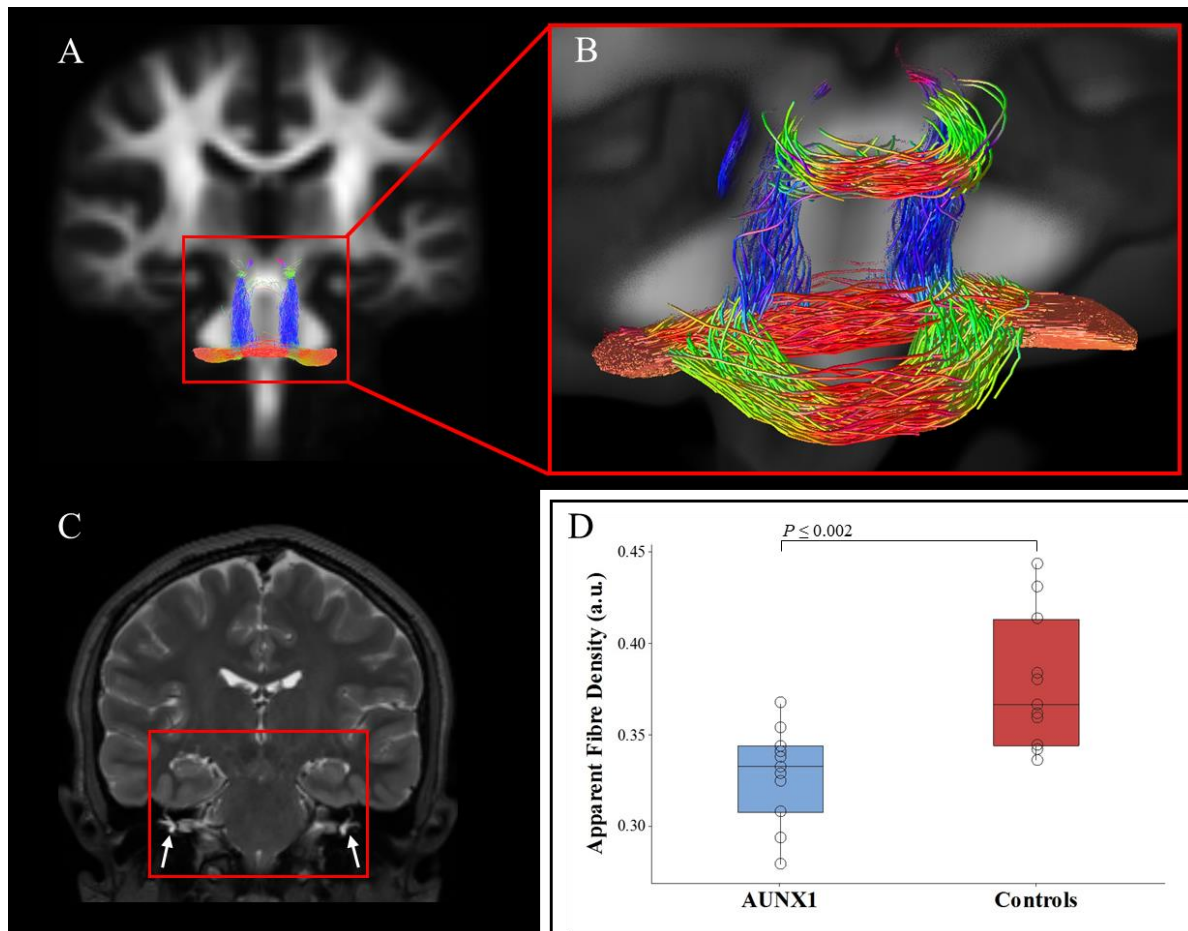


Figure 3.4 | Auditory brainstem tract analysis. (A) Shows a coronal slice of the tract of interest in the context of the whole-brain. The tract was produced by performing tractography between the auditory nerve and inferior colliculus bilaterally on the study specific template. (B) Magnification and 3-dimensional render of the auditory brainstem tracts, in which AFD values were compared between the AUNX1 subjects and normal hearing controls. Tract orientation is represented by the different colours and follows the directionally encoded colour convention; Left-right is red, anterior-posterior is green, and superior-inferior is blue. (C) Coronal T2-weighted MR image showing the corresponding area featured in (A). Both cochlea are visible in the T2-weighted image (arrows), however, the specific white matter tracts of the auditory brainstem are not. (D) Quantification of the spread of AFD results in the auditory brainstem tracts for the individuals with AUNX1 and normal hearing controls. AFD values are represented in arbitrary units (a.u.).

3.6.3 Hearing thresholds, acoustic reflexes and tympanometry

The AUNX1 group showed elevated hearing thresholds at all frequencies tested. The degree of hearing loss varied between affected individuals, however, an upward sloping audiometric configuration was a characteristic feature (Figure 3.5). In comparison, hearing thresholds for all the normal hearing controls were within the normal range (< 20 dB HL). Each octave frequency was significantly different between the two cohorts ($P \leq 0.002$) as were the four-frequency average hearing thresholds (AUNX1: 45.6 ± 8.7 dB HL; controls: 8.1 ± 5.2 dB HL, $P < 0.001$).

Middle ear muscle acoustic reflexes were absent at all test frequencies for the AUNX1 subjects and within normal limits for the controls (≤ 100 dB HL). Furthermore, all participants in the study had type A tympanograms, consistent with normal middle ear compliance.

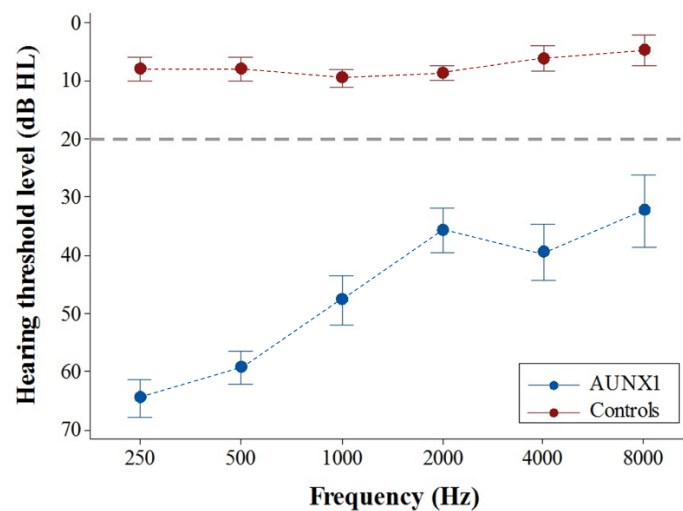


Figure 3.5 | Pure tone audiometry results for the AUNX1 subjects and normal hearing controls. Average hearing thresholds (± 1 standard error of the mean) at octave frequencies from 250 Hz to 8000 Hz are shown. The region above the grey dashed line represents the range of normal hearing. Each data point is significantly different between groups ($P \leq 0.002$).

3.6.4 Otoacoustic emissions

Distortion product otoacoustic emissions were present across the frequency range in all normal hearing and affected individuals except for one AUNX1 subject (see Table 3.1 for details).

3.6.5 Electrophysiology

3.6.5.1 Electrocochleography

Extratympanic ECoChG responses from subjects with AUNX1 showed marked differences compared to responses obtained from controls (Figure 3.6). Summating potentials were present in all but two AUNX1 subjects. Summating potential latencies (AUNX1: 0.9 ± 0.4 ms; controls: 0.8 ± 0.2 ms) and amplitudes (AUNX1: 0.2 ± 0.2 μ V; controls: 0.2 ± 0.1 μ V) were not significantly different between the two groups (latencies: $P = 0.55$; amplitudes: $P = 0.92$). The compound action potential was absent

bilaterally in half of the AUNX1 participants and the CAP amplitude was significantly lower compared to the controls in the remaining five (AUNX1: $0.2 \pm 0.2 \mu\text{V}$; controls: $0.8 \pm 0.4 \mu\text{V}$, $P = 0.01$). Where present, compound action potential latencies were similar between control and affected cohorts (AUNX1: $1.0 \pm 0.9 \text{ ms}$; controls: $1.5 \pm 0.2 \text{ ms}$, $P = 0.12$).

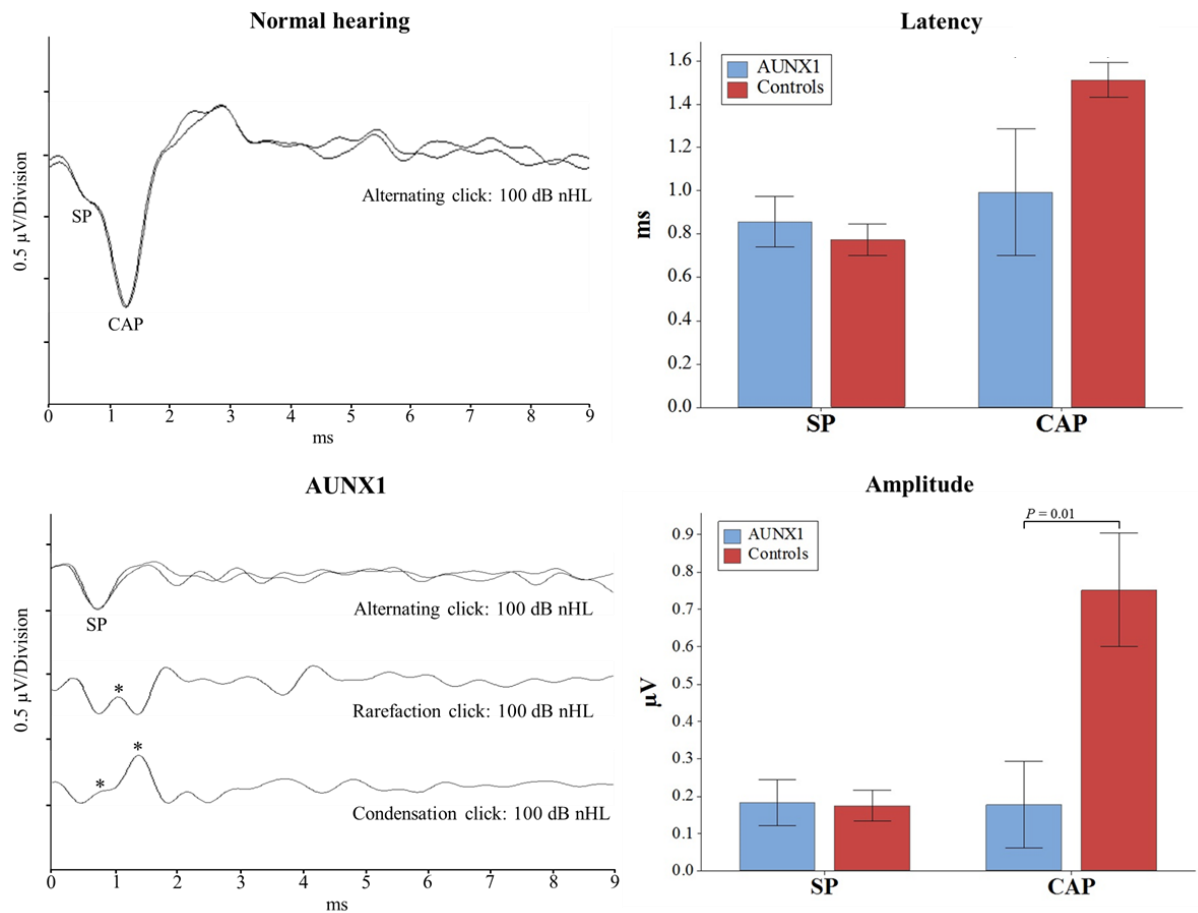


Figure 3.6 | Electrocochleography results. *Left:* Extratympanic ECoG waveforms obtained from one normal hearing control (top) and one representative AUNX1 subject (bottom). For the AUNX1 subject the asterisks mark the positive peaks of the cochlear microphonic, which are observable only in responses obtained from single polarity clicks (rarefaction/condensation). *Right:* Means and standard errors of the summing potential (SP) and compound action potential (CAP) peak latency (top) and amplitude (bottom) obtained from the AUNX1 group and normal hearing controls. The CAP amplitude was significantly lower in AUNX1 subjects.

3.6.5.2 Auditory brainstem response

Auditory brainstem responses were absent for all eleven AUNX1 participants (Table 3.1), while the cochlear microphonic, a potential corresponding to cochlear hair cell polarisation and depolarisation, was present in nine out of those 11 subjects. All control participants had repeatable ABRs with normal amplitudes and latencies.

3.6.6 Psychoacoustics: temporal resolution

The results of the gap detection task showed a significant difference between the two cohorts. On average, gap detection thresholds for subjects with AUNX1 were significantly longer (20.9 ± 8.8 ms) compared to controls (3.3 ± 1.0 ms, $P < 0.001$).

3.6.7 Speech perception

Word discrimination in quiet was significantly poorer in the AUNX1 group compared to the normal hearing controls (AUNX1: $28.8 \pm 27.4\%$; controls: $99.3 \pm 1.01\%$, $P < 0.001$; Figure 3.7A). Similarly, the ability to use interaural time and intensity differences to gain a perceptual advantage in the presence of spatially separated noise was significantly impaired in the AUNX1 cohort (Figure 3.7B). The perceptual improvement afforded to the listeners when the target speech and noise was spatially separated was $4.1 \pm 9.1\%$ for the AUNX1 subjects, whereas control participants gained an advantage of $47.5 \pm 15.4\%$ ($P < 0.001$).

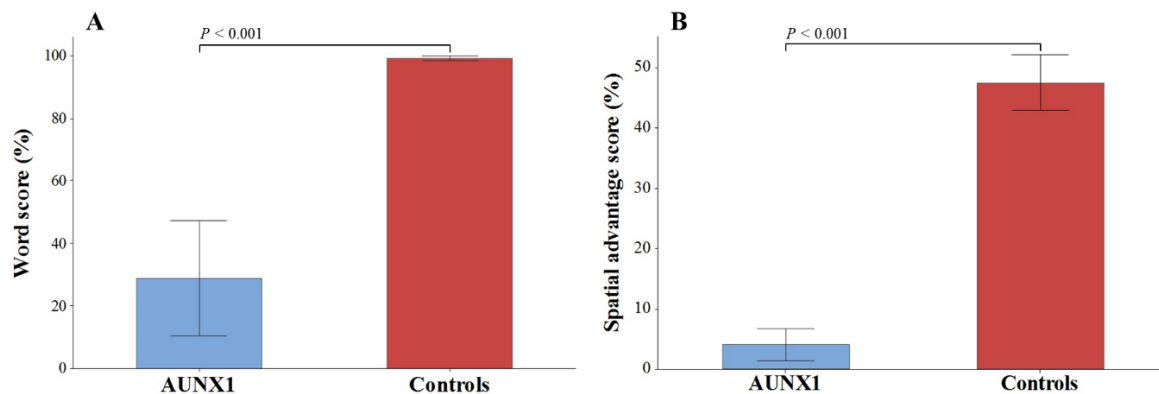


Figure 3.7 | Speech perception results. (A) Word-recognition scores in quiet for the AUNX1 subjects and normal hearing controls. (B) Spatial advantage scores for the AUNX1 subjects and controls. All data are the means $\pm$ 1 standard error of the mean.

3.6.8 Correlations between auditory measures

Pearson r correlation analyses were carried out to investigate the relationship between the auditory measures that were identified to be significantly different between the two groups (Table 3.2). This included the 4-frequency average hearing loss, speech in quiet, spatial advantage, temporal processing (gap detection), and CAP amplitude. For the individuals with AUNX1, speech perception in quiet was significantly correlated with hearing thresholds ($P = 0.02$), spatial advantage ($P = 0.02$), and gap detection ($P = 0.03$). That is, those individuals with the poorest speech perception ability also had the most significant hearing loss, most disrupted temporal processing ability and poorest binaural processing ability. No other significant correlations between the auditory measures were found.

Table 3.2 | Pearson *r* correlations showing relationship between auditory measures for the AUNX1 group

	4FA HL	Speech in quiet	Spatial advantage	Gap detection	CAP amplitude
Speech in quiet	$r = -0.700$ (0.02)				
Spatial advantage	NS (0.30)	$r = 0.706$ (0.02)			
Gap	NS (0.66)	$r = -0.659$ (0.03)	NS (0.2)		
CAP amplitude	NS (0.22)	NS (0.40)	NS (0.74)	NS (0.92)	

4FA HL = 4 frequency average hearing loss; r = correlation coefficient; NS = not significant. Significant P values are displayed in bold.

3.6.9 Correlations between auditory measures, AFD and clinical features

Pearson r correlation analyses were also performed to determine the relationship between the auditory measures, the AFD results and clinical features (age at assessment and disease duration) for the AUNX1 participants. Only the auditory assessments identified as being significantly different between the AUNX1 subjects and normal hearing controls were included in the analysis (Table 3.3). Results showed that AFD values were not correlated with any clinical features or auditory measures except for gap detection. AUNX1 subjects with poorer gap detection ability also had significantly lower AFD values in the VIII nerve region of the test ear ($P = 0.035$; Figure 3.8).

Table 3.3 | Pearson *r* correlations showing the relationship between auditory measures, AFD results and clinical features for the AUNX1 subjects (P values shown in brackets)

	Age at assessment	Disease duration	4FA HL	CAP amplitude	Gap detection*	Speech in quiet	Spatial advantage
AFD of significant regions	NS (0.803)	NS (0.729)	NS (0.651)	NS (0.817)	$r = -0.636$ (0.035)	NS (0.590)	NS (0.814)

*To determine the correlation between gap detection and AFD values, only the significant AFD values of each subject's test ear (see Table 3.1) were included in the correlation analysis. AFD = Apparent Fibre Density; 4FA HL = 4 frequency average hearing loss; r = correlation coefficient; NS = not significant. Significant P values are displayed in bold.

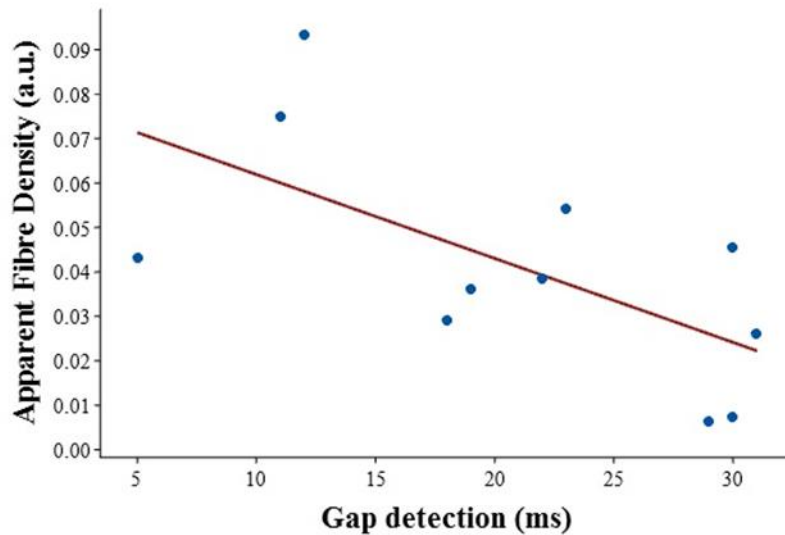


Figure 3.8 | Apparent Fibre Density vs. gap detection thresholds. Apparent Fibre Density values obtained from the VIII nerve region of the test ear and gap detection results for each individual with AUNX1. The line of best fit is shown in red ($r = -0.636$, $P = 0.035$)

3.7 Discussion

We present the first study that has applied the recently developed fixel-based analysis framework to investigate white matter microstructural changes within the brainstem. The findings of this study show that patients with AUNX1 have significant axonal degeneration throughout the white matter tracts of the auditory brainstem with the most extensive loss occurring in the VIII cranial nerve. Results are consistent with previously reported auditory and structural MRI findings, which suggest a post-synaptic lesion site affecting the auditory nerve afferent fibres (Wang *et al.*, 2003; Wang *et al.*, 2006; Zong *et al.*, 2015). Furthermore, we found that the degree of axonal degeneration of the VIII nerve was correlated with temporal resolution ability. Specifically, AUNX1 subjects with lower AFD values performed significantly poorer on the gap detection task. The results of the current study will be discussed in the context of (i) the lesion site and pathophysiological mechanism underlying the auditory neuropathy phenotype exhibited in AUNX1 (ii) auditory function as characterised by a comprehensive set of audiological assessments; and (iii) the relevance of the fixel-based analysis framework in the diagnosis and management of auditory neuropathy.

3.7.1 Site of lesion and pathophysiological mechanism in AUNX1

Objective measures of cochlear sensory hair cell function, acoustic reflex threshold testing, electrophysiological assessments and the AFD results were used in the current study to confirm the site of lesion in AUNX1. Normal outer hair cell function was confirmed in all but one AUNX1 subject by the presence of otoacoustic emissions. The subject with absent emissions (Table 3.1: subject #8) was older and had a longer disease duration than most of the other participants in the study. Therefore, the absent emissions in this individual are likely explained by normal age-related deterioration of the outer

hair cells, unrelated to AUNX1 disease progression. In general, it is not uncommon for otoacoustic emissions to deteriorate over time in individuals with auditory neuropathy (Starr *et al.*, 2001). Cochlear inner hair cell activation also appeared normal in the majority of AUNX1 subjects. This was evidenced by the fact that summing potential peak latencies and amplitudes were similar between the AUNX1 group and normal hearing controls. The compound action potential, however, was either absent or significantly reduced in amplitude, auditory brainstem responses featured no discernible waves/peaks, and acoustic reflexes were elevated beyond testing limits. Although these assessments are not specific to axonal degeneration, the results are consistent with a reduction in the number of auditory nerve afferent fibres being activated (Wang *et al.*, 1997; Starr *et al.*, 2003; Rance and Starr, 2015). Importantly, the AFD results showed distinct degeneration of white matter fibres within the internal auditory canal. Hence, the auditory-related findings support the AFD results and together they indicate a site of lesion affecting the axonal portion of the auditory nerve afferent fibres in AUNX1, while cochlear sensory function is spared.

The AFD results indicate the primary pathophysiological mechanism responsible for the auditory neuropathy phenotype in AUNX1 is the reduction of neural input caused by the degeneration of auditory nerve axons. Despite this evidence, it is likely that affected individuals also have a related loss of neural synchrony. For instance, axons undergoing degeneration can remain partially active and therefore produce inconsistent firing patterns when activated (Brown and Watson, 2002). It has also been suggested by Oertel *et al.* (2000) and Joris *et al.* (2004) that the convergence of multiple stochastic auditory nerve fibre inputs leads to enhanced temporal precision in the firing of the neurons within the cochlear nucleus of the auditory brainstem. Therefore, a loss of neuronal input could also reduce the temporal precision in the discharge of these neurons. Furthermore, due to the intricate relationship between axons and their myelin sheaths, it is unlikely that severe axonal loss occurs without any associated myelin sheath degeneration (Kalaydjieva *et al.*, 1998; Rapin and Gravel, 2003). In fact, demyelination features in many mitochondrial-related neurodegenerative conditions where it has a profound impact on neural conduction times (Dyck *et al.*, 1981; Starr *et al.*, 2003; Kovács *et al.*, 2004; Rance *et al.*, 2012).

The underlying pathomolecular mechanisms of axonal degeneration in mitochondrial disorders varies considerably. In most cases, failure of the mitochondria to maintain the energy requirements of the axon directly jeopardises its cellular integrity (Wallace, 1992; Beal *et al.*, 1993). Mutations in the *AIFM1* gene responsible for AUNX1 produces disruptions in mitochondrial respiratory chain complex I and oxidative phosphorylation (Hangen *et al.*, 2010; Sevrioukova, 2016). Both processes are vital to the formation of adenine triphosphate, the energy-carrying molecule used to drive many cellular processes, including the generation of nerve cell action potentials, propagation of nerve impulses, and the maintenance of myelin formation (Stevens and Fields, 2000; Stevens *et al.*, 2002). Defects of the mitochondrial respiratory chain are one of the most commonly inherited genetic disorders affecting

metabolism, with a reported incidence of 1/10,000 births (Wallace, 1992). In most of these disorders, axonal dysfunction is widespread and affected individuals often present with a host of other sensory and/or motor abnormalities. Further research is required to determine how such profound alterations in mitochondrial function can remain almost entirely specific to a particular sensory modality, such as the auditory system.

3.7.2 The effect of axonal degeneration on auditory function in AUNX1

All behavioural measures of auditory function, including sound detection, temporal processing (gap detection), binaural processing and speech perception were significantly impaired in the AUNX1 group compared to the normal hearing controls. In general, results were similar to those reported previously for individuals with other neurodegenerative conditions (Rance *et al.*, 2009; Rance *et al.*, 2012b; Rance *et al.*, 2012c; Santarelli *et al.*, 2015b). Out of all the auditory measures used in the current study, however, gap detection was the only assessment that showed a significant relationship to the degree of axonal degeneration in participants with AUNX1. Specifically, individuals with more severe VIII nerve deterioration, as characterised by the AFD metric, also had significantly poorer gap detection thresholds. Hence, the results suggest that the reduction in auditory neural input resulting from axonal degeneration likely diminishes the neural representation of the auditory stimulus within the central auditory nervous system, making it difficult for an affected individual to detect a difference in the presence of spontaneous neural activity (Zeng *et al.*, 1999; Zeng *et al.*, 2005). The results of the current study are supported by findings which show gap detection thresholds can be significantly elevated even in normal hearing listeners at reduced audibility levels (Nelson and Thomas, 1997; Zeng *et al.*, 2005). This temporal processing deficit, induced by a decrease in stimulus intensity, can be explained by the reduction of neural input; when the stimulus is presented at lower intensities fewer afferent fibres are activated, while neural synchrony remains unchanged (Paolini *et al.*, 2001). Compared to the other perceptual assessments used in this study, it is not immediately clear why the gap detection task was the only auditory measure significantly correlated with the degree of axonal degeneration. While it is possible that the ability to detect the presence of a gap in broadband noise is more sensitive to changes in auditory nerve density compared to the other behavioural auditory assessments used in this study, there is no direct evidence to support this assumption. Given the complex nature of speech, however, it is likely that speech perception ability is reliant on a greater subset of sub-cortical and cortical neurons of which regions responsible for temporal processing also play a significant part (Delgutte and Kiang, 1984; Frisina, 2001; Démonet *et al.*, 2015).

Speech perception ability was significantly impaired in all AUNX1 participants compared to the normal hearing control subjects. In quiet, and at reasonable sensation levels, AUNX1 participants were only able to correctly identify ~29% of the words that were presented to them. A significantly lower score than would be predicted from their hearing thresholds alone. Results are consistent with previously reported findings suggesting speech perception ability in auditory neuropathy is impaired irrespective

of the degree of hearing loss (Zeng and Liu, 2006a; Rance *et al.*, 2008; Rance *et al.*, 2012a). These findings are likely explained by the compromised temporal processing ability of the AUNX1 subjects. For instance, elevated gap detection thresholds can directly impact on the ability to distinguish voice onset times, the period that passes between the release of a stop consonant and the onset of voicing (Rance *et al.*, 2012c; Rance and Starr, 2015). Discrimination of voice onset times is essential to differentiate between voiced and unvoiced stop-consonants in both English and Mandarin (Rochet and Yanmei, 1991; Chao and Chen, 2008). Hence, it is likely that an inability to integrate and perceive these important speech cues contributes to the poor speech perception ability exhibited in AUNX1.

Binaural processing ability is also significantly impaired in AUNX1 compared to normal hearing controls. In general, both normal hearing listeners and listeners with cochlear sensory hearing loss have access to interaural timing and intensity cues (binaural cues), which can provide a perceptual advantage when listening to speech in the presence of spatially separated competing noise (Hawley *et al.*, 2004; Arbogast *et al.*, 2005; Glyde *et al.*, 2013). In this study, controls could consistently identify an average of ~48% more words when speech and noise were spatially separated, whereas AUNX1 subjects gained almost no benefit (~4% improvement in word recognition). Similar results have been reported for individuals with the auditory neuropathy phenotype due to Friedreich's ataxia and Charcot-Marie-Tooth (Rance *et al.*, 2012c). Again, loss of temporal precision within the central auditory nervous system likely accounts for the reduced capacity to use spatial cues to discriminate target speech from competing noise. Specifically, as discussed by Rance *et al.* (2012c), the disruption in interaural timing comparisons, required for the localisation of sounds, is likely to be the most significant contributor to the spatial-processing deficit experienced by AUNX1 subjects.

In regard to hearing thresholds, most individuals with AUNX1 have a distinctive audiometric configuration, in which low frequency thresholds are considerably poorer than the high frequencies (Wang *et al.*, 2003; Wang *et al.*, 2005; Zong *et al.*, 2015). While in cochlear sensory loss hearing thresholds are elevated predominantly due to damaged inner and outer hair cells, this is unlikely to be the case for individuals with AUNX1. Especially given the assessments of inner and outer hair cell integrity (summating potential amplitudes, and cochlear microphonics/otoacoustic emission results, respectively) were not significantly different in the AUNX1 subjects compared to normal hearing controls. Despite the lack of correlation between the degree of axonal degeneration and hearing thresholds, a possible alternate explanation for the representative pattern of hearing loss observed in AUNX1 is the preferential degeneration of low frequency afferent fibres, thus reducing their ability to relay low frequency intensity information to the central auditory nervous system. In support of this hypothesis are the notable morphological differences between low and high frequency afferent fibres that may make the former more susceptible to mitochondrial dysfunction. For example, fibres that transmit low frequencies are longer, a larger calibre, and require more myelin (Liberman and Oliver, 1984; Anniko and Arnesen, 1988; Köppl *et al.*, 2000). Hence, it is likely that these fibres have an overall

higher energy consumption and therefore require more mitochondria to maintain their cellular processes. In many other mitochondrial-related neurodegenerative conditions longer and larger neurons are preferentially affected for precisely this reason (McLeod, 1971; Berciano *et al.*, 1986; Jitpimolmard *et al.*, 1993; Sancho *et al.*, 1999; Starr *et al.*, 2003; Zhu *et al.*, 2012).

3.7.3 Relevance of the fixel-based analysis framework in the diagnosis and management of auditory neuropathy

In general, recent developments in diagnostic assessments have improved our knowledge of the pathophysiological mechanisms and lesion sites underlying auditory neuropathy. For instance, electrophysiological patterns of neural potentials obtained by transtympanic ECoChG can provide important information regarding the integrity of the inner hair cells and the activation of auditory nerve afferent fibres (Santarelli *et al.*, 2013; Santarelli *et al.*, 2015a; Santarelli *et al.*, 2015b). Diffusion MRI and fixel-based analysis techniques can further contribute to our current understanding of this heterogenous condition. The AFD metric is particularly applicable as it provides a way to investigate, characterise and quantify potential degeneration within the white matter tracts of the auditory system *in-vivo*. Up until now, post-mortem histopathological analysis of temporal bones was the only way to assess the integrity of post-synaptic structures within the auditory system. The AFD metric has the potential to provide further insights into the idiosyncratic nature of the auditory neuropathy phenotype and may be especially useful in quantifying the extent of dysfunction in patients with post-synaptic sites of lesion. This capability could directly impact recommendations for intervention by improving the accuracy in predicting cochlear implantation outcomes.

3.7.4 Limitations

We acknowledge our study has several limitations that need to be discussed. Firstly, the study would benefit from including an additional control group of individuals with cochlear sensory hearing loss matched in severity to those individuals with AUNX1. Adding such a group would provide further insights into the extent of neural degeneration occurring in individuals with AUNX1. Secondly, the relatively small sample size limits the potential of observing interactions with small effect sizes between the AFD metric and auditory measures. Finally, reverse-phase encoded images were not acquired for each participant and therefore the images could not be corrected for susceptibility-induced imaging distortions. Such distortions can introduce artefacts, particularly at air-tissue interfaces, which may produce greater variance in the fixel-based analysis.

3.7.5 Conclusion

Our findings have confirmed the pathophysiological process and lesion site underlying the auditory neuropathy phenotype in individuals with AUNX1. While AFD results show significant degeneration of the vestibulocochlear VIII nerve and white matter tracts of the auditory brainstem in affected

individuals, objective auditory assessments indicate relatively normal inner and outer hair cell function. Degeneration of auditory nerve afferents results in far fewer fibres being activated in response to an auditory stimulus, which in turn reduces the neural input to the nuclei within the central auditory nervous system. Consequently, individuals with AUNX1 have severe functional hearing deficits as characterised by behavioural assessments of temporal processing (gap detection), hearing thresholds and speech perception. Whether neural synchrony is also disrupted in the remaining auditory fibres is not known, however, it is a probable contributing factor. Importantly, our results show dMRI and associated voxel-based analysis techniques are viable tools to assess the integrity of the auditory tracts *in-vivo* and can complement existing auditory assessments in identifying lesion sites and pathophysiological processes in auditory neuropathy. Further research is required to determine whether AFD values of the auditory brainstem tracts can be used to help predict cochlear implant outcomes in affected individuals.

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Chapter 4: Investigating white matter microstructure of the VIII nerve and its relationship to auditory function

4.1 Introduction

Since the term ‘auditory neuropathy’ was first coined by Starr et al. (1996) significant advances have been made in understanding the different aetiologies, risk factors and pathophysiological processes responsible for the AN phenotype. While transient neonatal insults, such as hyperbilirubinaemia and hypoxia, are likely to be some of the most common causes (Amatuzzi et al., 2011; Shapiro & Nakamura, 2001; Sininger & Oba, 2001), AN has also been linked to numerous syndromic and non-syndromic neurodegenerative conditions. These include, Friedreich’s ataxia (Rance et al., 2010), CMT disease (type 1 and type 2) (Rance et al., 2012d; Starr et al., 2003), riboflavin transported deficiency (Menezes et al., 2016), dominant optic atrophy (Santarelli et al., 2015c), Mohr-Tranebjaerg Syndrome (Merchant et al., 2001), and AUNX1 (Wang et al. (2006); Chapter 3). Additionally, AN has also been reported to occur spontaneously, in the absence of any apparent health conditions (Starr et al., 2000). While there are many aetiologies, with differing sites of lesion responsible for the AN phenotype, currently there is a limited battery of assessments available to adequately define the condition. As such, this has made the diagnosis and management of AN challenging.

Temporal bone studies, animal models and the use of objective auditory assessments, such as OAEs, ABRs and ECoChG, have provided considerable insights into the sites of lesion responsible for the AN phenotype for many of the known aetiologies and risk factors. A range of structures and physiological processes in both the peripheral and central auditory system have been shown to be affected and sites of lesion vary between aetiologies. Oxygen deprivation, for example, causes a selective loss of inner hair cells, whereas the outer hair cells, spiral ganglion neurons and auditory nerve afferent fibres remain intact (Amatuzzi et al., 2011; Amatuzzi et al., 2001; Shirane & Harrison, 1987). Comparatively, hyperbilirubinaemia or kernicterus causes degeneration of the spiral ganglion neurons while the function of the organ of Corti is unaffected (Chisin et al., 1979; Santarelli & Arslan, 2002; Shapiro & Nakamura, 2001). Moreover, numerous studies have shown that degeneration of the auditory nerve afferent fibres and/or myelin sheath can occur in neurodegenerative conditions, while the sensory structures of the cochlea are spared (Menezes et al., 2016; Merchant et al., 2001; Santarelli et al., 2015a; Satya-Murti et al., 1980; Starr et al., 2003; Zong et al., 2015). Often, the AN phenotype in these individuals is a concomitant finding, occurring in conjunction with peripheral or motor neuropathies.

In general, AN lesion sites can be divided into those that occur at or before the inner hair cell synapse (pre-synaptic), and those that affect more central, post-synaptic structures, such as the auditory nerve dendritic terminals, spiral ganglion neurons, the axonal portion of the auditory nerve, and/or myelin sheath (Rance & Starr, 2015). This distinction is important because cochlear implantation, which is currently the most widely adopted intervention strategy to treat AN, has been shown to provide

consistently favourable outcomes for individuals with pre-synaptic sites of lesion (Breneman et al., 2012; Jeong et al., 2007; Rance & Barker, 2009; Rodriguez-Ballesteros et al., 2003; Santarelli et al., 2011). As discussed in detail in section 1.6, outcomes are significantly more variable for post-synaptic cases, however; some affected individuals achieve good speech perception outcomes, while others attain no functionally useful hearing at all (Kutz et al., 2011; Mason et al., 2003; Miyamoto et al., 1999; Rance & Starr, 2015; Santarelli et al., 2015c). The reason for this variability in outcomes can be explained, in part, by the mode of stimulation of the cochlear implant. For instance, the electrode array of an implant bypasses the pre-synaptic structures of the auditory system, such as the inner and outer hair cells, and directly stimulates the spiral ganglion neurons and auditory nerve axons (Fayad et al., 1991; Fayad & Linthicum, 2006; Linthicum et al., 1991). Hence, in pre-synaptic cases, delivery of the electrical signal from the implant to the central auditory system is unperturbed by the lesion site, while in post-synaptic patients electrical transmission may be affected by the structures affected *and* the extent of disruption (Rance & Starr, 2015). This hypothesis is supported by the findings of Santarelli et al. (2015c), who investigated cochlear implant outcomes in individuals with dominant optic atrophy due to mutations in the optic atrophy 1 gene (*OPA1*), resulting in neurodegeneration which manifests in retinal ganglion cell degeneration, and a loss of the dendritic terminals of the auditory nerve. The researchers found that most individuals with *OPA1* achieved relatively good cochlear implantation outcomes. The more severely affected individuals, however, who had longer disease durations, had significantly poorer post-implant speech perception results. A finding which is likely explained by the involvement of the spiral ganglion neurons and auditory nerve axons in *OPA1* disease progression (Kjer et al., 1983). Hence, in less severe *OPA1* patients the cochlear implant can bypass the post-synaptic site of lesion at the dendritic terminals, however, as the condition affects more central structures, the electrical stimulation from the cochlear implant is no longer able to be successfully transmitted.

Although current auditory assessments can provide important information regarding the site of lesion, they are not yet able to identify the site of lesion *and* quantify the extent of disruption in affected individuals. As discussed, this is especially problematic for individuals with post-synaptic lesion sites. Newly developed dMRI techniques have the potential to provide insights for this issue. For instance, the fixel-based analysis framework can provide a way to investigate the white matter microstructure and quantify changes in fibre density in individuals with neurodegenerative conditions. This technique has been successfully used to characterise white matter degeneration in AUNX1 (Chapter 3), and other neurodegenerative conditions, such as Alzheimer's disease (Mito et al., 2018), multiple sclerosis (Gajamange et al., 2018), and motor neuron disease (Raffelt et al., 2012b).

The present study, investigated whether the Apparent Fibre Density metric, could be used to examine the extent of disruption in the auditory nerve in individuals with the AN phenotype resulting from a range of aetiologies. Moreover, imaging findings were correlated with the results from auditory assessments to determine whether the AFD metric is sensitive to varying degrees of neural disruption.

The extent of neural dysfunction could then be interrogated in relation to the degree of hearing impairment, as characterised by a range of objective and behavioural hearing assessments.

4.2 Methods

4.2.1 Human research ethics committee approval

Approval to carry out the following study design in Australia was obtained through the Human Ethics and Research Ethics Committee of the Royal Victorian Eye and Ear Hospital, Melbourne, Victoria (project reference number: 15/1254H; see Appendix 2). The study protocol was undertaken in strict accordance to the ethical standards defined in the 1964 Declaration of Helsinki. All participants received information regarding the nature and purpose of the study after which they provided written informed consent and were eligible to withdraw at any time.

4.2.2 Participants

Nine individuals with confirmed bilateral AN (4 females and 5 males) and 16 normal hearing, age-matched controls (7 females and 9 males) took part in the study. Ages ranged from 8 – 31 years and the ages of the normal hearing controls were matched within ± 12 months of a participant with AN. Hence, a two-sample *t*-test did not show a significant difference between the mean age of the two groups (AN: 16.4 ± 6.5 years; controls: 15.8 ± 5.6 years; $t(14) = 0.3$, $p = 0.79$). Participants with AN were recruited through The University of Melbourne's Audiology Clinic where the AN phenotype was initially identified or confirmed by an experienced clinical audiologist. Where known, clinical and demographic details for the AN participants are detailed in Table 4.1. Individuals in the control group were recruited by word of mouth via staff colleagues, family members and friends. All control participants had normal hearing, and none showed clinical signs of AN. Audiological test results for both AN and control participants are shown in Table 4.2. Control participants were matched for age and gender to those in the AN group. All subjects that took part in the study underwent MRI scanning and audiometric testing.

4.2.2.1 Inclusion criteria

Inclusion in each study was confirmed via clinical evaluation. Inclusion in either the normal hearing control group or AN group was based on each participant's prior medical history, and confirmation of normal or abnormal auditory function using the relevant combination of hearing tests described in section 4.2.3 and 4.2.4. A diagnosis of AN was based on the characteristic clinical presentation including an absent or disordered ABR with either a present cochlear microphonic and/or extant OAEs.

4.2.2.2 Exclusion criteria

Participants were excluded from the study if they had any of the following:

- Severe cognitive deficits and/or a history of brain injury or trauma as detailed in their medical records or advised by the participant and confirmed by the participant's physician.

- Contraindications to receiving an MRI scan, as detailed in the MRI screening form in Appendix 3.
- A pathology affecting the transmission of sound through the middle ear bilaterally, such as otitis media, otosclerosis etc.

4.2.2.3 Randomisation

Subject randomisation was not possible due to the relatively limited availability of participants with AN. As a result, convenience sampling was utilised and numbers in each group were determined by participant availability.

4.2.3 Audiological assessment

4.2.3.1 Pure tone audiometry

Otoscopy was performed at the beginning of each test session to check for wax occlusion or foreign bodies. Each participant underwent audiometric assessment using a calibrated Interacoustics AD226 audiometer. Testing was carried out in either a sound-treated booth or in a quiet room where a Digitech pro sound level meter was used to ensure noise levels were < 40 dB SPL. Air conduction thresholds were obtained at octave frequencies (250 Hz – 8 kHz) and a four-frequency average (4FA; 0.5, 1, 2, and 4 kHz) was subsequently calculated. Normal hearing ability was determined to be a 4FA hearing threshold of < 20 dB HL.

4.2.3.2 Otoacoustic emissions

Distortion product otoacoustic emissions were carried out to assess outer hair cell function. Testing was conducted using a GSI Audera evoked potential system. Otoacoustic emission data for each participant was collected on each ear at 0.5-, 1-, 2-, 3- and 4 kHz. Stimuli were presented at intensity levels of 65 dB SPL for F1 and 55 dB SPL for F2 (test ratio of 1.20). If no response was obtained at this level stimulus intensity was increased to 70 dB SPL for both F1 and F2. A response was considered present only if the response amplitude was ≥ 6 dB for three consecutive frequency bands.

4.2.4 Electrophysiological assessments

4.2.4.1 Auditory brainstem response

Neural activity and conduction time within the auditory nerve and brainstem was assessed using ABR testing with a GSI Audera. Auditory brainstem response testing was performed bilaterally for all the participants, except four controls in whom responses were recorded monaurally due to equipment-related issues. The stimuli, a 100 μ s broadband click, was delivered via a single ER-4 insert earphone to the test ear. Placement of the AMBU Neuroline 720 silver/silver chloride surface electrodes were as follows; ground electrode placed on low forehead, inverting electrode placed on test-ear mastoid, and non-inverting electrode on high forehead. Each ear was assessed separately at an intensity level of 90 dB nHL – 100 dB nHL and a stimulus rate of 8 Hz. Each auditory brainstem recording was averaged

from a total of 2000 stimulus presentations. Response repeatability was determined by recording two separate averages of the waveform for each ear with opposite polarity clicks (condensation/rarefaction). The cochlear microphonic and Waves I, III & V were identified where possible. Subsequently, inter-peak latencies were calculated from absolute latencies, and the Wave I and V amplitude was also recorded.

4.2.5 Psychophysical assessments

4.2.5.1 Gap detection

The ability to discern short, rapid fluctuations in an auditory stimulus is an essential component in the processing and understanding of speech. Moreover, the capacity to resolve intervals, and the duration of auditory stimuli is vital in identification of consonant-vowel syllables (Wright et al., 1997). Previous research has shown significant prolongation in gap detection thresholds in individuals with AN (Starr et al., 1991; Zeng et al., 2005; Zeng et al., 1999). Hence, the inclusion of the gap detection task in this study was based on this previous research, described in section 1.5.4, showing that gap detection ability is often significantly disrupted in individuals with AN and distinct from the perceptual deficits exhibited by individuals with normal hearing and cochlear sensory hearing loss alone (Rance, 2005; Zeng et al., 2005).

An adaptive, three-alternative, forced-choice procedure with a two-down and one-up approach was utilised for the gap detection assessment (Levitt, 1971; Zeng et al., 2005). Stimuli were delivered via ER-4 insert earphones to the better hearing ear as determined by each participant's 4FA. For each trial three sounds were played (one target/affected signal and two standard/unaffected signals) to the participant in a random order (3-alternative) and the participant was required to pick the target signal (forced-choice). The gap detection assessment began with a large difference between the target signal and the standard stimuli to give the participant the best possible chance to correctly identify the target signal. After two consecutive, correct responses, the difficulty of the task increased as the difference between the target signal and standard stimuli became smaller (two-down). If the participant incorrectly responded once, the difference increased (one-up). In all cases, the stimuli were presented to the participant at a sensation level of ≥ 30 dB above their 4FA hearing threshold. Using this procedure gap detection thresholds were established by measuring the smallest temporal gap that each participant could detect in a burst of noise. The standard and target stimuli were almost identical broadband noise bursts, except for the fact that the targets contained a period of silence that varied in duration (between 0 – 60 ms).

4.2.6 Speech perception

4.2.6.1 Open-set speech perception testing

Open-set speech perception was assessed using the consonant-nucleus-consonant (CNC) word test (Peterson & Lehiste, 1962) in quiet (+20 dB SNR) and in noise (0 dB SNR). This test uses monosyllabic words phonemically balanced to Australian English. In the noise condition, 4-talker babble was presented concurrently with the CNC words to the better hearing ear (based on the 4FA hearing threshold). Sound files were played through the on-board soundcard (Conexant ISST) of a Hewlett Packard laptop using Windows Media player and delivered to the participant through an ER-4 insert earphone. For participants with normal hearing or a mild hearing loss the stimuli were presented at an intensity of 85 dB SPL. For participants with a moderate-to-profound hearing loss, the intensity level was increased to make the stimuli audible, however there was a maximum presentation level of 105 dB SPL. Each participant was asked to repeat the CNC words to the best of their ability, and the examining audiologist scored each word according to the phonemes that were correctly repeated. A half-list consisting of 25 words was presented in both quiet and noise.

4.2.6.2 Binaural speech perception

The listening in spatialised noise-sentences test (LiSN-S) was administered to determine each participant's ability to integrate interaural timing cues in auditory signals in order to gain a spatial advantage (Cameron & Dillon, 2007). Participants were required to discriminate a simple target sentence from a competing speech noise signal. The speech reception threshold was determined for each participant, which is a measure of the signal to noise ratio at which a participant can perceive 50% of the sentences delivered correctly. The speech reception threshold was determined in four different conditions, which differ based on the location of the noise source and vocal pitch of the target speaker. Test conditions were as follows: Different voices (DV) at $\pm 90^\circ$, DV at 0° , same voice (SV) at $\pm 90^\circ$, and SV at 0° . Three comparative metrics are also computed including: (i) Talker advantage, which represents the improvement in the speech reception threshold (in dB) gained by using pitch or 'talker' cues; (ii) Spatial advantage which represents the advantage gained by using spatial cues, and; (iii) Total advantage, which is a measure of the benefit gained from both talker and spatial cues. The stimuli were presented to the participants through Sennheiser HD215 headphones. For participants with a hearing loss the prescribed gain amplifier software, available through the LiSN-S, was used to increase the speech stimulus intensity to a level appropriate based on their audiometric thresholds (0.250 – 8 kHz). When using the prescribed gain amplification option, gain is provided according to the National Acoustics Laboratories-Revised Profound (NAL-R) prescription method (Glyde et al., 2013).

4.2.7 Magnetic resonance imaging assessment

4.2.7.1 MRI data acquisition

MRI data was acquired using a 3 Tesla Siemens Magnetom Skyra system (Siemens Medical Solutions, Erlangen, Germany), with a 32-channel head coil receiver. Diffusion-weighted images were acquired using a twice refocused, spin-echo, EPI sequence with the following parameters: repetition/echo time = 8400/110 ms, 2.5 mm isotropic voxels, FoV = 240 x 240 mm, matrix size = 96 x 96, and acceleration factor = 2. A total of 64 single-shell diffusion weighted images ($b = 3000 \text{ s/mm}^2$) and 8 non-diffusion weighted images ($b = 0 \text{ s/mm}^2$) were acquired with a total acquisition time of ~10 minutes. Diffusion-weighted images were visually assessed for motion artefacts and entire datasets were excluded if excessive motion was identified.

4.2.7.2 Data pre-processing

All pre-processing steps were carried out using commands implemented within MRtrix3Tissue (<https://3tissue.github.io/>), a fork of MRtrix3 (Tournier et al., 2019). Pre-processing of dMRI data included denoising (Veraart et al., 2016a; Veraart et al., 2016b), removing Gibbs-ringing artefact (Kellner et al., 2016), eddy-current correction and motion correction (Andersson & Sotiropoulos, 2016; Smith et al., 2004), EPI susceptibility distortion correction (Andersson et al., 2003) and bias field correction (Tustison et al., 2010). For EPI susceptibility distortion and eddy-current correction, the MRtrix3Tissue commands interfaced with FSL (Jenkinson et al., 2012), an external software package for dMRI processing. To improve alignment of anatomical structures in the template building step the dMRI data was regridded using cubic b-spline interpolation to an isotropic voxel size of 1.3 mm (Raffelt et al., 2012).

Subsequently, SS3T-CSD (Dhollander & Connelly, 2016) was used to compute FODs based on the 3-tissue (WM, GM, and CSF) group averaged response functions (Dhollander et al., 2019; Dhollander et al., 2016). The response function estimation and SS3T-CSD algorithms are both entirely unsupervised and their outputs are directly dependent on the dMRI data itself. All 3 tissue compartments were then used to estimate a spatially smoothed varying multiplicative field for global and local (bias field) intensity normalisation. Computed FOD images from each participant were used to generate a study-specific, unbiased FOD template. Both linear and non-linear registration techniques were employed to facilitate this as described previously by Raffelt et al. (2012a) and Raffelt et al. (2011). Whole-brain probabilistic tractography (iFOD2 fibre tracking algorithm (Tournier et al., 2010)) was performed on the study-specific unbiased FOD template producing 20 million streamlines. In order to reduce reconstruction biases the 20 million streamlines obtained with the probabilistic tractography were filtered down to 2 million using the spherical-deconvolution informed filtering of tractograms (SIFT) algorithm developed by Smith et al. (2013).

4.2.8 Fixel-based analysis

4.2.8.1 Apparent Fibre Density

As part of the fixel-based analysis framework, the AFD metric was used in the current study to examine the white matter microstructure of the auditory system. The AFD value was determined by estimating each fixel's contribution to the entire dMRI signal in each voxel using SS3T-CSD (Dhollander & Connelly, 2016). Subsequently, the AFD value for each subject was assigned to a fixel template mask, which allowed for the AFD metric in corresponding fixels to be examined in specific regions and compared between groups.

4.2.8.2 Region of interest analysis

Given the significant heterogeneity in aetiology, possible lesion sites, and extent of dysfunction in the AN participants in this study, a fixel-based analysis of the whole-brain or brainstem was not performed. Instead, individual AFD values of the VIII nerve were examined. This region was selected based on previous findings showing a significant reduction in AFD in these regions for individuals with AUNX1 (Chapter 3).

The VIII nerves were readily identified on the study-specific FOD template as distinct protrusions exiting the brainstem at the ponto-medullary junction (see Chapter 2 for details). Single fibre regions of the VIII nerves were manually demarcated on the FOD template using the fixel overlay tool in *Mrview* (the graphical user interface of MRtrix3) as a guide. Subsequently, mean AFD values were calculated across the VIII nerve regions for all subjects.

4.2.9 Statistical analyses

All statistical analyses were carried out using Minitab (version 19 for Windows). A p -value of < 0.05 was considered to be significant unless otherwise stated.

4.3 Results

Table 4.1 | Clinical and demographic characteristics of auditory neuropathy subjects. Predicted lesion site and locus is based on each subject's aetiology.

Subject	Gender	Age at assessment (years)	Predicted lesion site	Predicted Locus	Aetiology/risk factors	Comorbidities	Duration since AN diagnosis (years)	Genetic details
#1	M	10	Pre-synaptic	Inner hair cells	Hypoxia	None	10	Unknown
#2	M	15				None	15	Unknown
#3	M	30				None	4	Unknown
#4	F	16	Post-synaptic	Dendrites & axons	RTD2	None	8	SLC52A2 mutations
#5	F	18				None	3	Unknown
#6	F	21				Motor neuropathy	16	SLC52A2 mutations
#7	F	11		Axons	FRDA	None	2	Unknown
#8	M	9		Axons & myelin	CMT2C	Motor neuropathy	3	TRPV4 mutations
#9	M	18	Unknown	Unknown	Unknown	None	18	Unknown

RTD2: riboflavin transported deficiency type 2; FRDA: Friedreich's ataxia; CMT2C: Charcot-Marie-Tooth disease type 2

Table 4.2 | Audiological data for the auditory neuropathy (Subjects 1-9) and control participants (Subjects 10-25)

Subject	Aetiology	PTA (4FA (db HL))	ABR Wave V latency (ms)		Ear tested	CNC words (% phonemes correct)		Gap detection (ms)	Hearing aid/ALDs
		L/R	L/R	L/R		+20 dB SNR	0 dB SNR		
#1	Hypoxia	40/32.5	-/-	6.1/6.0	Right	78.7	24	11	Bilat. HAs
#2	Hypoxia	65.8/59.2	-/-	Absent	Right	52	9.3	15	Bilat. HAs
#3	Hypoxia	40.8/49.2	+/+	Absent	Left	37.3	0	8	No benefit
#4	RTD2	23.3/37.5	+/+	Absent	Left	96	14.7	4	No benefit
#5	RTD2	73.3/86.7	+/+	Absent	Left	0	0	*	No benefit
#6	RTD2	76.7/77.5	+/+	Absent	Left	1.3	0	42	Bilat. HAs
#7	FRDA	20.8/21.7	+/+	6/5.8	Left	97.3	26.7	17	Remote mic
#8	CMT2C	11.7/7.5	+/+	5.6/5.8	Right	92	13.3	6	Remote mic
#9	Unknown	60/69.2	+/+	Absent	Left	48	0	8	Bilat. HAs
#10	Control	3.8/7.5	+/+	5.5/5.7	Left	80	26.7	4	-
#11	Control	2.5/1.3	+/+	^/5.5	Right	94.7	30.7	4	-
#12	Control	11.3/10	+/+	5.7/^	Right	90.7	13.3	3	-
#13	Control	2.5/5	+/+	5.6/^	Left	96	28	4	-
#14	Control	2.5/2.5	+/+	5.6/5.6	Right	94.7	34.7	4	-
#15	Control	5/5	+/+	5.7/5.8	Right	96	40	2	-
#16	Control	10/10	+/+	5.5/5.6	Right	94.7	29.3	2	-
#17	Control	3.8/2.5	+/+	5.8/5.7	Right	95.1	34.7	2	-
#18	Control	10/10	+/+	^/5.8	Right	96	45.3	2	-
#19	Control	3.8/3.8	+/+	5.3/5.5	Right	90.7	21.3	2	-
#20	Control	15/3.8	+/+	5.6/5.5	Right	94.7	37.3	2	-
#21	Control	6.3/-1.3	+/+	5.4/5.3	Right	97.3	23	3	-
#22	Control	8.8/7.5	+/+	5.6/5.5	Right	97.3	24	2	-
#23	Control	7.5/7.5	+/+	5.4/5.5	Right	90.7	30.7	4	-
#24	Control	15/11.3	+/+	5.5/5.3	Right	97.3	21.3	2	-
#25	Control	0/-1.3	+/+	5.4/5.4	Left	98	32	2	-

*Unable to perform task because the stimulus was not audible to the participant. ^Data not collected. RTD2: riboflavin transporter deficiency type 2; CMT2C: Charcot-Marie-Tooth type 2C; PTA: pure tone audiometry; 4FA: 4 frequency average; OAEs: otoacoustic emissions; CNC: consonant-nucleus consonant; SNR: signal-to-noise ratio; ms: milliseconds; dB: decibel; Hz: Hertz; ALDs: assistive listening devices; FM: frequency modulated; HAs: hearing aids.

4.3.1 Audiometry

Hearing thresholds, as measured by PTA, were elevated for all but one AN subject (subject #8) and varied significantly between individuals and across aetiologies (Figure 4.1). As a group, hearing thresholds were significantly poorer for the AN participants compared to the normal-hearing controls at octave frequencies between 0.250 – 8 kHz. A two-sample *t*-test was used to compare the 4FA hearing thresholds between groups. The 4FA thresholds were significantly poorer in the left ear (AN: 45.8 ± 24.1 dB HL; controls: 6.6 ± 4.5 dB HL; $t(8) = 4.8$, $p \leq 0.001$) and right ear (AN: 49.0 ± 26.5 dB HL; controls: 5.4 ± 4.1 dB HL; $t(8) = 4.9$, $p \leq 0.001$) in the AN group compared to the normal hearing controls. The 4FA values for each participant are shown in Table 4.2. All control subjects had hearing thresholds < 20 dB HL at each of the frequencies tested.

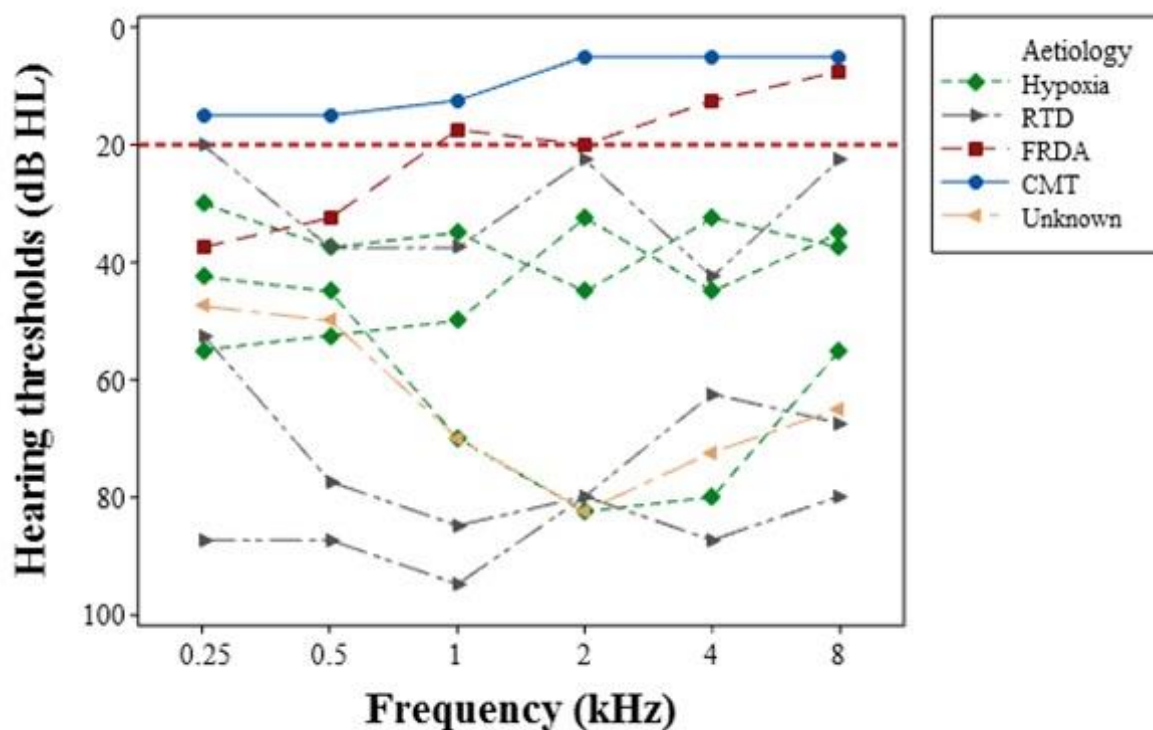


Figure 4.1 | Average hearing thresholds displayed according to aetiology. Thresholds were elevated for most AN subjects but varied considerably. The region above the red dashed line represents the range of normal hearing.

4.3.2 Otoacoustic emissions

Distortion product otoacoustic emissions were present at octave frequencies between 500 Hz and 4 kHz in all the controls and in seven out of the nine AN participants (Table 4.2).

4.3.3 Auditory brainstem response

Auditory brainstem responses were absent bilaterally for six out of the nine participants with AN. Repeatable responses were obtained from the subject with FRDA (Figure 4.2: subject #7), CMT2C (Figure 4.2: subject #6), and one of the subjects who suffered oxygen deprivation at birth (Figure 4.2:

subject #1). The ABR waveform morphology in these individuals, however, was poorer compared to controls and latencies were prolonged (Figure 4.2). Comparatively, ABRs were present with normal waveform morphologies and latencies for all the normal hearing control subjects. Wave V latencies for each participant with recordable responses are included in Table 4.2. Furthermore, the CM was identified bilaterally in eight of the nine AN participants.

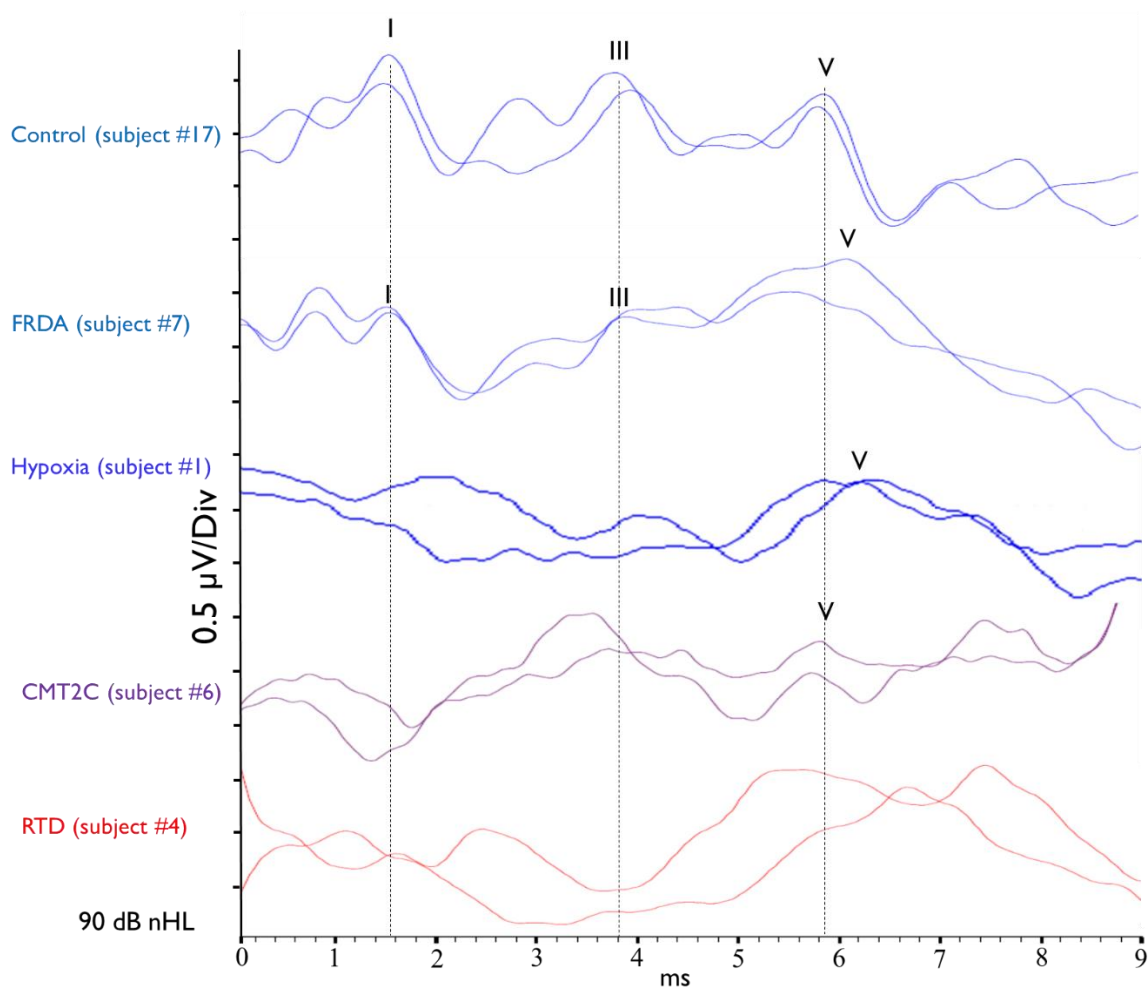


Figure 4.2 | Auditory brainstem response recordings. The varied responses from five participants are shown. ABRs were measured using acoustic click stimuli presented at 90 dB nHL at a rate of 33.1 Hz. The top tracing was obtained from a normal hearing control subject (subject #17). The dotted lines correspond to Wave I, III and V from this participant. Also included are the waveforms obtained from the AN participants with recordable ABRs, including the subject with Friedreich’s Ataxia (FRDA; subject #7), neonatal hypoxia (subject #1), and Charcot-Marie Tooth type 2 (CMT2C; subject #6). For comparison the results from one of the AN participants without an ABR is also included (riboflavin transporter deficiency (RTD; subject #4)). Variability of the waveforms is apparent between the different AN participants.

4.3.4 Temporal resolution

4.3.4.1 Gap detection

For one of the nine AN subjects (subject #5) the gap detection task was not audible, even at the maximum presentation level, and therefore this subject was unable to complete the task. For the

remaining eight AN subjects mean gap detection thresholds were significantly poorer (13.9 ± 12.2 ms) compared to normal hearing controls (2.8 ± 0.9 ms; $t(7) = 2.6$, $p = 0.04$). Individual gap detection results are included in Table 4.2.

4.3.5 Speech perception

4.3.5.1 Open set speech perception

As a group, speech perception scores were significantly impaired in the AN participants compared to matched normal hearing controls. Mean CNC word scores were lower for the AN group in both quiet (AN: 62.8 ± 34.2 ; controls: 94 ± 4.4 ; $t(7) = 2.8$, $p = 0.03$) and in noise (AN: 11 ± 10.7 ; controls: 29.1 ± 8.0 ; $t(8) = -4.2$, $p < 0.001$).

4.3.5.2 Binaural speech perception (LiSN-S)

Three out of the nine AN subjects were not able to perform the binaural speech perception task because the target speech was not audible to them. Speech reception thresholds were significantly higher for the AN group compared to the controls in each of the four simulated listening conditions of the LiSN-S: DV90° (AN: -4.3 ± 4.7 dB; controls: -14.8 ± 2.4 dB; $t(6) = 5.2$, $p \leq 0.002$); SV90° (AN: -1.6 ± 4.6 ; controls: -13.9 ± 3.1 ; $t(5) = 5.6$, $p \leq 0.003$); DV0° (AN: -1.8 ± 2.8 dB; controls: -8.2 ± 3.3 dB; $t(7) = 4.2$, $p \leq 0.004$), and; SV0° (AN: 1.3 ± 1.2 ; controls: -1.5 ± 1.1 ; $t(8) = 4.8$, $p \leq 0.001$) (Figure 4.3). The findings show that the participants with AN required a higher SNR in order to correctly identify 50% of the target speech compared to the control group. Furthermore, the ability to use pitch or ‘talker’ cues, as measured by the talker advantage score, was also significantly impaired in the AN group. The mean improvement gained by using talker cues was 3.0 ± 2.6 dB for the AN group compared to 7.1 ± 4 dB achieved by the controls ($t(14) = -2.8$, $p = 0.01$). Similarly, the ability to use spatial cues, as measured by the spatial advantage score, was also significantly impaired for the AN group compared to the normal hearing controls (AN: 3.6 ± 3.1 dB; controls: 12.1 ± 2.1 dB; $t(6) = -6.2$, $p \leq 0.001$).

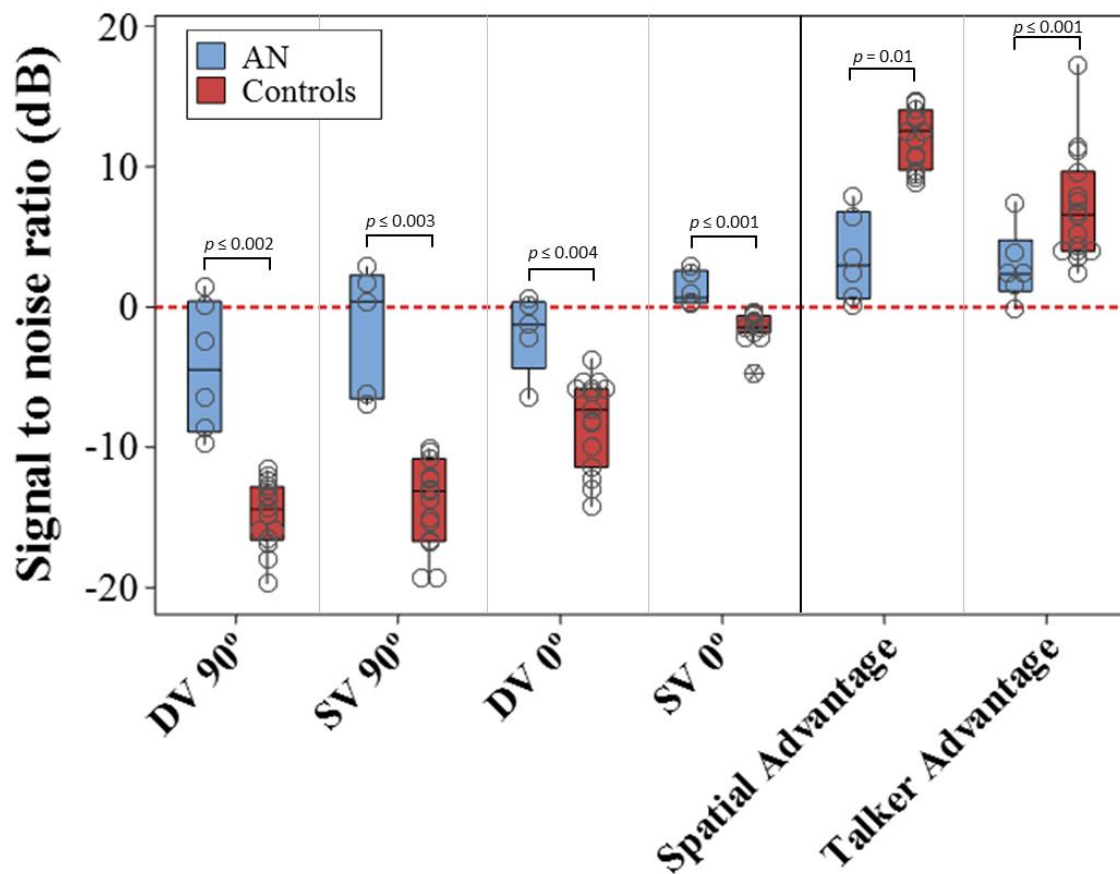


Figure 4.3 | Listening in spatialised noise-sentences test (LiSN-S) results. Boxplots showing speech reception thresholds for the AN group and the normal hearing controls in the four listening conditions simulated by the LiSN-S; DV90°: target speech and noise (babble) is spoken by different talkers and is spatially separated by $\pm 90^\circ$; SV90°: target speech and noise spoken by the same talker and spatially separated by $\pm 90^\circ$; DV0°: speech and noise is spoken by different talkers and is presented from the same direction at 0° azimuth; SV0°: speech and noise is spoken by the same talker and is presented from the same direction. The result of two comparative measures, talker advantage and spatial advantage, are also included. Talker advantage refers to the benefit gained (in dB) when the target speech is spoken by a different voice compared to the noise. The spatial advantage measure refers to the benefit gained when the speech and noise are spatially separated. Individual results are shown as circles and the dashed red line indicates 0 dB signal-to-noise ratio. DV: different voices; SV: same voice.

4.3.6 Apparent Fibre Density analysis of the VIII vestibulocochlear nerve

While the AFD values obtained from the VIII nerve region varied substantially for both groups, overall the results were significantly lower in the AN group compared to the normal hearing control subjects (AN: 0.04 ± 0.01 ; controls: 0.1 ± 0.02 ; $t(13) = -5.94$, $p < 0.001$) (Figure 4.4). Furthermore, within the AN group, individuals with RTD showed the most significant axonal degeneration, as characterised by the AFD metric, compared to the other aetiologies ($t(4) = -3.9$, $p = 0.02$).

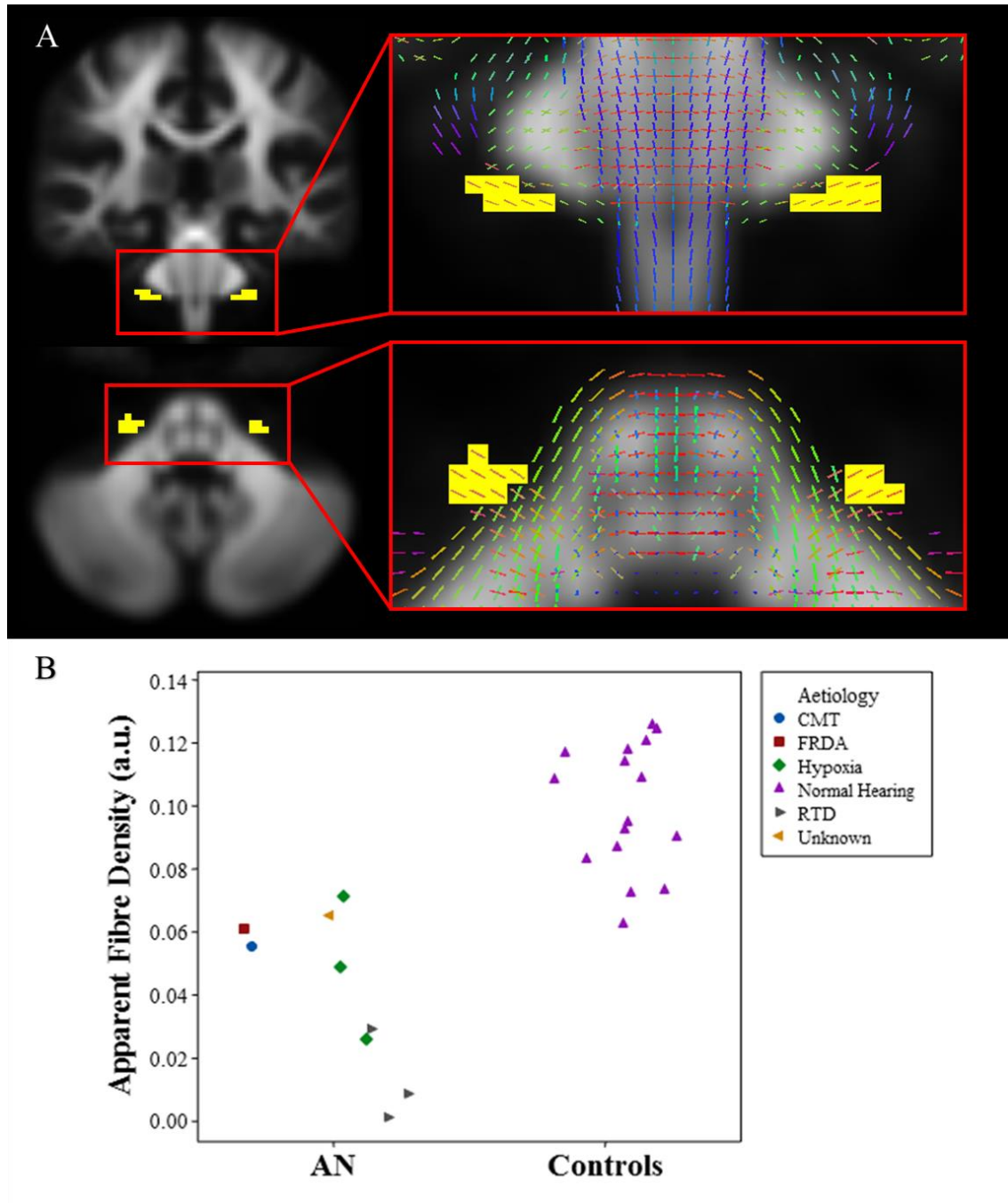


Figure 4.4 | Apparent Fibre Density results. (A) *Left:* Coronal (top) and axial (bottom) slice of the dMRI template image showing the whole brain with the regions of interest selected for further analysis (single fibre voxels of the VIII nerve) marked in yellow. *Right:* Magnification of brainstem region showing the fixels selected for AFD analysis. The fixels shown are colour coded according to the dMRI directionally encoded colour convention. (B) Quantification of the spread of AFD results computed from the VIII nerve regions (shown in (A)). The different aetiologies responsible for the AN phenotype are shown. Apparent Fibre Density values are represented in arbitrary units (a.u.)

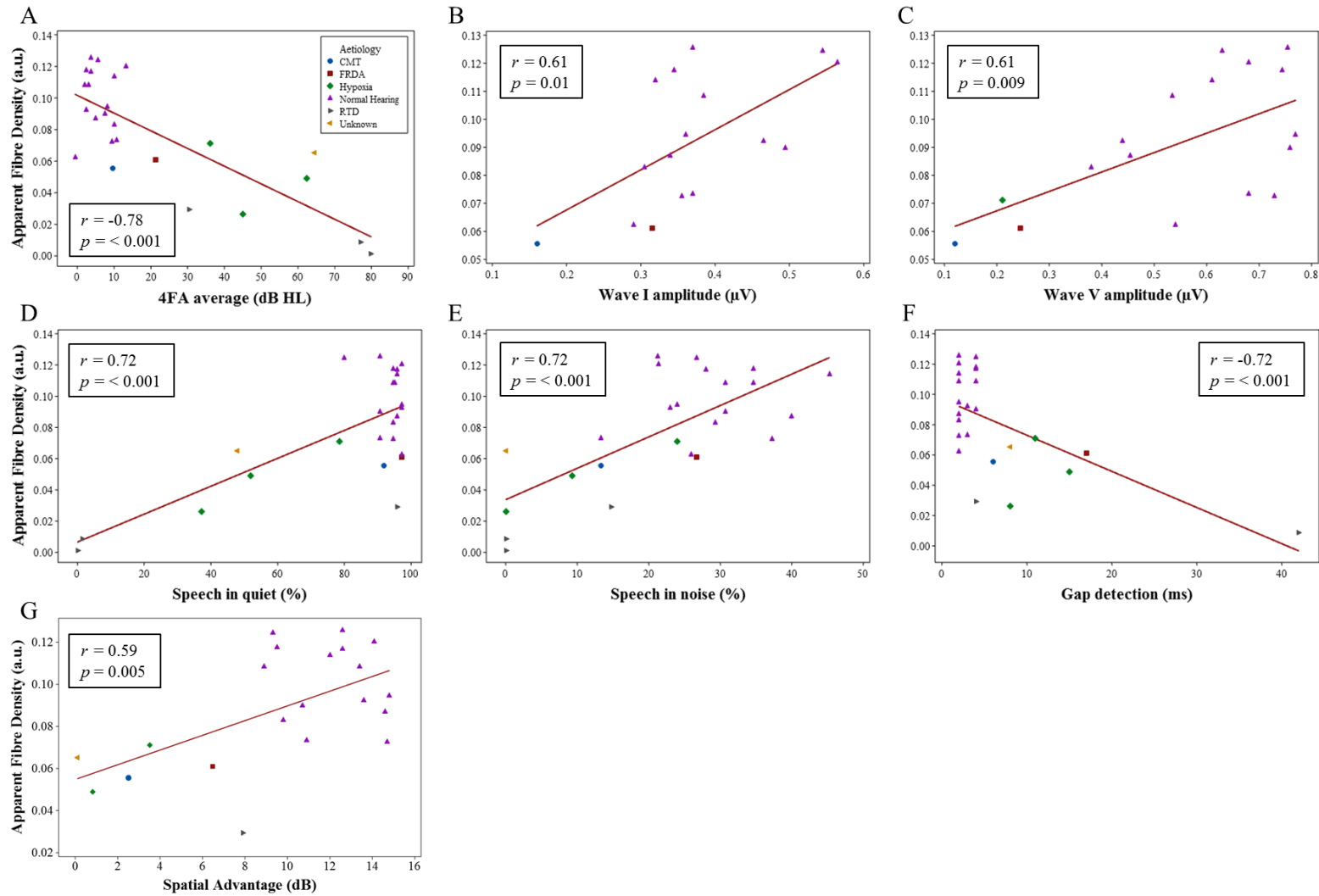


Figure 4.5 | Correlation graphs between AFD results and (A) 4-frequency average hearing loss, (B) ABR Wave I amplitude, (C) ABR Wave V amplitude, (D) Speech in quiet (%), (E) Speech in noise (%), (F) gap detection, and (G) spatial advantage (dB). Participants are grouped according to aetiology (legend is shown in graph (A)). The red line shown in each graph is the line of best fit and the box shows the regression fit and significance value for each comparison.

4.3.7 Relationship between auditory measures and AFD results.

Spearman correlation analyses were carried out to investigate the relationship between the AFD metric and the results obtained from the auditory measures, including PTA results (4FA hearing loss), speech perception in quiet and in noise, LiSN-S spatial advantage score, ABR amplitudes (wave I and V), as well as gap detection (Figure 4.5). Significant negative correlations were detected between AFD results and 4FA hearing loss ($r = -0.78$, $p < 0.001$), and gap detection ($r = -0.72$, $p < 0.001$). Findings indicate that individuals with lower VIII nerve fibre density also had poorer sound detection thresholds, and a greater degree of impairment in their temporal processing ability. Significant positive correlations were also found between the AFD metric and speech in quiet ($r = 0.72$, $p < 0.001$), speech in noise ($r = 0.72$, $p < 0.001$), the LiSN-S spatial advantage score ($r = 0.59$, $p = 0.005$), as well as the ABR amplitude of wave I ($r = 0.61$, $p = 0.01$), and wave V ($r = 0.61$, $p = 0.009$). These results suggest that individuals with lower axonal density of the VIII nerve have poorer speech perception ability in both quiet and noise, impaired ability to integrate binaural difference cues, and lower ABR wave I and V amplitudes.

4.4 Discussion

This study examined auditory function and axonal integrity in individuals with AN due to different aetiologies. To evaluate the degree of auditory impairment several behavioural and objective assessments were utilised, including PTA, otoacoustic emissions, ABRs, as well as tasks designed to examine binaural processing, temporal discrimination (gap detection) and speech perception ability. The auditory results obtained from the AN group in the current study are in agreement with previously published findings investigating auditory function in AN (Rance & Starr, 2015; Rapin & Gravel, 2003). That is, hearing thresholds obtained from affected individuals ranged significantly in severity, with some subjects achieving results within the normal range while others had a profound loss. Similar results have been reported throughout the literature (Menezes et al., 2016; Rance et al., 2002; Rance et al., 2012e; Starr et al., 1996). Otoacoustic emissions were present bilaterally in the majority of AN subjects, indicative of normal outer hair cell function. Two participants with a history of neonatal hypoxia, however, had altogether absent emissions. In general, it is not uncommon for OAEs to deteriorate over time in AN, especially in cases where affected individuals have been fitted with hearing aids (Starr et al., 2001). Auditory brainstem responses, on the other hand, were absent bilaterally in six out of the nine subjects with AN, and in the remaining three, ABR waveforms were severely delayed and/or reduced in amplitude. Auditory brainstem response waveform morphology has been shown to vary depending on the aetiology responsible for the AN. For instance, in cases where the AN phenotype is caused by auditory nerve demyelination, ABR latencies are typically prolonged, whereas in cases of deafferentation latencies remain unaffected but the amplitude is significantly diminished (Rance et al., 2012b; Rance et al., 2012d; Rance et al., 2012e). Furthermore, individuals with AN showed a significant reduction in the ability to use interaural difference cues to gain a perceptual advantage when listening

to target speech in the presence of competing noise. This was reflected by their reduced advantage scores obtained from the LiSN-S, which are in agreement with results obtained from individuals with CMT and FRDA by Rance et al. (2012e). Finally, affected individuals also showed significantly poorer speech perception and temporal processing ability compared to normal hearing controls, which are hallmark symptoms of AN and consistent with numerous previous studies (Rance et al., 2009; Rance et al., 2012d; Rance et al., 2012e; Santarelli et al., 2015b; Zeng et al., 2005).

Whereas auditory function in AN has been previously studied in significant detail, this is the first study to use dMRI-based quantitative analysis techniques to investigate neurodegenerative changes within the VIII nerve region in individuals with AN due to various aetiologies. A similar dMRI-based quantitative method based on the diffusion tensor model has, however, been used in a previous study to investigate changes in the VIII nerve in individuals with unilateral cochlear sensory hearing loss (Vos et al., 2015). These researchers showed a small but significant reduction in fractional anisotropy in affected individuals compared to normal hearing controls. Results suggest that prolonged auditory deprivation leads to axonal degeneration within the VIII nerve.

The results of the current study demonstrated a significant loss of axons in the VIII nerve in individuals with AN. Although the AFD results varied considerably between all participants, overall, they were significantly lower in the AN group compared to the normal hearing control group. Findings are consistent with post-mortem histopathological studies of human temporal bones from individuals with AN, which show significant neural degeneration of the cochlear nerve, while peripheral sensory structures of the auditory system, such as the outer and inner hair cells, remain largely intact (Bahmad et al., 2007; Hallpike et al., 1980; Merchant et al., 2001; Sagers et al., 2017; Spoendlin, 1974; Starr et al., 2003). Furthermore, the variability in AFD results are consistent with the range of aetiologies included in this study, which reflects different lesion sites and pathophysiological processes underlying the AN phenotype. For example, the individuals with RTD showed the most significant axonal degeneration in the VIII nerve region when compared to the other participants. While no temporal bone studies exist that can corroborate these results for RTD, our findings are consistent with the known pathomolecular process underpinning the symptoms observed in this condition. That is, a deficiency in the uptake of riboflavin prevents the mitochondrial respiratory chain complex from functioning normally, which is likely to reduce the viability of cells with high-energy demands, such as those responsible for the propagation of action potentials in the nervous system (Jaeger & Bosch, 2016). Furthermore, the current results are consistent with reported findings from motor and sural nerve biopsies which show that substantial axonal degeneration is the cause of the progressive pontobulbar palsy and sensory ataxia commonly observed in patients with RTD (Foley et al., 2014; Jaeger & Bosch, 2016). The participants, however, with CMT disease (type 2) and FRDA, which are both neurodegenerative conditions resulting from axonal loss, had AFD results similar to the normal hearing control subjects. Given the neurodegenerative nature of their conditions it might be expected that these

individuals would exhibit significant axonal degeneration within the VIII nerve region, similar to results obtained from individuals with RTD in this study, and AUNX1 reported in Chapter 3. There are, however, further factors that need to be considered. For instance, while temporal processing ability was significantly impaired in these two individuals, both had extant ABRs, which suggests that the function of the auditory nerve was not as severely disordered in these subjects compared to the majority of other AN participants. Moreover, these two individuals have had a shorter duration of disease compared to the other participants in the study suggesting that the progression of symptoms was still in the early stages. Finally, the AFD results for the three individuals who suffered a hypoxic event at birth varied, with one subject showing a similar AFD value as the control participants, whereas the other two showed a reduction in AFD. While hypoxia is typically thought to damage only the inner hair cells, it is likely that over time, without input and stimulation from the inner hair cells, the auditory afferent fibres also degenerate (Amatuzzi et al., 2011; Amatuzzi et al., 2001; Salvi et al., 1999). Overall, these reported findings provide some insight into the sensitivity of the AFD metric, indicating that the results are consistent with the other available tests, including the electrophysiology data and predicted sites of lesion, for each of the participants.

An examination of the relationship between auditory measures and AFD values for each participant in the study showed that those with fewer VIII nerve axons also had more impaired auditory function, as measured by the various behavioural and objective auditory assessments performed. This was particularly the case for hearing thresholds (4FA hearing loss), which showed the most significant and strongest correlation with the AFD metric. Findings suggest that the PTA results are the most precise predictor of VIII nerve axonal density. The correlation between neural density of the auditory nerve and hearing thresholds is clear in this study and is consistent with findings of human post-mortem histopathological analysis reported in the literature (Merchant et al., 2001; Satya-Murti et al., 1980; Starr et al., 2003). Importantly, a recent study by Sagers et al. (2017), who performed post-mortem histopathological analysis on the cochleae obtained from 30 individuals with a wide range of aetiologies resulting in AN, also showed that the degree of neural degeneration was significantly correlated with PTA results and word recognition scores in all affected individuals. Additionally, while a decline in neuronal density within the cochlea has also been shown to occur as a normal part of age-related cochlear sensory hearing loss (Makary et al., 2011), the degree of neural degeneration in these individuals has not been shown to correlate with their hearing thresholds (Sagers et al., 2017). This suggests that neural degeneration is not the sole reason for the elevated hearing thresholds exhibited by the elderly, and that other factors, such as degeneration of the cochlear hair cells, is likely to play a significant role.

The relationship between the AFD metric and the other auditory assessments also warrants further discussion. The results obtained in the current study indicate that individuals with fewer VIII nerve fibres have lower peak-to-peak ABR amplitudes (Wave I and V). While this is consistent with the fact

that the ABR amplitude reflects the population of neural elements present throughout the auditory brainstem pathway, it is also possible that reduced stimulus sensation level due to the presence of elevated hearing thresholds (as measures by PTA) had some influence on amplitude measures. The three participants with AN who had recordable ABRs in this study, however, had hearing thresholds ranging from normal to only a mild hearing loss and therefore it is unlikely that ABR amplitudes were significantly reduced due to a reduction in sensation level. Moreover, these findings are consistent with the reduction in ABR amplitudes reported to occur in individuals with neurodegenerative conditions known to result in axonal loss within the auditory system (Rance et al., 2008; Rance et al., 2012d). Similarly, individuals with fewer VIII nerve fibres also exhibited poorer temporal discrimination ability, as measured by the gap detection task. Again, while a reduction in sensation level, due to elevated hearing thresholds, has also been shown to impair temporal processing ability (Rance et al., 2009; Zeng et al., 2005), the AN participants in this study performed considerably poorer than would be expected for individuals with cochlear sensory hearing loss of an equivalent degree (Nelson & Thomas, 1997; Rance et al., 2009). Therefore, the extent of temporal processing impairment observed in the AN participants is more likely to be explained by the loss of VIII nerve fibres, rather than their elevated hearing thresholds. The consequence of the loss of afferent fibres, as measured by the AFD metric, is likely to result in a reduction in neural input from the auditory nerve fibres to the auditory brainstem nuclei. This has been shown to directly impair temporal processing ability (Paolini et al., 2001; Starr et al., 2003), and is congruent with the intensity-related performance on gap detection tasks demonstrated by normal hearing listeners. That is, a substantial elevation in gap detection thresholds can be induced when stimuli are presented to normal listeners at reduced sensation levels (Zeng et al., 1999). This intensity-related effect simulates a reduction in neural input, indicating that when fewer neurons are activated, temporal processing ability can suffer. Furthermore, as discussed in section 1.5.4, it is possible that coherent activation of multiple auditory nerve afferents can enhance the temporal precision of the cells within the auditory brainstem nuclei (Joris et al., 2004; Oertel et al., 2000). Therefore, a loss of afferent fibres may subsequently reduce the temporal encoding ability of these cells, directly impacting temporal acuity in affected individuals.

While the other auditory assessments such as speech perception and binaural processing ability are also significantly correlated with the AFD results, it is likely that these deficits are in part a consequence of the disruption in temporal coding. For instance, elevated gap detection thresholds can directly impact on the ability to distinguish voice onset times, the period that passes between the release of a stop consonant and the onset of voicing (Rance et al., 2012e; Rance & Starr, 2015). Discrimination of voice onset times is essential to differentiate between voiced and unvoiced stop-consonants. Hence, it is likely that an inability to integrate and perceive these important speech cues contributes to the poor speech perception ability exhibited in affected individuals. Similarly, the disruption in temporal processing

ability is also likely to distort interaural timing comparisons, essential for binaural speech perception and localisation of sounds.

4.4.1 Limitations

The small sample size of the AN group was the most significant limiting factor of this study. The stringent inclusion and exclusion criteria meant that individuals with AN who had received a cochlear implant could not participate. Given cochlear implantation is the rehabilitation strategy of choice for individuals diagnosed with AN, this had a significant impact on recruitment numbers. Alongside the small sample size, the relatively narrow range of AN aetiologies in the current study was another limitation. A broader range of aetiologies, in which the site of lesion is known, would be beneficial in determining the accuracy of the AFD metric and could provide important information regarding differences in lesion sites between aetiologies. Another important consideration regarding the results obtained in this study is the fact that the VIII vestibulocochlear nerve is composed of both the auditory nerve (supplying the cochlea) as well as the superior and inferior vestibular nerves (supplying the vestibular organs). These nerves run within the internal auditory canal in close proximity to the facial nerve. The dMRI techniques used in this study are not able to distinguish between the different components of the VIII nerve and facial nerve, which therefore reduces the specificity of this technique. Given the results from the auditory measures along with no reported facial or balance deficits, it is likely that the reduced AFD results observed in the AN participants originated from degeneration of the auditory nerve, rather than from changes within the vestibular or facial nerves. It has, however, been shown that both auditory and vestibular pathways can be affected for some aetiologies responsible for the AN phenotype (Moser & Starr, 2016; Sheykholeslami et al., 2000), and therefore, comprehensive assessments of vestibular function may be useful in future research to determine whether both the auditory and vestibular pathways are affected. Furthermore, the study could have benefitted from including a control group with cochlear sensory hearing loss. This would allow AFD values to be examined in another group of individuals who also exhibit impaired hearing ability as a result of a different, but better understood, pathophysiological process (i.e. cochlear sensory damage). This could provide further details regarding the sensitivity and accuracy of the AFD metric in identifying axonal degeneration.

4.4.2 Conclusion

The results of this study provided important insights into the degree of VIII nerve axonal degeneration in individuals with AN due to a range of aetiologies, a result which may prove to be diagnostically useful in the future. To date, this is the first study to use dMRI-based quantitative analysis techniques to explore changes in the VIII nerve in individuals with the AN phenotype due to a range of different aetiologies. Previous research has relied on post-mortem histopathological analysis methods, which are vital for validation of *in-vivo* techniques, like those used in this study, but are of no prognostic value to

the affected individuals from whom samples are obtained. Hence, the AFD metric could have significant implications for patient counselling regarding rehabilitation options. This is particularly relevant to potential cochlear implant recipients where the AFD metric may provide valuable information regarding the integrity of the auditory nerve afferent fibres, which are responsible for relaying the signal from the cochlear implant electrode array to the rest of the central auditory nervous system. Further research, however, is still required to determine the accuracy of the AFD metric in determining the degree of neural degeneration in an affected individual, and to establish the extent of axonal loss, as reflected by the AFD measure, that corresponds to poor cochlear implant outcomes.

Chapter 5: General Discussion

The studies presented within this thesis investigated the use of non-invasive dMRI-based analysis techniques, including tractography and the fixel-based analysis framework, to map the auditory brainstem white matter pathways, and study the integrity of these subcortical structures in individuals with AN. The impetus for this thesis was borne out of the limitations in current diagnostic assessments in identifying lesion sites *and* the extent of disruption in affected individuals (Rance & Starr, 2015) and built on preliminary studies investigating the human auditory brainstem using dMRI techniques (Liu, 2015; Nayagam, 2016).

As discussed, current behavioural audiological assessments can provide important information regarding the functional impairments experienced by individuals with AN, and objective tests can be used to assess the integrity of the cochlear sensory cells, including the inner and outer hair cells, as well as the activity of the auditory nerve. Such assessments have elucidated some of the sites of lesion responsible for the AN phenotype, and both presynaptic and postsynaptic structures of the auditory system have been shown to be affected. At present, however, no available tests can identify *in-vivo/in-situ* the degree of neural degeneration in affected individuals, which has important implications regarding management strategies and rehabilitation outcomes. This is particularly relevant to cochlear implantation, which is currently the intervention strategy of choice for AN. Cochlear implantation outcomes, however, are largely dependent on the integrity of postsynaptic structures, such as the spiral ganglion neurons and auditory nerve axons, involved in transmitting the electrical signal from the cochlear implant to the rest of the central auditory nervous system. Hence, variable results have been reported for individuals with AN due to postsynaptic lesion sites, with some affected individuals attaining speech perception results similar to their cochlear sensory hearing loss peers, whereas others achieve no benefit at all. Electrically-evoked ABRs, when obtained via the implanted intra-cochlear electrode array of a cochlear implant, can provide a good indication of post-operative implant outcomes in affected individuals. Unfortunately, in this scenario, even if no responses are recordable, the implant surgery has already been completed and therefore no further action can be taken. Alternatively, electrically-evoked ABRs can also be recorded using a trans-tympanic electrode, however, results are often inconsistent and/or difficult to interpret (Gibson & Sanli, 2007; Nikolopoulos et al., 2000; Pau et al., 2006). Hence, there remains a paucity of reliable diagnostic assessments that can inform potential cochlear implant candidates with AN of their likelihood of obtaining good post-operative outcomes.

To this end, the potential of using dMRI-based analysis methods to investigate the subcortical white matter tracts of the auditory system was explored. Since virtually no research had been conducted in this area, our initial approach was to determine the feasibility of using these techniques to map the major subcortical auditory pathways occurring between the cochlear nerve and the inferior colliculus. These structures or ‘regions of interest’ were specifically selected due to being relatively easy to identify on

MR images obtained at typical magnetic field strengths of 3 Tesla. Other subcortical nuclei of the auditory system, such as the CN, SOC, and lateral lemniscus, were too small to be adequately rendered on anatomical and diffusion MR images at this field strength and therefore they were not included in our analysis. As discussed in detail in Chapter 2, the tract reconstructions obtained using SS3T-CSD and probabilistic tractography were consistent with the known anatomical structure of the auditory brainstem pathways. That is, the VIII nerve was clearly visible, as were the collateral connections between the CN and IC. Moreover, the major tracts running between the ipsilateral and contralateral inferior colliculi were also rendered according to the known anatomical structure. The findings of Chapter 2 paved the way for further research conducted in Chapter 3 and Chapter 4 using these higher-order dMRI analysis techniques. Comparisons between the traditional and widely adopted diffusion tensor model and the higher-order SS3T-CSD model, showed that the latter was far superior in delineating the tracts of the auditory brainstem. In fact, due to the high proportion of crossing fibres within the subcortical auditory pathways DTI failed to reconstruct any streamlines between the VIII nerve and IC. Attempts to further refine the SS3T-CSD technique using the Anatomically Constrained Tractography algorithm (Smith et al., 2015) were also unsuccessful, with the tracts appearing to contain many false positives and false negatives. Although the ACT framework has been used to improve tract reconstructions in other sensory and motor systems, this was the first time it was applied to study the white matter tracts of the auditory system. The ACT algorithm, however, did not improve the delineation of the subcortical auditory pathways in our research. Upon further analysis, limitations in the tissue segmentation step were identified as the reason for the poor results obtained. The ACT framework relies on the tools provided by FSL to segment a co-registered T1-weighted image. In this study, it was made apparent that the segmentation step could not accurately identify the entry point of the VIII nerve into the brainstem, incorrectly categorising this region as GM or CSF and excluding it from the downstream tractography analysis steps. Many other MRI-based segmentation tools exist, such as Freesurfer (<http://freesurfer.net/>) (Fischl, 2012) and BrainSuite (<http://brainsuite.org/>) (Shattuck & Leahy, 2002), however, minimal research has been undertaken to validate these segmentation tools for the brainstem region and therefore such alternate methods are likely to suffer from similar issues as the FSL tool used as part of the ACT algorithm in Chapter 2.

Another important finding obtained in Chapter 2 was the consistency in the auditory brainstem tract reconstructions observed between the three normal-hearing participants. While some variation between structures was observed, the pathways appeared largely similar, even for the youngest participant in the study (~5 months of age). Compared to studies performed on children or adults, dMRI of infants and babies presents a unique set of challenges owing to the smaller head size (increased risk of partial voluming), higher proportion of GM, increased risk of image distortion due to movement, and significantly larger water-content and lower anisotropy due to incomplete myelination of WM fibres (Groeschel et al., 2010; Ouyang et al., 2019; Pannek et al., 2014). Image quality is also further

compromised due to the small size of the brain/brainstem in babies, which makes baby dMRI data more susceptible to off-resonance distortions that occur at air/tissue and bone/tissue interfaces. Postnatal changes in brain development are the most significant from birth to ~2 years of age, a period in which the overall size of the brain increases substantially, reaching approximately 90% of the adult volume by 2 years of age (Pfefferbaum et al., 1994). The structural changes that the brain undergoes during this period is the result of many highly regulated molecular and cellular processes, which mediate synapse formation, axonal growth, and myelin formation (Innocenti & Price, 2005; Miller et al., 2012). Hence, these added challenges can reduce the quality and accuracy of diffusion models and subsequent dMRI-based qualitative and quantitative analyses. While further validation studies are required, the findings and data collected for the study presented in Chapter 2 demonstrate the benefits of using SS3T-CSD on single-shell data obtained from infants and babies, which produces white matter-FODs that appear similar those obtained in older individuals (Dhollander et al., 2018).

Having shown that the higher-order diffusion model, SS3T-CSD, is capable of accurately characterising the auditory brainstem tracts between the VIII nerve and IC, Chapter 3 explored the applicability of SS3T-CSD-based quantitative techniques to assess the brainstem tracts in individuals with AN. Given the significant variability in aetiologies known to cause the AN phenotype, a homogenous group of affected individuals known to have a genetically characterised condition called X-linked auditory neuropathy (Wang et al., 2003; Wang et al., 2006) were selected to participate in this study. In these individuals the pathomolecular process underlying their auditory symptoms involves the disruption of the mitochondrial respiratory chain complex I, through mutations of the *AIFM1* gene, which is thought to result in focal axonal degeneration (Zong et al., 2015). In support of this assumption are anatomical MRI findings, which show that a proportion of affected individuals develop auditory nerve hypoplasia (Zong et al., 2015). Hence, as discussed in detail in Chapter 3, the fixel-based analysis framework (Raffelt et al., 2017) was used to identify regions of white matter changes within the brainstem in individuals with AUNX1 compared to normal-hearing controls. Moreover, the white matter microstructure of these tracts was further examined using measures of AFD (Raffelt et al., 2012b). The results are the first to show that dMRI-based quantitative analysis methods, such as the fixel-based analysis framework, can be used to identify neurodegenerative changes in structures as small as the cranial nerves. These findings alone have significant implications and are relevant to research outside of the auditory system (Hodaie et al., 2010; Policeni & Smoker, 2008). Moreover, this is also the first study to use such techniques to study the neural dysfunction occurring in the auditory brainstem pathways in individuals with a non-syndromic type of auditory neuropathy known as AUNX1. Consistent with our initial hypothesis, the AFD results showed significant axonal degeneration within the auditory brainstem pathways running between the VIII nerve and IC in affected individuals compared to normal hearing control subjects. The AFD results were also consistent with objective auditory assessments, which suggested abnormal auditory nerve function in the presence of normal

cochlear sensory hair cell function. Furthermore, AFD values obtained from individuals with AUNX1 were significantly correlated with temporal processing ability, as measured by a gap detection task, suggesting that the degree of VIII nerve degeneration directly impacts the ability to resolve temporal aspects of an acoustic signal. Overall, the findings presented in Chapter 3 are in agreement with previous histopathological analyses of individuals with AN (Hallpike et al., 1980; Sagers et al., 2017; Starr et al., 2003), and with the phenomenological model of AN proposed by Zeng et al. (1999), in which both a loss of neural elements and loss of synchrony within the auditory system is predicted to explain the perceptual deficits experienced by individuals with the condition. The findings of this study also demonstrate the potential for dMRI-based quantitative analysis methods to aid in the identification of lesion sites and pathophysiological processes in AN.

Finally, Chapter 4 explored the potential for the AFD metric to provide information regarding the white matter integrity of the VIII nerve in individuals with AN due to various aetiologies. Some of these are considered to give rise to the AN phenotype due to a pre-synaptic site of lesion, such as neonatal hypoxia, while others are thought to disrupt post-synaptic structures, including FRDA, CMT2, and RTD. Participants in this study differed significantly not only in the underlying aetiology but also in the severity of their hearing impairment, their age, and the duration of their disease. Despite these variations, results of this study showed that, as a group, the participants with auditory neuropathy had lower AFD values compared to the normal hearing age-matched controls. The AFD results, however, varied significantly, with some affected participants showing results similar to their normal hearing peers. The observed variability in AFD results obtained was consistent with the heterogeneous nature of the AN group and the degree of VIII nerve degeneration reflected the severity of perceptual deficits, as characterised by a range of auditory assessments. For instance, the three individuals with RTD, a progressive hereditary neurodegenerative condition, exhibited the most significant VIII nerve degeneration, and were also among the most significantly affected in terms of their perceptual ability. Comparatively, the individuals with the neurodegenerative conditions FRDA and CMT disease, exhibited minimal VIII nerve degeneration as measured by AFD. These two participants, however, were also younger, at earlier stages of disease progression, had fewer perceptual difficulties and less auditory nerve disruption, as evidenced by the ABR results. For participants who suffered neonatal hypoxia, the AFD results were again more variable. While hypoxia is thought to exclusively damage the inner hair cells (Amatuzzi et al., 2011; Amatuzzi et al., 2001; Salvi et al., 1999), it is probable that over time, due to a lack of stimulation, the auditory nerve afferent fibres become atrophied and subsequently degenerate. Evidence that this is likely to occur comes from animal studies of noise-induced changes to cochlear sensory structure, in which auditory afferent fibres are shown to degenerate as a result of damaged inner hair cell pre-synaptic ribbons (Furman et al., 2013; Liberman et al., 2016). Overall, these findings build on those obtained in Chapter 3 and suggest that the AFD metric may prove to be a useful

adjunct to existing auditory assessments in the characterisation of AN, which may improve diagnostic specificity and guide rehabilitation options for affected individuals in the future.

5.1 Clinical implications

The findings from the studies contained within this PhD thesis have several implications for clinical practice. Firstly, the fixel-based analysis and AFD metric could provide important information regarding the integrity of postsynaptic structures, such as the auditory nerve axons and brainstem pathways, in individuals with AN, which has the potential to impact decisions surrounding rehabilitation strategies. This is especially relevant to choices regarding cochlear implantation, as outcomes are reliant on at least some intact and functional postsynaptic structures. Secondly, the AFD metric may also be of significant prognostic value for individuals with long-term (post-lingual) auditory deprivation who are considering cochlear implantation. Similar to individuals with AN, post-operative speech perception outcomes for this cohort has also been shown to vary considerably and this variability is thought to be related to the degree of neural degeneration of post-synaptic auditory structures (Briaire & Frijns, 2006; Incesulu & Nadol Jr, 1998; Tyler & Summerfield, 1996). Finally, tractography-based delineation of the auditory brainstem pathways may provide important information for neurosurgical planning and could be used as a guide for the placement of auditory brainstem implants.

While the clinical implications are promising, there are a number of factors which need to be considered before an experimental technique, such as the fixel-based analysis framework, can be implemented as a clinical diagnostic tool to accompany the currently adopted test battery in the diagnosis of AN. Firstly, the MRtrix3 software package used to analyse the dMRI datasets within the three studies of this thesis is currently only available for research purposes. As such, MRtrix3 would require Therapeutic Goods Administration (TGA) approval before it could be used for clinical purposes in Australia. This is of course feasible; however, it would require considerable funding and further research to validate the product. Secondly, the fixel-based analysis framework and AFD metric need to be applicable to infants (<5 months of age), as early diagnosis and intervention is essential to ensuring speech and language development equivalent to infants with cochlear sensory hearing loss can be achieved. While SS3T-CSD-based probabilistic tractography is capable of delineating the WM pathways from the VIII nerve to the IC in a baby 5 months of age, as shown in Chapter 2, further studies are required to determine if this is also possible in infants (<1 month). The same applies to the fixel-based analysis and AFD metric, which have not yet been used to investigate potential lesion sites/extent of dysfunction in affected infants. Finally, appropriate management guidelines are also required for individuals with AN who are identified as poor candidates (i.e. individuals with severe axonal degeneration as identified by the AFD metric).

5.2 Future directions

The results reported in Chapter 2 emphasise the need for validation studies, using neuronal tracers or microdissection techniques, to confirm the accuracy and validity of dMRI-based tractography techniques in delineating the subcortical pathways of the auditory system. Currently, no such evidence exists, making it difficult to interpret qualitative changes in white matter connectivity that may be occurring within the auditory system in a given individual. While this is also an issue for tractography studies of other sensory and also motor neural systems (Maier-Hein et al., 2017; Pujol et al., 2015; Schilling et al., 2019; Thomas et al., 2014), validation studies are particularly salient for the subcortical auditory tracts due to their small diameter and complex configurations, which can directly affect the accuracy of tractography-based reconstructions. For example, several studies have shown that tractography algorithms frequently produce a greater number of false positives and false negatives for smaller, more complex pathways in the brainstem compared to the larger neuronal structures within the brain (Ford et al., 2013; Maffei et al., 2018; Maffei et al., 2015; Mori & Tournier, 2013).

The results obtained in Chapter 2 also emphasise the importance of obtaining dMRI data with a sufficiently high spatial resolution, which improves the ability to identify the subcortical nuclei of the auditory system (Sitek et al., 2019). With the development of higher-order models and improved access to MRI scanners with higher field strengths there has been a renewed interest in the application of dMRI to study smaller, more complex white matter pathways, like those of the auditory system. As shown by Sitek et al. (2019) and García-Gomar et al. (2019), MRI scanners with higher field strengths (i.e. 7 – 11T) can produce imaging data of higher quality with much greater spatial resolution (isotropic voxel size of ~ 1.05 mm) compared to the 3T scanners typically available for clinical use, improving the ability to observe smaller structures within the brain and brainstem. Such improved spatial resolution is likely to allow for a greater number of auditory subcortical tracts to be reconstructed using tractography, which could subsequently enable more comprehensive and tract-specific investigation of potential changes between clinical populations to be carried out. While collecting dMRI data using ultra-high field strengths, however, introduces its own unique set of problems, such as increased image distortions due to B_0 field inhomogeneity and eddy-currents, as well as a rise in chemical shift artefacts, future developments are likely to find suitable ways to ameliorate such issues (Vargas et al., 2018; Zanin et al., 2019). Moreover, access to these types of MRI scanners is currently limited, but this is also likely to change in the future as the need for such equipment grows.

The results from Chapter 3 and Chapter 4 are promising, and show the potential of dMRI-based quantitative analysis tools to improve decisions surrounding rehabilitation options, particularly in regard to cochlear implantation, in individuals with AN. Further research, however, is required to determine how AFD values correlate with post-operative speech-perception ability and if the technique is accurate enough to predict whether affected individuals will receive sufficient benefit from an

implant. Furthermore, while the amount of surviving neural elements, such as spiral ganglion neurons and auditory afferent axons, is linked to post-operative implant outcomes (Linthicum & Anderson, 1991), it is possible that other additional factors, such as electrode placement and stimulation patterns, are also involved, and hence further investigation is required to determine the extent such factors contribute to poor outcomes (Finley & Skinner, 2008).

With the improvement of both MRI equipment and dMRI analysis techniques, it may be possible to assess each of the internal auditory canal components separately, including the cochlear nerve, superior vestibular nerve, inferior vestibular nerve, and facial nerve. As current methods of diagnosis are based on the visual assessment of these four nerves and a comparative analysis of size, improved techniques may enhance the ability to diagnose cochlear nerve hypoplasia or other abnormalities affecting the vestibular or facial nerves. This may also improve the prognostic value of the dMRI quantitative analysis in determining the potential success of cochlear implantation.

Finally, while the fixel-based analysis framework and AFD metric can identify regions undergoing neurodegenerative changes, more advanced imaging tools, with the ability to examine structures within the cochlea at the cellular level are also required. Currently, methods such as two photon microscopy (Yang et al., 2012) or micro-optical coherence tomography (Iyer et al., 2016) have shown the ability to image the cochlear sensory hair cells in animal studies. These techniques, however, are not suitable for use on humans since they require the instruments to be inserted through the tympanic membrane and round/oval window to a location inside the cochlea. With future advancements, however, this experimental work, may further elucidate some of the disease mechanisms in humans and has the potential to improve our understanding and management of different diseases that affect the auditory system, including AN.

Conclusions

This thesis explored the use of dMRI-based qualitative and quantitative analysis techniques to study the auditory subcortical white matter tracts *in-vivo* in individuals with AN through three distinct studies. Together, the findings indicate that higher-order diffusion models, such as SS3T-CSD, in conjunction with probabilistic tractography algorithms are capable of delineating the subcortical pathways between the VIII vestibulocochlear nerve and the IC. Furthermore, dMRI quantitative analysis techniques, such as the fixel-based analysis framework and especially the AFD metric, have the potential to provide clinically valuable information regarding cochlear implant suitability in individuals diagnosed with AN, irrespective of aetiology.

More generally, the development of dMRI-based qualitative and quantitative analysis techniques has revolutionised the study of white matter pathways within the brain and brainstem. Prior to its inception, our knowledge and understanding of neural connections and underlying processes in neurodegenerative

disease progression were based on animal studies and human post-mortem investigations using neuronal tracers and histopathological analysis techniques. Currently, however, a relatively short MRI scan can capture enough information that the white matter pathways of different sensory and motor systems can be studied in detail *in-vivo* without the need for contrast agents or other invasive procedures. While relatively limited research exists that document the use of such techniques to study changes within the auditory system, especially the subcortical auditory pathways, the studies contained within this thesis highlight the considerable potential of these techniques to provide clinically useful information not currently available with established diagnostic assessments. The clinical data provided by these methods has the potential to guide intervention recommendations for a given individual, which could directly impact patient outcomes.

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Appendices

Appendix 1: Review article

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Review Article

Review: Using diffusion-weighted magnetic resonance imaging techniques to explore the microstructure and connectivity of subcortical white matter tracts in the human auditory system



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ABSTRACT

Since its inception 30 years ago, diffusion-weighted magnetic resonance imaging (dMRI) has advanced to become a common component of routine clinical MRI examinations. Diffusion-weighted magnetic resonance offers a way to measure anisotropic diffusion *in-vivo*, which has led to the development of techniques capable of characterising the orientation of diffusion within living tissue. These modelling techniques can be used to investigate the microstructure and connectivity of white matter tracts within the human brain. Such techniques have been used to study many neural networks within the human body. There is, however, a notable paucity of research utilising dMRI techniques to investigate the white matter tracts of the auditory brainstem. In this review we provide a brief introduction to the basic principles of dMRI analysis and consider some of the difficulties associated with applying dMRI techniques to study the auditory pathways of the brainstem. We also consider aspects of current dMRI methodologies relevant to the auditory brainstem to inform future research in this area.

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1. Introduction

Knowledge of the anatomical structure and organisation of the

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human auditory system has largely been inferred from post-mortem studies of experimental animal models (Heid et al., 1997; Kaas and Hackett, 2000; Robertson et al., 1987; Scott and Johnsrude, 2003). Historically, neuronal tracing methods have been used to study the axonal pathways and their connections within the subcortical and cortical tracts of the auditory system. These methods involve the injection of a histochemical tracer into target brain regions in a living subject. Synaptic connectivity can then be examined in the brain post-mortem (Köbber et al., 2000). Such methods are invasive and require demanding surgical procedures which can only be performed in a laboratory setting, rendering them unsuitable for use in human studies (Molnár et al., 2006).

Alternative non-invasive, electrophysiological assessments such as the auditory brainstem response (ABR) and cortical auditory evoked potentials have been used both clinically and experimentally to measure aspects of different neural components of the auditory system. Although these provide a useful assessment of the degree of hearing (or hearing loss), they are somewhat more limited in the information they can provide as to lesion location, particularly in cases where subjects have substantial sensory hearing loss (Hood, 1998). Furthermore, these assessments provide little insight into the microstructure or connectivity of neural fibres and are therefore limited in their ability to precisely identify sites of dysfunction within the auditory pathway. More targeted and sensitive diagnostic assessments could contribute to a more accurate identification of lesion sites, assessment of disease processes and possible neural pathway changes in a number of hearing disorders, including auditory neuropathy (Rance and Starr, 2015), tinnitus (Lockwood et al., 1998; Möller, 2003; Möller et al., 1992), axonal neuropathies, and demyelinating disorders (Rance et al., 2012; Rance and Starr, 2015). Using improved methods to identify gross structural changes in white matter connectivity, it may be possible to provide and/or develop more targeted management strategies for patients in these disease cohorts.

1.1. Overview of diffusion-weighted magnetic resonance imaging

Diffusion-weighted magnetic resonance imaging (dMRI) is currently the only available method to assess and study the microstructure of white matter of the brain in detail. Additionally, dMRI allows this to be achieved *in-vivo*, non-invasively and without the use of ionising radiation. Diffusion-weighted MRI itself is a data acquisition strategy, which produces images that are sensitive to the orientation and magnitude of water diffusion within a given tissue (Basser et al., 1994b). The white matter tracts of the brain are composed of coherently oriented and tightly packed bundles of myelinated axons. Thus, the diffusion process in these tracts is highly hindered or even restricted, and anisotropic - as water diffuses more freely along the orientation parallel to the axons but is much more hindered along orientations perpendicular to them. Conversely in cerebrospinal fluid, for example, the diffusion process is considered unhindered and isotropic (Beaulieu, 2002). By capturing the diffusion-weighted MR signal for several different orientations, an orientational diffusion profile can be obtained within each imaging voxel.

Whilst the term "dMRI" refers to the specific diffusion-sensitive image contrasts obtained through the application of diffusion gradients (pulsed magnetic field gradients sensitive to molecular diffusion), the similar sounding popular term "diffusion tensor imaging" (DTI) refers to a type of modelling technique used to model dMRI datasets (Basser et al., 1994a). Diffusion tensor imaging was proposed over 20 years ago as the first rotationally-invariant model to assess the anisotropic diffusion properties of white matter (Basser et al., 1994a). Diffusion tensor imaging is a simplified

model of the 3-dimensional diffusion process, which aims to characterise diffusivity, including to identify the main diffusion orientation, within each voxel (a 3-dimensional pixel; the smallest component of the image). These orientations can subsequently be used to create virtual maps of continuous white matter pathways within the brain and brainstem. Techniques to generate such virtual white matter pathways are most commonly referred to as tractography methods (see Jeurissen et al., (2017) for review). Although numerous different tractography approaches exist, many (but not all) of the algorithms currently in use are often classified as being either deterministic or probabilistic. Deterministic tractography algorithms typically reconstruct white matter tracts by stepping along a single (pre-computed) orientation from voxel to voxel to generate trajectories known as 'streamlines', that can be used collectively to infer larger fibre bundles. Tractography is typically initiated from user defined seed points or regions, and streamlines are produced until some termination criterion is fulfilled (i.e. anisotropy threshold, curvature constraint, etc.). Probabilistic tractography algorithms consider a range of fibre orientations in each voxel based on particular uncertainties in the data, processing or modelling assumptions. Hence, rather than consistently tracing the same path for a given seed point, each successive streamline will differ according to the estimated orientations and uncertainty of the data (Jeurissen et al., 2017; Mori and Tournier, 2013). Through the application of such algorithms, tractography maps can be generated for the whole-brain or for specific white matter tracts using a regions-of-interest (ROIs) based tractography approach. However, it is important to be aware that mapping white matter pathways in this manner is not selective for afferent or efferent connections, as it is not possible to differentiate between these fibre types using dMRI (Van Hecke et al., 2015). Moreover, these techniques do not provide the ability to identify or interrogate the function of synapses or provide details regarding the functionality of a given pathway.

In addition to providing orientation information for dMRI tractography, the diffusion tensor can also provide quantitative indices that are sensitive to certain properties of the local microstructure of white matter tracts. The most common of such indices are the mean diffusivity (MD), which reflects the average amount of freedom of diffusion in each voxel and the fractional anisotropy (FA), which provides a measure of how much the diffusion process in each voxel deviates from isotropy. These metrics have been widely used to detect changes and/or differences in underlying tissue microstructure. For example, a loss of axons, or other types of axonal damage are often reflected in the FA and MD values obtained from the DTI model. Diffusion tensor imaging measures can be computed for the whole-brain and/or specific regions-of-interest (ROIs). Additionally, the measures can also be compared in a voxel-wise fashion across the whole brain (voxel-based analysis), which then requires image registration to match spatial positions across subjects (Ashburner and Friston, 2000). These assessments differ from tractography-based analyses as they do not attempt to reconstruct WM fibre connections within the brain or brainstem; they are purely focused on comparing quantitative indices. Each technique has its own set of advantages and disadvantages. Choosing the most appropriate, depends on numerous factors including: the research question, the group size or sizes (i.e. single subject vs. group analysis), and whether there is a hypothesis regarding the site or extent of change (Van Hecke and Emsell, 2016).

Despite its widespread use in brain research, DTI has significant limitations (Jones et al., 2013). Some of these stem from the fact that the microstructure of interest is about 3 orders of magnitude smaller than typical voxel sizes (micrometres versus millimetres). The diffusion signal measured in each voxel is the ensemble average due to all combined microstructure in the voxel. This intra-

voxel heterogeneity of microstructure (white matter tract orientations as well as other tissues) is typically referred to as the partial volume effect. Diffusion tensor imaging reconstructions in particular have been shown to provide erroneous fibre directions in voxels that contain multiple distinct orientations of “crossing” axon tracts, as well as fanning, merging, kissing, bending or other complex configurations (Behrens et al., 2007). Such erroneous fibre orientation estimation can seriously compromise the reliability of subsequent fibre tracking based on such information. This is a significant limitation given that these types of multiple fibre orientations may occur in up to 90% of voxels containing white matter tracts (Behrens et al., 2003; Jeurissen et al., 2013). This shortcoming also has implications for the computation of DTI-based quantitative metrics. For example, studies have shown that voxel-averaged indices such as FA are confounded and erroneous, especially in areas of crossing white matter tracts, rendering interpretation of measured differences difficult and often inaccurate (Douaud et al., 2011; Pierpaoli et al., 2001). It should be noted that such voxel-averaged anisotropy measures can also be significantly affected by other partial volume effects (such as the proportion of anisotropic white matter (WM) to isotropic grey matter (GM) or cerebrospinal fluid (CSF) within a voxel), in addition to any “crossing fibre” effects.

Several alternative methods of modelling the diffusion process have been developed, which provide more anatomically accurate estimates of fibre tract orientations than the diffusion tensor (Farquharson et al., 2013; Wahl et al., 2009). Included in this extensive and growing list are techniques and models such as Q-ball imaging (Tuch, 2004), the diffusion orientation transform (Ozarslan et al., 2006), and a range of constrained spherical deconvolution (CSD) approaches (Dhollander and Connelly, 2016; Jeurissen et al., 2014; Tournier et al., 2007) to obtain a continuous so-called fibre orientation distribution (FOD). (For a more comprehensive description of the range of available dMRI analysis methods, see Tournier (2010)). These improved techniques and higher-order models have been specifically developed to provide alternative and more accurate and specific measures of white matter fibre density (Raffelt et al., 2012, 2017) that are not as easily confounded by fibre crossings. Mixture or multi-compartmental models such as multi-shell, multi-tissue CSD (MSMT-CSD; Jeurissen et al., 2014) and single-shell 3-tissue CSD (SS3T-CSD; Dhollander and Connelly, 2016) are also better able to deal with partial volume effects, improving the precision of tract reconstructions in WM voxels in the presence of other tissues and fluid. Such models are able to achieve this by obtaining response functions for different tissue types (e.g., WM, GM and CSF) and using this information to model the constituent tissue components within a given voxel. Emergence of some of these new methods of fibre modelling require diffusion data obtained with more advanced dMRI acquisition techniques. For example, some methods require the acquisition of high angular resolution diffusion imaging (HARDI; Tuch et al., 2002); this entails the acquisition of dMRI images using a significantly larger number of different diffusion gradient orientations, with far higher diffusion weighting (i.e. higher b-values) than required for DTI processing. Such an acquisition scheme captures a more detailed and accurate profile of the complex diffusion process within each voxel. As a result, image datasets are of better quality, containing much greater angular resolution information, enabling reconstruction using more advanced models such as CSD and Q-ball. As these techniques are further developed it is expected they will provide additional insights into the structural pathways and interconnectivity of the human auditory system, and thus offer other possible diagnostic applications for hearing disorders. As such, dMRI may prove to be an essential adjunct to the diagnostic assessments already

available, particularly in cases where the pathology remains undetermined following routine audiometric testing.

2. Brief anatomical review of the central auditory nervous system

The human auditory system has a complex arrangement of interconnected subcortical nuclei and cortical regions. The afferent white matter tracts of the auditory system commence at the level of the cochlea, with myelination starting to appear as the fibres enter the osseous spiral lamina (Liberman and Oliver, 1984; Spoendlin and Schrott, 1989). Afferent fibres from the cochlea travel via the VIII vestibulocochlear cranial nerve to bifurcate and synapse on more specialised cell types within the cochlear nucleus (CN). The CN is considered to be the first processing and relay station within the auditory system. The CN itself cannot be directly visualised on structural MR images; however, its location can be gauged based on the surrounding structures, which can be easily identified on high-resolution T2-weighted structural images. For example, the CN is located at the junction between the medulla and the pons at the cerebellopontine angle with an inferior aspect located at the entry point of the VIII nerve. The CN lies on the superficial edge of the inferior cerebellar peduncle, and at the anterior edge of the foramen of Luschka (Abe and Rhoton, 2006). Fig. 1 shows the approximate locations of a number of the auditory subcortical nuclei on T2-weighted structural images.

Afferent projections from the cochlear nuclei diverge, with fibres innervating all other nuclei of the auditory brainstem including the primary nuclei of the superior olivary complex (lateral superior olive; LSO, medial superior olive; MSO, and medial nucleus of the trapezoid body; MNTB), lateral lemniscus (LL) including the ventral and dorsal divisions, inferior colliculus (IC), and medial geniculate body (MGB). Furthermore, tracts do not exclusively run ipsilaterally. The majority of tracts decussate to innervate nuclei located in the contralateral brainstem, and commissural connections are also present at the level of the CN, LL, IC, MGB and the primary auditory cortex (AI). Fig. 2 shows a simplified schematic of the auditory system starting at the level of the inner hair cells in the cochlea, and including the subsequent primary ascending pathways. Along these pathways the auditory signal is processed to enhance temporal and spectral aspects of the signal for integration and analysis at higher levels. The subcortical nuclei also mediate the interaction of auditory inputs with other sensory modalities (Pickles, 2012). From the MGB the auditory radiations carry the filtered and processed acoustic information to the primary auditory cortex.

3. Use of dMRI for studying the auditory brainstem

To date, research utilising dMRI to study the auditory brainstem has been relatively limited. Maffei et al. (2015) provide a brief discussion on the delineation of auditory brainstem tracts using dMRI on a human cadaveric specimen. Importantly, the focus of their review, is predominantly on the use of tractography techniques to reconstruct the auditory radiations. In general, the paucity of available research in the area is likely due to the inherent difficulties in studying auditory tracts *in-vivo*. The subcortical tracts of the auditory brainstem are thin, have a complex structure, diverge and converge frequently and run in very close proximity to several other brainstem tracts, including those of the middle and superior cerebellar peduncle, medial lemniscus and corticospinal tracts. This complexity suggests that dMRI acquisitions might require a high spatial resolution to accurately capture the tracts of the auditory brainstem. The importance of spatial resolution in studying the brainstem pathways has for instance been evidenced

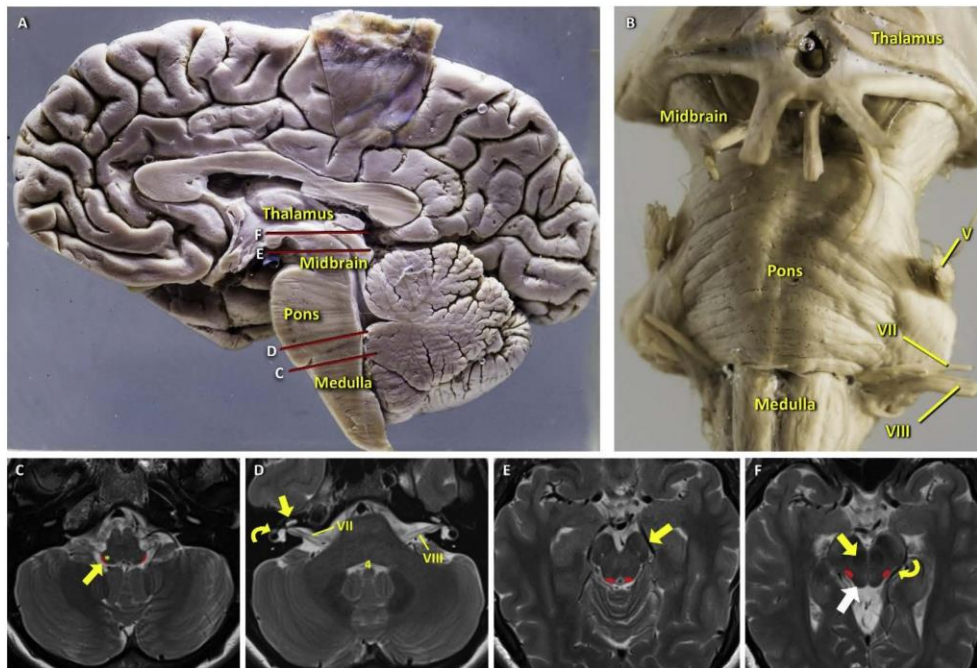


Fig. 1. Anatomical overview of prominent features of the auditory system. (A) Postmortem specimen showing a sagittal section through the midline of the encephalon. The divisions C, D, E and F correspond to the axial T2-weighted high-resolution images acquired in-vivo from a different subject (Fig. 1 C–F) showing some of the prominent features of the auditory pathway. (B) Anterior view of a postmortem specimen of the brainstem with notable divisions and features labelled. (C) Axial section at the pontomedullary junction showing the approximate location of the cochlear nucleus (red). The arrow identifies the foramen of Luschka and the location of the inferior cerebellar peduncle is shown by the asterisk (*). (D) Axial section at the level of the pons showing the cochlea (straight arrow), the semicircular canals (curved arrow), and the fourth ventricle (4). Cranial nerves VII and VIII are also labelled. (E) Axial section at the level of the midbrain showing the location of the inferior colliculi (red) and the posterior cerebellar artery (arrow). (F) Axial section at the level of the thalamus showing the location of the medial geniculate body (red), the red nucleus (straight yellow arrow), the superior colliculus (white arrow) and the basal vein (curved yellow arrow). The T2-weighted images were acquired from a single subject using a 3 T S scanner, with a 32-channel head coil receiver and the following parameters: voxel size $0.5 \times 0.5 \times 2.0$ mm, repetition/echo time = 4750/101 ms, flip angle = 150° , and acquisition time = ~5 min. Photography of wet specimens courtesy of Dr David Nayagam. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in a study conducted by Ford et al. (2013). They evaluated the impact of acquiring dMRI data using different spatial resolutions on several larger brainstem fascicles. It was found that tract reconstructions were artificially inflated and contained greater false positives when the spatial resolution was reduced from 0.33 mm^3 to 2 mm^3 . This issue, in part, arises from the challenge for tractography algorithms to identify between different tracts that traverse a single voxel (i.e. depending on the spatial resolution of the scan, a single voxel may contain fibre components from numerous tracts; Mori & Tournier, 2013; Alexander, 2005). This means that a tractography algorithm might start in part of the auditory tract, but deviate from the correct route and end up in a neighbouring tract (e.g. the corticospinal tract). To complicate matters further, it is likely that several other acquisition parameters such as angular resolution (i.e., the number of gradient directions), signal-to-noise ratio (SNR), and diffusion weighting(s), as well as the ability of diffusion modelling techniques to deal with complex fibre arrangements, all play an important role (Alexander, 2005).

Diffusion-weighted MRI studies of other brainstem tracts have also identified additional limitations that are likely to be encountered in studies of the auditory brainstem pathways. For example, conventional MRI and dMRI (T1-weighted, T2-weighted, FA maps etc.) rarely produce enough contrast for the subcortical nuclei of the brainstem to be easily and accurately identified (Deoni et al.,

2005; Ford et al., 2013; Nagae-Poetscher et al., 2004). This can make precise ROI placement problematic to the detriment of the accuracy of subsequent ROI-based analysis. Higher field strengths (e.g., 7 or 11 T) can improve contrast between nuclei, however such scanners are rarely available for diagnostic purposes. This issue is compounded in studies examining small subcortical nuclei, such as those found in the auditory system.

Another notable limitation is the inherent inability of tractography algorithms to identify synapses. This means both deterministic and probabilistic tracking algorithms are particularly prone to producing tract-reconstruction errors in pathways that contain multiple synapses, like those of the auditory brainstem (Mori & Tournier, 2013; Wakana et al., 2004). Errors are particularly likely to occur where two separate tracts, in close proximity, are running in parallel; when one of these tracts diverges at a synaptic terminal, tracking algorithms are unable to correctly differentiate which of the tracts diverge. Unfortunately, it is unlikely that a solution for this problem will eventuate in the near future, however, increased spatial resolution might assist in reducing the amount of errors that occur as a result of this.

3.1. Review of past dMRI studies of the auditory brainstem

A small number of studies have applied various imaging and

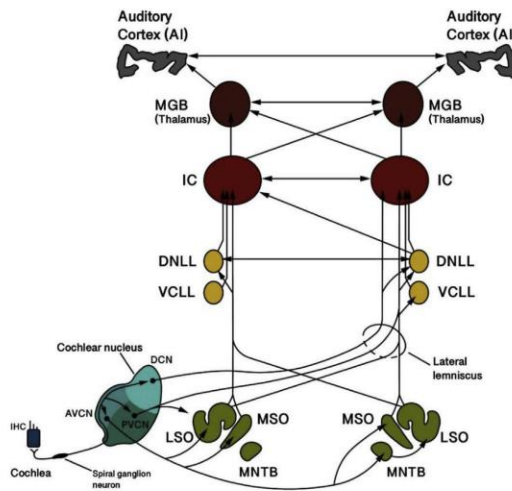


Fig. 2. Simplified schematic of the auditory system showing the major ascending pathways. Minor projections, including many of the inhibitory pathways are not included. The ascending pathway begins at the level of the cochlea with afferent fibres synapsing onto the inner hair cells (IHC). Collectively the AVCN (anterior ventral cochlear nucleus), PVCN (posterior ventral cochlear nucleus) and DCN (dorsal cochlear nucleus) constitutes the cochlear nucleus; the LSO (lateral superior olive), MSO (medial superior olive) and MNTB (medial nucleus of the trapezoid body) constitutes the superior olivary complex; the VCLL (ventral complex of the lateral lemniscus), DNLL (dorsal nucleus of the lateral lemniscus) forms the lateral lemniscus; IC (inferior colliculus); MGB (medial geniculate body). Modified from Pickles (2012).

analysis methodologies to extract quantitative metrics as well as produce connectivity maps of auditory subcortical and/or cortical pathways. Chang et al. (2004) were among the first researchers to examine the auditory pathway using DTI. The authors manually delineated regions-of-interest (ROIs) for each of the subjects in native space (i.e. using each subject's own diffusion-weighted MR images). Large circular ROIs with an average size of 49.5 mm² were used to separately delineate the superior olivary complex, trapezoid body, LL, IC and auditory radiations. However, no details were provided regarding the method used to identify each of the subcortical nuclei, which are typically not readily distinguishable on MR images. Diffusion characteristics of the ROIs were compared between a group of individuals with sensory hearing loss and a cohort of healthy controls. The study showed a reduction in FA values in each of the individuals with sensory hearing loss compared to their normal hearing counterparts, however the regions affected differed across subjects. Chang et al. (2004) suggested the reduction in FA within affected regions was due to axonal degeneration and/or loss of myelin, which is expected to occur because of auditory deprivation. These results are further supported by findings published by Lin et al. (2008) and Wu et al. (2009), who reported significant changes in FA values at the level of the LL and IC in individuals with hearing loss as compared to normal-hearing controls. Both studies applied a similar methodology to investigate white matter microstructure in individuals with sensory hearing loss and cochlear nerve deficiency (a condition in which the cochlear nerve is small or altogether absent). Fig. 3 shows the position of the ROIs on the dMRI datasets. The corresponding T1-weighted image typically has low contrast in this region (as shown in Fig. 3(b)), indicating the additional contrast and tract orientation information provided by dMRI, despite the

typically lower spatial resolution of the latter (i.e. voxel sizes in dMRI images are often about 2–2.5 mm³). The authors used the added contrast and orientation information provided by the diffusion data to guide the placement of the ROIs within the brainstem. Overall, the comparison of these two images emphasises the difficulty in identifying small subcortical nuclei using these different MR modalities individually, given their respective limitations.

The studies discussed focused on specific regions within the auditory brainstem to compare quantitative indices (i.e. fractional anisotropy, mean diffusivity etc.). Tractography was not incorporated in the analysis and hence, the tract may have not been delineated as accurately as would be possible by leveraging the tractography process. The type of ROI-based analysis utilised has both advantages and disadvantages over other analysis methods. In general, it is a relatively simple technique, which is less reliant on specific parameter settings than tractography or voxel-based analysis and does not require extensive know-how to implement. It does, however, require a good understanding of the anatomy and some prior knowledge about the affected regions (Van Hecke and Emsell, 2016). Therefore, a significant challenge and potential limitation of this technique is in the manual demarcation of the ROIs. Particular care has to be taken to ensure ROIs do not contain contaminants (i.e. other structures than those of interest) as these can alter the sensitivity of subsequent analysis. For instance, it has been shown that variation in the placement of ROIs on subject data can have substantial effects on FA and MD values observed (Astrakas and Argyropoulou, 2010; Pajevic and Basser, 2003; Vos et al., 2011). In comparison, the use of tractography can make the identification of specific tracts or regions of interest easier, more automated, and less precision is required in delineation of seeding and inclusion regions to guide tractography (Van Hecke and Emsell, 2016). For example, to delineate a specific tract or region, fewer roughly delineated seeding and inclusion regions are generally required when using tractography as the tracking algorithm can be relied upon to select the appropriate tracts that run between two or more identified regions and exclude those that are part of other structures. This benefits precision and reproducibility.

Variations in ROI placement can also have a significant impact when assessing individual or group differences. Hence, when performing such comparisons, it is recommended to spatially normalise the individual imaging data (Van Hecke and Emsell, 2016). Spatial normalisation allows all individual data to be registered into a common space, which allows for direct comparison of ROI delineation and enables more consistent ROI placement. Despite this advantage, detractors of spatial normalisation argue that such techniques result in a loss of detail due to spatial smoothing, often the consequence of interpolation of the imaging data. Hence, spatial normalisation can cause an increase in partial volume effects and reduce the accuracy of localising abnormal brain regions (Jones et al., 2005; Smith et al., 2006). Finally, both Wu et al. (2009) and Lin et al. (2008) used FA maps to aid identification of subcortical nuclei. It is, however, noted that it may be advisable to mark regions-of-interest independently of the diffusion measures being studied to avoid any bias in their placement (Jones and Cercignani, 2010).

The majority of published dMRI studies have acquired image datasets using an MRI scanner with a field strength of 3T. Prats-Galino et al. (2012) instead reviewed high spatial resolution diffusion data (0.43 × 0.43 × 0.43 mm) of a human *ex-vivo* brainstem specimen. The data was acquired with a 7T BioSpec 70/30 horizontal MRI scanner using a quadrature volume coil. This data was used to map the main auditory tracts running between the CN and LL using a DTI-based tractography method. The rationale for the higher field strength was to obtain images with much greater

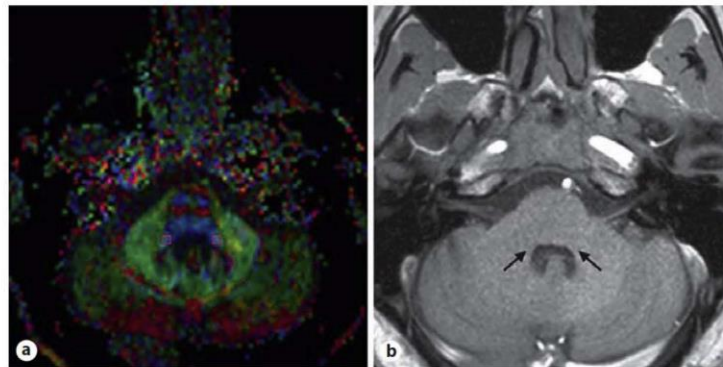


Fig. 3. (a) An example of the FA map at the level of the LL. The FA map is generated from the dMRI data and shows all the measured regions of anisotropic diffusion. Diffusion was modelled using the diffusion tensor. The colours represent the different directions of diffusion; Left-right is red, anterior-posterior is green, and superior-inferior is blue. The red squares are the regions-of-interest. Images were acquired on a 3 T scanner. Diffusion-weighted magnetic resonance imaging data was acquired using a single-shot echo planar imaging sequence along 12 noncollinear directions with a b-value of 1000 s/mm². (b) The corresponding T1-weighted structural MRI. The arrows indicate the region of the LL. FA: fractional anisotropy; LL: lateral lemniscus; dMRI: diffusion-weighted magnetic resonance imaging. Reproduced from Wu et al. (2009) (reprinted with permission of S. Karger AG, Basel). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

spatial resolution (Soria et al., 2011), while still retaining a reasonable SNR of the dMRI signals. It should be noted, however, that although higher field strengths improve image quality for structural MR images, this is not necessarily the case for diffusion imaging. In fact, with an increase in magnetic field strength there is a subsequent increase in image distortion by B_0 field inhomogeneity and eddy currents, as well as a rise in chemical shift artefacts (Vargas et al., 2018). These factors can negatively impact the quality of the acquired diffusion-weighted images (Choi et al., 2011; Curran et al., 2016), as well as the inherent spatial resolution of certain areas (in particular those which suffer more from these artefacts and distortions). Prats-Galino et al. (2012) reported tract reconstructions were largely consistent with anatomical reports; however, no quantitative data was obtained from the image datasets. Furthermore, the reconstruction of detailed connectivity maps including merging and branching tracts was also not possible (Fig. 4). These limitations were likely partially due to the inherent shortcomings of the DTI-based analysis methods that were employed. Additionally, because of lengthy scanning times the images were acquired only from human post-mortem specimens, restricting their real-world applications and limiting the contrast-to-noise ratio of the diffusion signal profiles. This is an important consideration for clinical applications, where patients may be limited in their capacity to lie still in an MR scanner for extended periods of time, either as a result of their age, disease progression, or both.

Berman et al. (2013) compared DTI based tractography to a Q-ball based fibre tracking algorithm in delineating auditory radiations in 25 subjects. The DTI tractography was performed on both 30-direction dMRI and 64-direction HARDI acquisition scheme, whereas Q-ball tractography was performed only on the HARDI data. To account for the variability inherent in manual ROI identification, the authors applied a specialised volume segmentation and white matter parcellation method to each participant's T1-weighted MR image. ROIs were based on the resulting labels obtained from this process. The starting ROI was located within Heschl's gyrus and the target ROI included the MGB. T1-weighted images were co-registered to the $b = 0$ (non diffusion-weighted) images obtained as part of the diffusion acquisition. In this study, an anatomical exclusion region (i.e. disallowing streamlines to

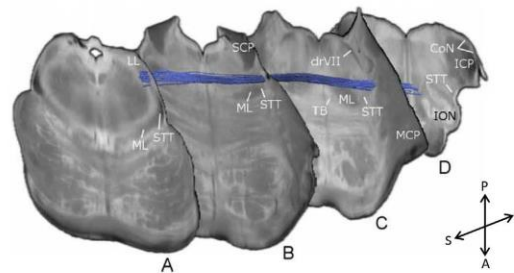


Fig. 4. Reconstruction using DTI-based tractography of the auditory fibres running between the cochlear nucleus (CoN) and lateral lemniscus (LL). Four high-resolution 7 T T1-weighted rostral-caudal sections of the brainstem are shown. Four anatomical directions are provided as a guide: I: inferior, S: superior, A: anterior, and P: posterior. (A) Pontomesencephalic junction. (B) Mid pons. (C) Lower pons. (D) Upper medulla. A spin echo diffusion tensor imaging sequence was used to acquire the diffusion-weighted data along 30 directions with a b-value of 1000 s/mm². The diffusion data was modelled using the diffusion tensor and tracts were reconstructed using diffusion tensor tractography. drVII: descending root of the facial nerve; ICP: inferior cerebellar peduncle; ION: inferior olivary nucleus; MCP: Middle cerebellar peduncle; ML: Medial lemniscus; SCP: superior cerebellar peduncle; STT: spinothalamic tract; TB: trapezoid body. Reproduced with permission from Prats-Galino et al. (2012).

traverse this area), based on neuroanatomical knowledge, was also incorporated. Evidence suggests that constraining tractography in this and other ways can improve the precision and accuracy of tract reconstructions (Wakana et al., 2007), however exclusion ROIs should be used sparingly as to avoid tailoring tracts too much to prior expectations (Caan, 2016). In this context, Berman et al. (2013) manually identified and excluded several large white matter tracts within the cortex from the tractography analysis to minimise errant fibre trajectories. Their findings showed that the diffusion tensor model consistently failed to accurately delineate the auditory radiations due to the presence of the inferior longitudinal fasciculus, a much larger white matter tract that crosses the auditory radiations. In contrast, the Q-ball based tractography method successfully delineated the auditory radiations with good accuracy. The authors

concluded that both a HARDI acquisition and more sophisticated diffusion models is necessary when assessing the auditory radiations, in order to account for crossing fibre tracts. Although the focus of this research was on the auditory radiations, this issue is likely to be exacerbated in tract-reconstructions of the subcortical auditory tracts due to the complex nature of the neuronal architecture. Hence, the findings of Berman et al. (2013) are also relevant to dMRI studies of the auditory brainstem.

Utilising both functional MRI (fMRI) and DTI, Javad et al. (2014) reconstructed the auditory radiations connecting the auditory cortex to the IC and MGB. Sound, pitch and melody areas of the auditory cortex were identified using fMRI and separately delineated as ROIs. Subcortical ROIs were identified on T1-weighted structural images. The inferior colliculi were identified as distinct circular regions on axial views and as obvious protrusions on sagittal planes. The location of the MGB was approximated based on surrounding anatomical landmarks, such as the substantia nigra and lateral geniculate body. To account for the crossing fibre problem, a multi-tensor ball-and-stick model was used (Behrens et al., 2007). Once tractography was performed, tracts were transformed from native space into template space (Montreal Neuroscience Institute template) for group comparisons. Tractography results showed good correlation to a 3-dimensional brain atlas based on myeloarchitectonic and cytoarchitectonic mapping (Burgel et al., 2006; Rademacher et al., 2002) (Fig. 5). The findings of the study suggest that fMRI could also be used to aid the identification of subcortical nuclei in the auditory system, which would allow for more accurate tract reconstruction with dMRI-based tractography methods. Furthermore, different fMRI paradigms could be utilised to pinpoint functionally distinct components within the auditory pathway. The one notable limitation of the described study was in the duration of the scan, which lasted approximately 100 min, restricting its use in a clinical setting.

To illustrate the level of detail that is achievable using a higher order modelling approach, we present an example of constrained spherical deconvolution (CSD) guided tractography within the auditory brainstem of a single living human subject in Fig. 6. Magnetic resonance image acquisition was performed on a 3 T scanner using a 32-channel head coil. Whole-brain dMRI data were acquired using a twice refocused, spin-echo, echo planar imaging (EPI) sequence for 64 gradient directions at $b = 3000 \text{ s/mm}^2$. The diffusion data was acquired using a voxel size of

$2.5 \times 2.5 \times 2.5 \text{ mm}^3$. Denoising and Gibbs ringing artefact removal was performed on the diffusion data (Kellner et al., 2016; Veraart et al., 2016a, 2016b). The data was also corrected for motion and eddy-current induced distortions (Andersson et al., 2016; Andersson and Sotiropoulos, 2016; Smith et al., 2004). The white matter fibre orientation distributions (FODs) as well as GM and CSF compartments were computed using single-shell 3-tissue CSD (SS3T-CSD; Dhollander and Connelly, 2016) with the 3-tissue response functions for WM, GM and CSF estimated directly from the data themselves (Dhollander et al., 2016). Tracts were seeded from the left CN to bilateral inferior colliculi using iFOD2, a probabilistic FOD guided tractography algorithm (Tournier et al., 2010). The cochlear nuclei and inferior colliculi were manually demarcated as the regions-of-interest based on their ease of identification on structural T1- and T2-weighted MR images. Results show good consistency with anatomical reports of the central auditory pathway (Pickles, 2012). Notably, the reconstructed fibres show a thick contralateral connection between the CN and IC; the weaker ipsilateral projections from the CN to the IC are also captured (Moore, 1991). Emerging CSD techniques such as these permit the computation of quantitative metrics that differ from the common DTI indices, including a fibre population specific measure of fibre density (Raffelt et al., 2012, 2017); such further analyses, however, have not been performed in this case.

4. Methodological considerations for dMRI studies of the auditory brainstem

In this review we provide a brief discussion of some of the available literature on the topic of dMRI of the auditory brainstem. The studies discussed here have applied various dMRI acquisition strategies, models of diffusion, tractography methods and quantitative analysis techniques in an attempt to investigate the white matter tracts of the auditory system. Development of acquisition and analysis methodologies in future research should be guided by the available literature and consider recent developments in dMRI techniques in order to maximise the accuracy and reproducibility of findings.

4.1. Image acquisition considerations

Imaging acquisition parameters require careful consideration

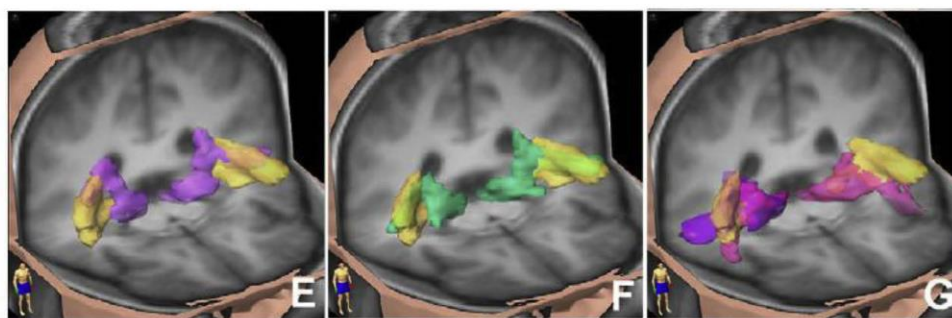


Fig. 5. Three-dimensional T1-weighted images of group data in MNI space. All images were acquired on a 3 T scanner. A spin-echo double refocused echo planar imaging sequence was used to collect the diffusion data over 64 diffusion-sensitising gradient directions with a b -value of 1400 s/mm^2 . Diffusion data was modelled using a multi-tensor ball-and-stick model and tractography was performed with a probabilistic tracking algorithm. (E) light purple: tract connecting the sound area to the IC and MGB; (F) green: tract connecting pitch area to the IC and MGB; (G) magenta: tract connecting the melody area to the IC and MGB. In E, F and G, tracts from the histologic brain atlas (Burgel et al., 2006) are shown in yellow. IC: inferior colliculus; MGB: medial geniculate body. Reproduced from Javad et al. (2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

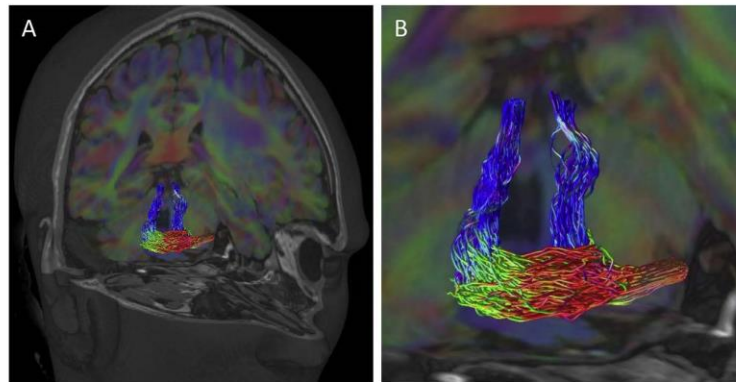


Fig. 6. Examples of a single-shell 3-tissue constrained spherical deconvolution tractography segmentation of the auditory brainstem in a single subject. Tracts were seeded from the left cochlear nucleus to bilateral inferior colliculi. Tract orientation is represented by the different colours and follows the directionally encoded colour (DEC) convention; Left-right is red, anterior-posterior is green, and superior-inferior is blue. Panchromatic sharpening was used to aid in the delineation of tracts and nuclei (Dhollander et al., 2015a, 2015b). (A) Three-dimensional render of a panchromatic sharpened T1-weighted image showing the position of the tract within the brain. A close-up of the tract is shown in (B). Images were generated using MRtrix3 software (www.mrtrix.org). Informed consent was obtained for the acquisition of these images (HREC approval 15/1254H, Royal Victorian Eye and Ear Hospital). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

for dMRI studies of the auditory brainstem. Selection of appropriate parameters will be dependent on what question(s) are being asked of the data and the dMRI analysis methods being utilised. In the ideal situation acquisition parameters would include high spatial resolution, high SNR, high diffusion weighting (which provides angular contrast), and a large number of gradient directions (i.e. angular resolution). For *in-vivo* studies, however, acquisition time remains the limiting factor and therefore acquisition parameters need to be carefully selected to account for this. Here we consider some acquisition parameters particularly relevant to dMRI studies of the auditory brainstem *in-vivo*:

- As noted, the white matter fibre bundles of the auditory brainstem are of a thin calibre compared to other tracts occupying the same space. Therefore, to ensure the auditory brainstem pathways are discernible, data should be acquired using the smallest isotropic voxel size that the scanner and acquisition time permits. However, this typically also impacts on the SNR.
- In addition to being relatively thin, the white matter tracts of the auditory brainstem are also architecturally complex; the pathway has many bending, crossing, diverging, and converging tracts. Hence, to capture this detail it is recommended that data is acquired with high angular resolution, using the largest amount of diffusion gradient orientations that the available time and equipment permits.
- For the same reason stipulated above, it is recommended that the data is acquired using a high b-value (in the vicinity of 2500–3000 s/mm²; Tournier et al., 2013). Higher diffusion weightings increase angular contrast, which is beneficial for resolving complex fibre architectures (Alexander and Barker, 2005; Jones et al., 2013; Yeh et al., 2010). Beyond b-values of ~3000, however, the gain in angular contrast is easily offset by the associated reduction in SNR (Tournier et al., 2013).
- Following on from the previous point, the SNR is another important consideration. In general, when diffusion data is acquired using a higher spatial resolution (using smaller voxels) and higher b-values there is a subsequent decrease in the SNR (Jones et al., 2013). Poorer SNRs will result in

unreliable data, which reduces the accuracy of diffusion modelling techniques. Therefore, parameters must be carefully selected to ensure the SNR remains optimal. Furthermore, it is important to consider techniques capable of denoising diffusion data (up to a certain extent) after the image has been acquired (Veraart et al., 2016a, 2016b).

- For dMRI studies of the vestibulocochlear nerve, ways to mitigate off-resonance distortions should be considered. Off-resonance distortions occur at air/tissue and bone/tissue interfaces due to the difference in magnetic susceptibility between the tissue types. Such distortions can be caused by the mastoid air cells, which are located in the vicinity of the internal auditory canal. Hence, if not corrected for, off-resonance distortions can impact the quality of the diffusion data obtained from these regions (Peterson and Bammer, 2016). Off-resonance distortions can be mitigated in several ways: using multi-shot echo planar imaging rather than single-shot EPI, increasing the bandwidth, and/or increasing the parallel imaging factor (Jones, 2010; Liu et al., 2005; Peterson and Bammer, 2016).
- Ways to mitigate artefacts caused by cardiac pulsation are also particularly relevant to *in-vivo* dMRI studies of the brainstem. Cardiac pulsation is the motion of brain tissue caused by the pumping of blood through the arteries of the brain. Cardiac pulsation has been shown to be most significant in the brainstem and periventricular regions (Greitz et al., 1992) and can result in dMRI signal loss or dropout (Nunes et al., 2005). Cardiac pulsation can be accounted for during the acquisition phase by using cardiac gating. This feature ensures the dMRI data is acquired at particular phases of the cardiac cycle, in which the effect of pulsatile motion is significantly reduced (Nunes et al., 2005).

In conclusion, optimising acquisition is a balancing act. While, in theory, it is clear which parameters one would want to optimise, all parameters as well as the acquisition time share complex interactions. The longer the acquisition time, the greater the chance that subject motion is introduced, which must be considered. While motion can be corrected for up to a certain extent, its impact

might be particularly damaging when attempting to resolve small tracts.

4.2. Image pre-processing & dMRI modelling methods

Pre-processing, the procedure of correcting for artefacts present in the diffusion data after image acquisition, is an essential step in all dMRI processing pipelines. There are many suitable ways to deal with commonly encountered imaging artefacts such as, eddy current distortions, signal dropouts, subject motion, scanner vibrations and Gibbs ringing artefacts; for a comprehensive review please refer to Jones et al. (2013). The degree to which dMRI studies of the auditory brainstem are affected by these artefacts is likely to be similar, if not greater, than dMRI studies of other brain regions. Hence, pre-processing remains a vital component to dMRI studies of the auditory pathways.

Again, as a result of the complex fibre architecture of the auditory brainstem tracts, special consideration needs to be placed on the type of diffusion modelling techniques employed. Older models of diffusion, such as the diffusion tensor, will provide erroneous estimates of fibre orientations in these complex fibre configurations and far better alternatives are available nowadays. These alternatives include several higher-order models, some of which have been mentioned in this review, that are better able to deal with complex fibre arrangements and partial volume effects. A detailed discussion of the available higher-order models is outside the scope of the current review. Several relevant reviews are available: Dell'Acqua and Tournier (2018); Jones (2010); Jones et al. (2013) and Mori and Tournier (2013).

4.3. Tractography algorithms

Our final consideration is in regard to whether deterministic or probabilistic tractography is best suited to delineating the tracts of the auditory brainstem. In general, many of the inherent limitations encountered in tractography affect both deterministic and probabilistic tractography methods equally. Some of which include the inability to identify synapses, inability to differentiate afferent from efferent tracts, and constraints in delineating highly curved tracts (Jeurissen et al., 2017; Mori & Tournier, 2013). Additionally, the accuracy of both methods is reliant on the quality of the diffusion data, the acquisition parameters employed, and the type of diffusion model utilised. Despite the commonalities, probabilistic tractography algorithms may be better suited to reconstructing the tracts of the auditory brainstem because of their capacity to deal with areas of higher uncertainty of structure and challenging tract architecture. Probabilistic techniques are also more capable of dealing with noise (Bucci et al., 2013; Descoteaux et al., 2009). Utilising additional streamline filtering techniques such as anatomical constraints (Lemkaddem et al., 2014; Smith et al., 2012) and anatomical prior knowledge (Christiaens et al., 2014; Rheault et al., 2019) may also improve tracking accuracy. Further validation studies are ultimately required to determine the most appropriate tractography method for the delineation of the auditory brainstem tracts.

5. Summary

In summary, dMRI data and accompanying analysis methods offer a unique way to study the microstructure of white matter tracts within the human auditory system *in-vivo*. To date, there have been relatively few studies that attempt to validate the consistency and reliability of dMRI based analysis methods in delineating the central auditory pathways, particularly within the brainstem. In comparison, a much greater body of work exists for

other neural systems including the visual system (Chamberland et al., 2017; Kammen et al., 2016; Martinez-Heras et al., 2015) and the somatosensory systems (Ciccarelli et al., 2003; Thiebaut de Schotten et al., 2011). Further studies are necessary to ensure available methods produce tract reconstructions comparable to histological results. Overall, however, application of dMRI based analysis methods in the study of hearing disorders is growing, with dMRI techniques being used to investigate the effect of sensory hearing loss (Tarabichi et al., 2017; Vos et al., 2015), tinnitus (Crippa et al., 2010), vestibular schwannoma (Gerganov et al., 2011; Rueckriegel et al., 2016), ageing (Profant et al., 2014), and cochlear nerve deficiency (Wu et al., 2009) on the central auditory neural pathways. There is also the potential to use dMRI analysis techniques to predict cochlear implant outcomes (Huang et al., 2015), particularly in relation to those individuals who present with central pathologies of unknown aetiology (such as demyelinating or axonal neuropathies). As illustrated in this review, it is apparent that there are currently significant methodological differences across published research, which makes comparisons between studies challenging. Furthermore, it is evident that across the majority of the studies, presently, DTI is still the most prevalent method for reconstruction and analysis of white matter tracts within the auditory system. Given the limitations of the tensor model regarding 'crossing fibres' and partial volume as outlined above, this is of significant concern in the study of the auditory brainstem tracts, which are relatively small, diverge and converge frequently and share the same space as many larger fascicles. Advances in the field of dMRI have resulted in acquisition strategies such as HARDI and higher-order modelling techniques, which offer solutions to the crossing-fibre problem. Hence, moving forward it is recommended that these techniques be adopted to optimise the accuracy of fibre tract reconstruction.

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Appendix 2: Ethics approval & amendment



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HREC Number: 15/1254H
Project Title: Imaging the human auditory brainstem using MRI
Principal Investigator: Dr Bryony Nayagam

I acknowledge receipt of the revised Cover Sheet and Participant Information Consent Form.

I am pleased to inform you that the above project has received ethical approval from the Eye and Ear HREC and is consistent with the National Statement.

The HREC approval date is 11 January 2015, the HREC Number is 15/1254H, and approval is valid for five years.

The following documents have been approved:

- Project proposal, v1 dated 9 Nov 2015
- Participant Information and Consent Form, v1 dated 3 Nov 2015
- Participant Information and Consent Form Parent/Guardian, v1 dated 3 Nov 2015

The following researchers are approved for their described role in this project:

- Bryony Nayagam
- Farnoosh Sadeghian
- Gary Rance

The Royal Victorian Eye and Ear Hospital HREC is constituted and operates in accordance with the National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research (2007 and as updated) and in accordance with the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and the Health Privacy Principles in the Health Records Act 2001(Vic) and Section 95A of the Privacy Act 1988.

In order to comply with the National Statement and Good Clinical Practice requirements you are required to:

- submit an annual progress report for the duration of the project
- submit a comprehensive final report upon project completion
- notify the Secretariat of the project start date
- submit all proposed amendments to the project
- submit any adverse event reports

- report any unexpected developments in the project with ethical implications
- notify any changes of project Principal and all other Investigators
- advise if the project is completed prior to the anticipated date or is withdrawn
- preserve the confidentiality of information and records about research participants and only use information for the purpose specified in the approved protocol

Please note that any researcher named in the application did not participate in deliberations or decision making for this project.

Please use the HREC number in all future correspondence with the HREC Secretariat.

On behalf of the Committee, I wish you every success with your project.

Yours sincerely

A handwritten signature in dark ink, appearing to read 'K Baker', written in a cursive style.

Kerryn Baker
Secretary
Human Research Ethics Committee

13 January 2016

HREC Number: 15/1254H
Project Title: Imaging the human auditory brainstem using MRI
Principal Investigator: Dr Bryony Nayagam

I refer to your amendment dated 21 June 2017 requesting an amendment to personnel for project 15/1254H.

The HREC Chair approved the above amendment. This decision will be ratified at the next full HREC committee meeting in August 2017.

The following researcher/s were approved (subject to Eye and Ear appointments and scope of practice for researchers where required):

- Julien Zanin

Thank you for notifying the Committee.

Yours sincerely



Kerryn Baker
Secretary
Human Research Ethics Committee

28 June 2017

Appendix 3: MRI screening form

MELBOURNE BRAIN CENTRE

MAGNETIC RESONANCE IMAGING (MRI) SCREENING FORM

Name: _____
 Study: _____ SB Number: _____
 DOB: ____/____/____ Weight: ____kg Height: ____
 Gender: Male / Female Contact Phone Number: _____
 Contact Address: _____ P/Code: _____

1. Have you ever had surgery or an operation? No / Yes
 If yes, please indicate the date and type of surgery _____
2. Have you ever had a diagnostic imaging study or examination (MRI, CT, X-ray)? No / Yes
3. Have you ever had:

Epilepsy/Seizures:	No / Yes	Brain Infection:	No / Yes
Febrile Convulsions:	No / Yes	Psychiatric Disease:	No / Yes
Head Injury:	No / Yes	Other health issues:	No / Yes
4. Do you currently have any neurological symptoms (e.g. weakness, double vision)? No / Yes
5. Are you on any medication? No / Yes
6. Are you allergic to any medication? No / Yes
7. Do you have any history of renal disease? No / Yes

For female patients:

8. Date of last menstrual period: ____/____/____ or Postmenopausal? No / Yes
9. Are you pregnant or experiencing a late menstrual period? No / Yes

Further details:

For Office Use Only

Status: Control / Patient Time In: ____:____ Time Out: ____:____

Notes:

MELBOURNE BRAIN CENTRE



Certain implants, devices, or objects may interfere with the MR procedure. To help us to determine your suitability for an MRI scan and to ensure your safety, please complete the following checklist carefully.

Please indicate if you have ever had any of the following:

ITEM/DEVICE	NO	YES
Aneurysm Clips		
Cardiac Pacemaker / Defibrillator		
Neuro-stimulation system		
Implanted electrical device		
Vascular Surgery Clips		
Artificial Heart Valve		
Intra-ventricular or Spinal Shunt		
Metallic Stent / Filter or Coil		
Surgical staples, metallic sutures or metallic plates		
Eye Implant or Eye Operation		
An injury to your eye involving metal fragments?		
Cochlear or other Ear Implant		
Orthopaedic Devices (screws/rods/pins/plates/nails etc.)		
Any metallic fragment or foreign body		
Dentures (false teeth)		
Hearing Aid (Please remove before entering MR room)		
Piercing Jewellery		
Tattoo or Permanent Makeup		
Any other surgical procedures / operations / implants?		
If yes provide details:		
.....		
.....		



IMPORTANT INSTRUCTIONS

Before entering the MR room all metallic objects **MUST BE REMOVED**. These include hearing aids, dentures, glasses, partial plates, mobile phones, pagers, watch, hairpins, jewellery, body piercings, keys, coins, bank cards, magnetic strip cards, & pens.

Please consult the MRI Radiographer if you have any questions or concerns **BEFORE** you enter the MR room.

NOTE: You will be required to wear hearing protection during the MRI procedure.

I confirm that the above information is correct to the best of my knowledge. I have read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signed: _____ Date: _____

Parent or Guardian if under 18: _____ Date: _____

Witnessed: _____ Date: _____

MRI CONSENT FORM

Research studies carried out at Melbourne Brain Centre are designed to improve our knowledge and are not designed for clinical purposes.

After your scan a specialist will review the pictures; however this will not be done on the day of your study. You should be aware that sometimes even in completely healthy people, minor abnormalities are found. On the other hand, because the pictures are taken for a specific research purpose, not all abnormalities are necessarily seen. On extremely rare occasions, we might find an abnormality that is significant and which may need to be investigated further. If a significant abnormality is found, we will contact the researcher directly involved in your study.

Although such a finding is extremely unlikely, please take the time to consider carefully what it would mean to you. It would be entirely your choice as to what you might do with any such information. However, knowledge of an abnormality may affect your ability to do such things as work in certain professions, obtain life or health insurance, etc. If you do not want to know, then you are under no obligation to participate in this part of the study.

I confirm that I have read and understand the above information and that I have had the opportunity to ask questions. I confirm that I agree to have an MRI scan as part of this research study.

Signed: _____ Date: _____

Parent or Guardian if under 18: _____ Date: _____

Witnessed: _____ Date: _____

I have agreed to have an MRI scan as part of this specific research study, but I am aware that the data may also be useful for other studies in the future.

I therefore **AGREE / DO NOT AGREE** (please circle as appropriate) to the data being used for further research purposes.

Signed: _____ Date: _____

Parent or Guardian if under 18: _____ Date: _____

Witnessed: _____ Date: _____

